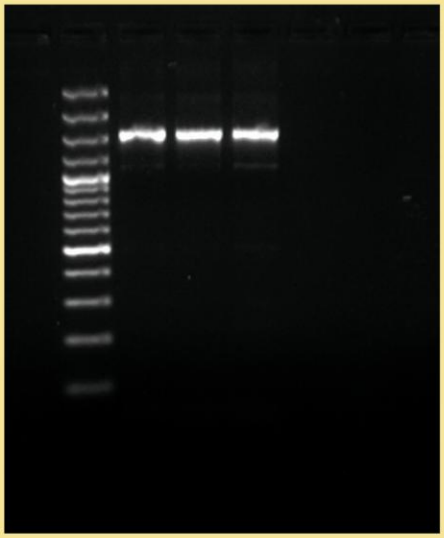
CIBA Special Publication : TM Series 2015 No. 1

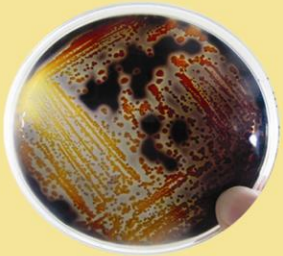
TRAINING MANUAL

on

Diagnosis, prevention and control of brackishwater finfish and shellfish diseases

31st August – 5th September, 2015





काकद्वीप शोध केन्द्र
भाकृअनुप–केन्द्रीय खारा जलजीव पालन अनुसंधान संस्थान
Kakdwip Research Centre
ICAR-Central Institute of Brackishwater Aquaculture (ICAR)
Kakdwip, South 24 Parganas, West Bengal- 743347

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Course Convener
Dr. Sanjoy Das

Edited by
Dr. Sanjoy Das
Dr. S. V. Alavandi

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CIBA

भाकृअनुप – केन्द्रीय खारा जलजीव पालन अनुसंधान संस्थान

ICAR - CENTRAL INSTITUTE OF BRACKISWATER AQUACULTURE ISO 9001:2008 CERTIFIED www.ciba.res.in

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डॉ. के.के. विजयन / **DR. K.K. Vijayan**

निर्देशक / **Director**

FOREWORD

Due to expanding world population, food security will be a major challenge for the future generations. Brackishwater aquaculture is the fast-growing finfish and shellfish farming sector in India and overseas, both in terms of production and export earnings. Sustainable growth of this sector ensuring economic viability, environmental friendly with a social relevance, is very much important in meeting food security of the present and the increasing Indian population. Frozen shrimp itself contributes around 64 % of export earning of India earned through export of fish and fishery products. But diseases problems and related crop losses in brackishwater aquaculture rearing systems very often act as major obstacle against growth of this sector. Various shrimp diseases caused by microbial pathogens in hatcheries and farms, such as pathogenic bacterial diseases, White spot disease (WSD), Early mortality syndrome (EMS), Infectious hypodermal and haematopoietic necrosis, etc. played havoc at different point of time reducing the aquaculture production leading to heavy economic losses. In India, during 2000-10 the economic loss due to white spot diseases alone was around ₹ 3000 crores, considering only the value of shrimp.

Both West Bengal and Odisha hold an important position in the brackishwater aquaculture production, considering vast areas under farming. Like other brackishwater aquaculture states, the shrimp production of West Bengal and Odisha also suffered a setback due to different diseases, especially WSD. Proper awareness on disease conditions and disease management, biosecurity measures, appropriate maintenance of soil and water quality and adequate awareness among farmers are very much important in prevention of diseases in these areas.

Scientists of Kakdwip Research Centre of Central Institute of Brackishwater Aquaculture is taking proactive involvement with stake holders of the region in the disease monitoring and disease management. The present training programme on

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'Diagnosis, prevention and control of brackishwater finfish and shellfish diseases' during 31st August to 5th September, 2015, is with the aim of creating awareness among the farmers of West Bengal and Odisha, on various aspects of diseases in brackishwater aquaculture system. I am also of strong opinion that this special publication brought out for the training programme will be extremely helpful for the trainees and related stakeholders. I hope the trainees will make best use of the training programme and manual and will gain both knowledge and experiences in combating different brackishwater aquaculture diseases.

I Appreciate my colleague scientists at the Kakdwip Research Centre of CIBA for conceiving the training programme. I wish all the best to all the trainees and hope the training programme will be successful.

Chennai

25th August, 2015

(K.K. Vijayan)

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डॉ. संजय दास / Dr. Sanjoy Das
वरिष्ठ वैज्ञानिक / Senior Scientist

PREFACE

Brackishwater aquaculture is highly potential food-producing sector of India characterized by its high growth rate and export earnings. But the occurrence of different diseases is one of the major limiting factors for the fast growth of this sector. The occurrence of White spot diseases (WSD) has paralyzed the shrimp industries of different brackishwater aquaculture producing states of India with number of outbreaks at different parts of country. In addition to these, other diseases such as Infectious hypodermal and haematopoietic necrosis (IHHN), Loose shell syndrome (LSS), Infectious myonecrosis, Hepatopancreatic parvovirus infection, White faecal syndrome, Running mortality syndrome, White patch disease, Monodon slow growth syndrome, Muscle cramp, etc. very often affect shrimp farms of different regions of India with production loss of varying degree. Moreover, the introduction of exotic species like *Penaeus vannamei* has potentiated the risk of transmission of different presently non-existent exotic diseases such as Early mortality syndrome, Taura syndrome, Yellow Head Diseases, etc. So, the thorough surveillance of all the diseases are highly required for prevention and control of diseases leading to better aquaculture productions and for all these, proper education and awareness among farmer are highly essential.

West Bengal and Odisha hold very important position as per brackishwater aquaculture production is concerned, but at the same different diseases especially WSD caused heavy damage to many shrimp farms of these regions. The present short-term training programme entitled 'Diagnosis, prevention and control of brackishwater finfish and shellfish diseases' is being organized to increase the awareness of different diseases among brackishwater aquaculture farmers of these regions and it will in-turn help the farmers in monitoring, prevention and control of diseases in their farms.

I am extremely grateful to Dr. K.K. Vijayan, Director, ICAR-CIBA for his precious suggestions and guidance in designing this training programme and also for the funding support. I take opportunity to thank Dr. S.V. Alavandi, HOD, AAHED for his valuable guidance and especially for the editorial work. We are also thankful to Dr. T.K. Ghoshal, Officer In-charge, Kakdwip Research Centre,

ICAR-CIBA for his constant support and encouragement in organizing the training programme and preparation of the manual. It is also my proud privilege to thank all the authors of the manual for spending their valuable time for preparation of the manuscript. My whole-hearted thanks are also due to Dr. Debasis De, Dr. Gouranga Biswas, Dr. Prem Kumar and Ms Christina Lalramchhani, who gave me moral strengths to complete this uphill task. We also sincerely acknowledge the helps received from all the permanent and contractual staffs of Kakdwip Research Centre for various kinds of co-operation in conducting the training programme. I have every hope that this training and training manual will be very much helpful to the farmers in controlling disease in their farms leading to higher production.

Sanjoy Das

Place: Kakdwip
Dale: 29.08.2015

(Sanjoy Das)
Course Convener

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An Overview of Brackishwater Aquaculture

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1.1. Introduction

Ever increasing human population coupled with decreasing availability of space for land-based food production system (such as agriculture, animal husbandry) has resulted shortage and inconsistent supply of quality food for large number of people across the globe. A realistic solution of the problem could be sustainable utilization of water resources as most of the global water resources are lying unutilized or underutilized. Amongst a variety of food items present in the aquatic system, fishes are considered as the most important group of the organisms suitable for human consumption. While capture fisheries is showing the signs of almost stagnation for more than a decade, aquaculture offers a vast scope of expansion.

India is the second most populous country in the world with a total population of 1.21 billion as in 2011 representing 17.5% of the world's population and occupying only 2.4% of the world's landmass. In line with the global trend, greatest challenge the country faces is to ensure food security of the largely undernourished protein starved population in rural as well as urban areas, especially in the context of declining land resources available for agriculture and animal husbandry. Hence fisheries, mainly aquaculture sector would have to emerge as the savior to meet increased food demand. With its limited resources of man and materials various central and state govt. agencies are making all out efforts at augmenting production by way of exploiting sustainably all potential sources of aquatic organisms in various kinds of water resources.

Indian aquaculture has demonstrated a six and half fold growth over the last two decades. Carp in freshwater and shrimps in brackishwater form the major areas of activity. Aquaculture in India is generally practiced with the utilization of low to moderate levels of inputs, especially organic-based fertilizers and feed. About 40% of the available 2.36 million hectares of freshwater resources and 13% of a total potential brackishwater resource of 1.24 million hectares is under use at present. This offers vast scope for both horizontal and vertical expansion of these sectors. As aquaculture plays vital role in socio-economic development in terms of income and employment, environment friendly aquaculture has been accepted as a tool for rural development. It also has huge potential for foreign exchange earnings.

Initiatives have also been taken up to use the unutilized and underutilized resources in several regions of the country. Issues like investment in fish and prawn hatcheries, establishment of aquaculture estates, feed mills, R&D support and ancillary industries have been given special emphasis to strengthen the pace of growth of the sector.

1.2. Origin and development

Brackishwater farming in India is an age-old traditional system confined mainly to the '*bheries*' (manmade impoundments in coastal wetlands) of West Bengal, '*gheris*' in Odisha, '*pokkali*' (salt resistant deep water paddy) fields in Kerala, '*khar lands*' in Karnataka and '*khazans*' in Goa coasts. These systems have been sustaining production of 500–750 kg/ha/year with shrimp contributing 20–25% with no additional input, except that of trapping the naturally bred juvenile fish and shrimp seed during tidal influx. In this traditional method, low lying areas near the banks of saline water rivers and creeks are encircled by peripheral dyke and tidal water is allowed to enter the impoundment along with natural seeds of various species of shrimps, crabs and fish. Water is retained with periodical exchanges during lunar cycles and the animals are allowed to grow.

Realizing the importance of shrimp farming in Indian economy, Central Inland Fisheries Research Institute (CIFRI) under the Indian Council of Agricultural Research (ICAR) established first Experimental Brackish water Fish Farm at Kakdwip, West Bengal in 1973. This was followed by inception of All-India Coordinated Research Project on Brackish water Fish Farming in 1975 by the ICAR with centres in West Bengal, Odisha, Andhra Pradesh, Tamilnadu, Kerala and Goa. At the same time, shrimp seed production studies were initiated by the Central Marine Fisheries Research Institute (CMFRI) of ICAR. Commercial scale shrimp farming started gaining roots only after 1988–1989 and the semi-intensive farming technology demonstrated production levels reaching 4–6 tons/ha. Area under shrimp farming increased substantially during 1990–1994 with remarkable growth rate till 1995 as the boom period of commercial-scale shrimp culture and the bust came in 1995-96, with the outbreak of viral disease. The fact that most of the farmers were new to commercial scale and high intensive shrimp farming, the general ignorance of good aquaculture practices and the lack of suitable extension services, led to a host of problems. Later with the advent of bio-secured closed culture technology using better management practices, semi-intensive and intensive shrimp farming again started to regain its lost glory during early years of the present century. Farmed shrimp production increased from 102940 tons in 2001-02 to record production of 144346 tons in 2006-2007 and operating at around 100000 tons over the years. The state wise area and production of shrimp is as follows:

Table 1: Areas (ha) under shrimp cultivation by state

State	1990	1994	1999	2014
West Bengal	33815	34400	42525	48410
Orissa	7075	8500	11332	6302
Andhra Pradesh	6000	34500	84269	36123
Tamil Nadu	250	2000	2670	7804
Kerala	13000	14100	14595	12917
Karnataka	2500	3500	3540	394
Goa	525	600	650	31
Maharashtra	1800	2400	970	1486
Gujarat	125	700	997	2359
Total	**65090**	**100700**	**161548**	**115826**

Table 2: Area (ha) under farming, production and percent of total production during 2013-2014

State	Area under farming	Production (mt)	Percent of total production
West Bengal	48410	52581	19.4
Orissa	6302	14532	5.4
Andhra Pradesh	36123	159083	58.7
Tamil Nadu	7804	25815	9.5
Kerala	12917	5175	1.9
Karnataka	394	664	0.2
Goa	31	63	0.02
Maharashtra	1486	3513	1.3
Gujarat	2359	9393	3.5
Total	**115826**	**270189**	**-**

Source: MPEDA, 2014

Brackishwater aquaculture development in India was mostly oriented till 2009 to tiger shrimp, *Penaeus monodon* culture only. Other shrimp species like *P. indicus*, *P. merguiensis*, *P. penicillatus*, *P. japonicus* and *P. semisulcatus* are not yet cultured on large commercial level. As tiger shrimp farming became regressed by viral diseases since 1995 and profitability was decreasing due to abnormal hike in input cost and decreasing unit sale value, Indian farmers were looking an alternative. In 2009, the Coastal Aquaculture Authority of India (CAA) permitted the entrepreneurs to introduce a new species, *P. vannamei* (Pacific white leg shrimp) in India with prescribed guidelines. Before introduction, risk analysis was carried out by Central Institute of Brackishwater Aquaculture (CIBA) and National

Bureau for Fish Genetic Resources (NBFGR) after pilot scale initiation in 2003. At the same time CAA is very keen in the bio security and approval for culture of *P. vannamei*. Since its introduction, vannamei farming showed rapid (Fig. 1).

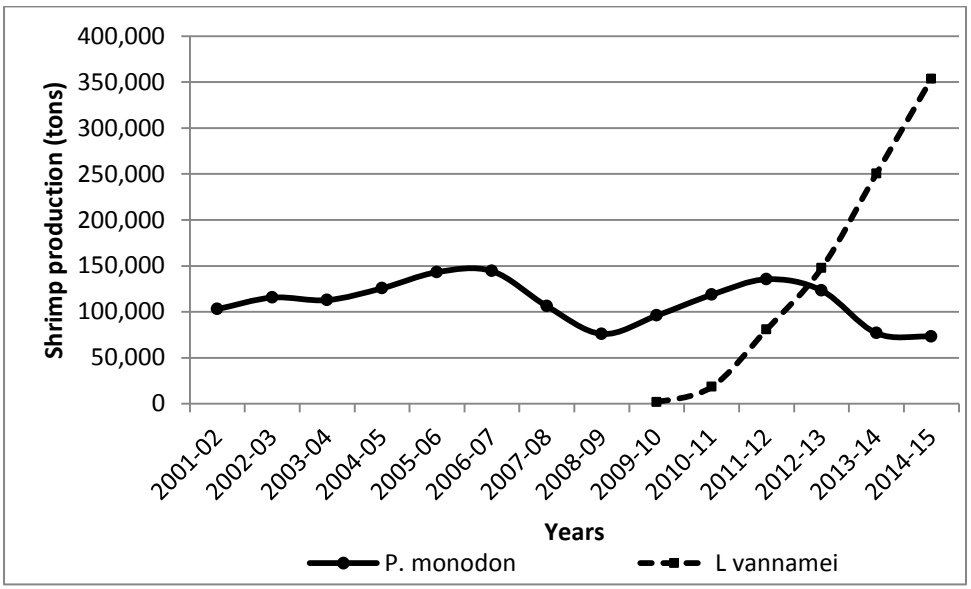

Fig. 1: Shrimp production in India from aquaculture farms

Among Indian states, Andhra Pradesh is leading in *P. vannamei* farming contributing nearly 80% of Indian production. Table 3 depicts state wise area under culture and production of *P. vannamei* (Pacific white leg shrimp) in India.

Table 3: State wise area under culture (ha) and production (tons) of *P. vannamei* in India

States		2009-10	2010-11	2011-12	1012-13	1013-14	2014-15
West Bengal	Area	0	0	0	0	130	326
	Production	0	0	0	0	479	395
Orissa	Area	0	0	25	46	485	2340
	Production	0		100	436	2907	11866
Andhra Pradesh	Area	264	2739	7128	20198	49764	37560
	Production	1655	16913	75385	133135	210639	276077
Tamil Nadu & Pondicherry	Area	0	34	397	1511	5087	5037.1
	Production	0	109	2863	8595	26281	32687.8
Kerala	Area	0	0	0	0	0	5.8
	Production	0	0	0	0	0	11.75
Karnataka	Area	0	0	72	154	157	124.76
	Production	0	0	232	484	517	623.2
Goa	Area	0	0	0	1	29	27.2
	Production	0	0	0	15	67	88.2
Maharashtra	Area	10	94	127	439	908	1274.51
	Production	30	508	941	1503	3291	4901.04
Gujarat	Area	9	64	88	366.71	707	3545.4
	Production	46	717	1195	3348.19	6326	26763
Total	**Area**	**283**	**2931**	**7837**	**22715.71**	**57267**	**50240.77**
	Production	**1731**	**18247**	**80717**	**147516.2**	**250507**	**353413.1**

Source: MPEDA (Kochi)

There is huge potential for mud crab farming in the country. Still there is no organized aquaculture of mud crab for supporting the export trade. Major reason is the non-availability or inconsistent availability of crab seeds for farming. Technology for seed production, culture and fattening of green mud crab *Scyllla serreta* has been developed by CIBA. Some farmers are practicing crab fattening in the coastal regions with considerable success. After the inception of crab hatchery by the Rajiv Gandhi Centre for Aquaculture (RGCA) in Nagapattinam district in Tamil Nadu, hatchery produced crab seeds are now available. Some progressive farmers have started crab grow-out farming with better performance of hatchery produced seeds compared to wild ones.

High value carnivorous fishes like Asian seabass (*Lates calcarifer*), snappers (*Lutjanus sp.*) and herbivorous/omnivorous fishes like Striped grey mullet (*Mugil cephalus*), Tade mullet (*Liza tade*), Parsia (*Liza parsia*), Milk fish (*Chanos chanos*) and Pearl spot (*Etroplus suratensis*) are available for farming in the Indian coastal ecosystem. In addition to this, fishes like *Mystus gulio*, are also being cultured. Successful technology has been developed for the seed production of Asian seabass under controlled conditions and farming by CIBA since 1997. Some enterprising farmers in Tamilnadu and West Bengal have taken up seabass culture. Monoculture of Asian seabass is in practice in those areas where cheap trash fish is available in plenty. Polyculture of Asian seabass following 'predator-prey culture' system using tilapia as prey material has also been tried with considerable success. In addition to this an avenue has come by successful breeding and seed production of cobia (*Rachycentron canadam*) using land based pond reared brooders by CIBA. Cobia farming in India is gaining momentum. Controlled breeding of grouper (*Epinephelus tauvina*), striped grey mullet and pearl spot has also been successful. Mullets, milkfish and pearl spot have shown promises for commercial aquaculture in inland saline soil / water areas. Production potential ranging from 0.5 to 3 tons/ hectare/ year has been demonstrated from such waters.

1.3. Farming systems

There are five different shrimp aquaculture practices mentioned in the literature, ranging from traditional to ultra-intensive techniques, but the most common techniques followed in India are traditional, extensive, semi-intensive, and intensive. These three categories are divided, according to their stocking densities (shrimp/ m^2), and the extent of management over grow-out parameters, i.e. level of inputs (Table 4).

Traditional culture practices dependent completely on the natural tidal entry for seed, food and water exchange. Furthermore, traditional systems are often characterized by polyculture with fish or by rotation with rice, e.g. in the *bheris* of West Bengal and *pokkalis* of Kerala in India. In this method of aquaculture low

lying areas near the banks of saline water rivers and creeks are encircled by peripheral dyke and tidal water is allowed to enter in the impoundment along with natural seeds of various species of shrimps, crabs and fishes. Water is retained with periodical exchanges during lunar cycles and the animals are allowed to grow. After 3–4 months harvesting is done partially during lunar cycles. Productivity in this system ranged between 500–750 kg/ha, of which about 30 percent is contributed by prawns/ shrimps and 70 per cent by mullets

Extensive shrimp aquaculture is primarily used in areas with limited infrastructure. Producers rely on the tides to provide most of the food for the shrimp and as a means of water exchange. Feed for shrimp is naturally occurring, in some cases fertilizers or manure is added to promote algal growth. Low stocking densities result in modest yields. Land and labour are the principal inputs, which keeps operational cost at a minimum. The extensive farming is commonly known as improved traditional farming. This system involves construction of peripheral canals/ ponds of size ranging from 1–5 ha. Shrimp seed at the rate of 15000 – 20000/ha are stocked. Water management is done by tidal effect. The average yield is 1500 – 1700 kg/ha, including fin fishes. In most of the cases, the stock is left at the mercy of nature and the predators. Supplementary feeding is not generally practiced as the entire production system is dependent on utilization of natural productivities. However some farmers use oil cakes and rice bran as supplementary feed.

Semi-intensive cultivation involves stocking densities beyond those that the natural environment can sustain without additional inputs. Consequently these systems depend on a reliable shrimp post larvae (PL) supply, and a greater management intervention in the pond's operation compared to extensive ponds. Semi-intensive shrimp aquaculture relies on water pumps to exchange up to 25% of pond volume daily; however, mostly closed culture is practiced at present. With stocking rates of 6-20 shrimp PL per m^2, fertilizers are applied to augment natural food in the ponds. Supplementary feeding is done using formulated feeds preferably in pelletized form. Maximum annual yields range from 2 to 6 tons per hectare. The risk of crop failure increases with increasing farming intensity, which is mainly due to the impact on water quality exerted by the high stocking densities and supplementary feeding. All of the costs associated with semi-intensive production are much higher relative to those for extensive production, including a more complex system of ponds, installation of a pump system to regulate water exchange, skilled management, labour, purchased feed and seed stock, and increased energy usage for aeration and other purposes. The higher the culture intensity, the higher the capital required and the higher the risks involved. Thus, the increased capital inputs required for semi-intensive culture often preclude its

adoption by small-scale producers. Tiger shrimp farming and low density pacific white leg shrimp farming in India falls under this category.

Intensive grow-out systems utilize ample supplies of clean sea / estuarine water, adequate infrastructure, and well-developed hatchery and feed industries. Intensive shrimp farming introduces small enclosures (down to 0.1 ha), high stocking densities (20-50 hatchery-produced shrimps/m^2), around-the-clock management, very high inputs of formulated feeds, and aeration. Aeration, the addition of oxygen to the water permits much higher stocking and feeding levels. Yields range from 7 to 15 tons per hectare per year. The risk of disease can be serious in intensive culture, especially if water discharge from one pond or farm is taken into another to be reused. Most of the *P. vannamei* farming in India is conducted in this method using specific pathogen free (SPF) seeds under strict biosecurity protocol.

Table 4: Farming practices based on level of management, stocking density and production followed in India:

	Traditional	**Extensive**	**Semi-intensive**	**Intensive**
Pond size (ha)	0.1-50	1-10	0.2-2	0.1-1
Stocking	Natural	Natural + artificial	Artificial	Artificial
Stocking density (seed/m^2)	Unregulated	2-6	6-20	20-50
Seed source	Wild	Wild + Hatchery	Hatchery + wild	Hatchery
Annual production (ton/ha/yr)	< 0.6	0.6-1.5	2-6	7-15
Feed source	Natural	Natural	Natural + Formulated	Formulated
Fertilisers	No	Yes	Yes	Yes
Water exchange	Tidal	Tidal + pumping	Pumping	Pumping
Aeration	No	No	Yes	Yes
Diversity of crops	Polyculture	Monoculture, polyculture rarely	Monoculture	Monoculture
Disease problems	Rare	Rare	Moderate to frequent	Frequent
Employment (persons/ha)	1-2	2-3	3-4	4-5

1.4. Human resource development through brackishwater aquaculture

Employment opportunities in coastal areas have increased greatly with the development of shrimp farming. According to an estimate, the average labour requirement in shrimp farming has been estimated to be about 600 labour

days/crop/ha. In contrast, labour days/crop/ha in paddy cultivation is 180, which is much lower than shrimp farming. Case studies carried out at a sea-based farm in the Nellore District of Andhra Pradesh showed an increase of 2–15 percent employment and 6–22 percent income for farm labourers following the establishment of shrimp farms. Hatcheries and feed mills in the brackish water sector are also providing substantial employment opportunities. Jobs generated in the main and supporting sectors of the shrimp aquaculture sector was estimated to be over three lakhs in India.

1.5. Laws and regulations

There are many laws and regulations, which are relevant to aquaculture adopted at state level, several key laws and regulations relevant to aquaculture at the central level. Those include the century-old Indian Fisheries Act (1897), which penalizes the killing of fish by poisoning water and by using explosives; the Environment Protection Act (1986) with provisions for all environment related issues; Water (Prevention and Control of Pollution) Act (1974) and the Wild Life Protection Act (1972). The Supreme Court prohibited the construction / set up of shrimp culture ponds except traditional and improved traditional types of ponds within the Coastal Regulation Zone (CRZ) on 11th December, 1996. It also ruled that an authority should be constituted to protect the ecologically fragile coastal areas, sea shore, water front and other coastal areas and specially to deal with the situation created by the shrimp culture industry in the coastal states / union territories. To perform the functions indicated by the Supreme Court, Coastal Aquaculture Authority was formed in accordance with the Environment (Protection) Act. The Authority, to which specific responsibilities for aquaculture have been allocated, falls under the administrative control of the Ministry of Agriculture.

1.6. Institutional setup

Division of Fisheries under the Department of Animal Husbandry, Dairying, and Fisheries of the Ministry of Agriculture, Govt. of India is the nodal agency for planning, monitoring and the funding of several centrally sponsored developmental schemes related to fisheries and aquaculture in all of the Indian States. Most of the states possess a separate Ministry for Fisheries and also have well-organized fisheries departments, with fisheries executive officers at district level and fisheries extension officers at block level, who are involved in the overall development of the sector. Centrally sponsored schemes are implemented through 422 (Fish Farmer's Development Agency) FFDAs in freshwater sector and 39 (Brackishwater Fish Farmer's Development Agency) BFDAs in the maritime districts substantially contributes to brackishwater aquaculture development. The ICAR under the Department of Agricultural Research and Education, which in turn is within the Indian Ministry of Agriculture, has a Division of Fisheries, which undertakes the

R&D on aquaculture and fisheries through a number of research institutes. There are about 400 Krishi Vigyan Kendras (KVKs) operated through State Agricultural Universities, ICAR Research Institutes and NGOs, most of which also undertake aquaculture development activities. The MPEDA functioning under the Ministry of Commerce, besides its role in the export of aquatic products also contributes towards the promotion of coastal aquaculture. Many other organizations like Department of Science and Technology, Departments of Biotechnology, University Grants Commission also support or conduct R&D in the subject. Various NGOs and private organizations contribute substantially in this context.

1.7. Research and Development

Eight fisheries research institutes are there under ICAR, the nodal agency for aquaculture research in India, of which CIBA, in Chennai is responsible for research on brackishwater aquaculture. These institutes have their regional centres located in different agro-ecological regions to undertake research on problems of regional importance. Research programmes are set depending on national priority and regional necessity, farmers' feedback is also given due emphasis. Fisheries colleges under different State Agriculture Universities, as well as other universities and organizations also undertake aquaculture research.

The institutes transfer the developed techniques and technology through research publications and on-farm demonstrations. To disseminate the emerging technologies, electronic media also play vital role.

1.8. Way forward

Exports will remain the mainstay of the sector for years to come. Institutional agencies focused towards this end must seek to examine the scope for diversification of markets, communicate to exporters and processors the niche markets that exist for exclusive markets for value added fish and shrimp. Non-tariff barriers to trade will continue to assume different forms and dimensions. It is the versatility and capability of the seafood industry to adapt that will enable them to survive such onslaughts on their territory. Domestic markets for fish and fish products not only provide an entirely new opportunity for growth but also can act as a buffer in case of gluts in the international markets. Institutional agencies such as National Fisheries Development Board (NFDB) have an onerous task on hand for enabling the domestic markets for fish to establish itself. Mud crab farming is one of the avocations started in the brackishwater sector recently to enhance the production of mud crabs as well as to uplift the socio-economic condition of coastal rural population. In the brackish water sector there were issues of waste generation, conversion of agricultural land, salinization, and degradation of soil and the environment due to the extensive use of drugs and chemicals and destruction of mangroves. Efforts towards adoption of improved farming

technologies like recirculatory aquaculture system (RAS), improved polyculture, integrated multitrophic aquaculture (IMTA) may make brackishwater aquaculture more environmentally acceptable.

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2

Health Management in Aquaculture: An Overview

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Aquaculture is considered as one of the fastest growing food sectors with current growth rate of 6.2% per annum. The sector involves farming of finfishes, crustaceans, molluscs and aquatic plants. Fish production through aquaculture is estimated to be 66.6 million tons, out of the 158 million tons of total fish production in 2012 (FAO, 2014). Among the cultured species, crustaceans are a valuable commodity both in domestic and international market. According to FAO, 2014, globally crustacean production accounts for 9.7% (6.4 million tons) of food fish aquaculture production by volume and 22.4 percent (USD 30.9 billion) by value. In India, farmed crustaceans contribute 2,99,926 metric tons and it represents 7.13% of the total farmed fish production of the country (FAO, 2014).

Despite the spectacular growth in aquaculture, the economic viability and sustainability of the sector had been threatened by frequent occurrence of diseases and the infectious diseases have become the major constraints in production. Brackishwater aquaculture in India, which is dominated by shrimp, has also been plagued by disease problems causing heavy economic loss and negative impact on the sustainable growth of aquaculture in the country. A multitude of factors have contributed to the widespread disease problems faced by aquaculture today. Aquaculture sector has undergone rapid expansion, intensification and diversification and the processes have to heavily depend upon large-scale movement of live aquatic animals and animal products such as broodstock, post-larvae, feed, etc. It has been recognised that this increased movement of live aquatic animals and their products has played a significant role in the spread and establishment of diseases in many aquaculture systems. Diseases in aquaculture, especially of cultured shrimp, are caused either by infectious or non-infectious aetiology. Majority of the diseases are caused by infectious agents such as viruses, bacteria, fungi and parasites. However, syndromes of non-infectious aetiology as well as cryptic diseases (diseases caused by obscure or unknown aetiology) are also significantly affecting aquaculture production.

Disease problems of substantial economic significance have been recorded in various aquaculture systems globally. Typical example is the shrimp aquaculture in several countries in Asia, South America and Africa affected by white spot disease (WSD) caused by white spot syndrome virus (WSSV), which resulted in heavy production losses. The disease continues to cause heavy mortality resulting in

heavy economic loss to the farmers and valuable foreign exchange for the countries. According to Oidtmann and Stentiford (2011), WSSV has become an endemic barrier to production in all known production zones through its global distribution with traded crustaceans. It is estimated that up to 40% of tropical shrimp production (> USD 3 billion) is lost annually, mainly due to viral pathogens. Besides WSSV, an emergent disease, acute hepatopancreatic necrosis disease (AHPND), originally known as early mortality syndrome, has spread through Southeast Asia to Vietnam, Malaysia, Thailand and to reach as far as Mexico in early 2013. The disease has caused ~60% drop in shrimp production in the affected region compared with 2012 and the global estimate of the losses per year is USD 1 billion. All these resulted in the prediction by FAO that global supply of shrimp would contract by 15% in 2015. In India, since 1994, diseases have emerged as the significant factor impacting production and thereby affecting the economic viability and sustainability of aquaculture. In a study conducted during 2006-08, it was observed that the gross national losses in the country due to shrimp diseases was 48717 metric tons of shrimp, valued ₹1022 crore and employment of 2.15 million man-days (Kalaimani et al. 2013). As the diseases have become the most important limiting factor determining aquaculture production, to achieve economic viability and sustainability in aquaculture, an effective strategy of health/disease management is crucial.

2.1. Health management strategies

The major objective of an effective health management protocol is to ensure not only improved production, but also to reduce the risk associated with diseases. According to the established protocol, a comprehensive health management focuses on all critical control points along the production pathway. This includes all levels of aquaculture activities from the production unit (hatchery, pond, tank, cage etc.), farm, local (district/zone), state to the national and international level. In other words, the management strategies can be formulated to deal with the disease problems broadly at two levels: At micro-level, this starts from hatchery/farm, where better management practices (BMPs) are practiced such as effective biosecurity measures including the stocking of pathogen-free stock and proper application of approved inputs and following scientific management practices including water quality and feed management. At a macro level, the strategy involves international and national perspectives in terms of implementing regulatory measures with respect to tranboundary movements of aquatic animals and products such as import risk analysis, quarantine and certification.

2.2. Components of health management

2.2.1. Farm/hatchery-level (Micro-level)

Although a variety of pathogens have been recorded and attributed as the cause of diseases outbreaks, diseases in aquaculture is largely an expression of complex interaction between host, pathogen and environment (as depicted in the Fig. 1). In this background, health maintenance or management at farm or hatchery-level is one of the most important aspects of successful aquaculture development and management. In this, accurate and timely diagnosis of diseases, health monitoring and application of various management measures play critical roles.

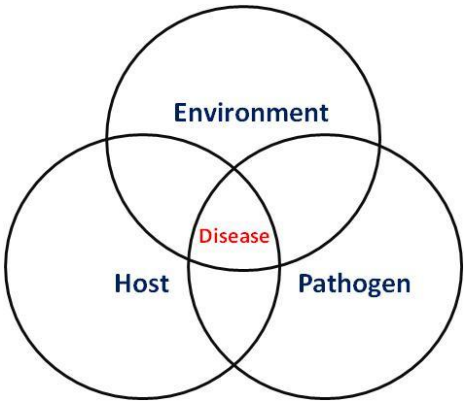

Fig.1. Interacting triangles depicting the relationships among host, disease agent and environment leading to diseases.

2.2.1.1. Diagnostics

It is a well recognised fact that control and prevention of diseases in aquaculture is a function of management, and diagnosis of diseases forms the fundamental step in any health management programme. In aquaculture, failure in the accurate and timely diagnosis of diseases would lead not only to large-scale mortality but also to indiscriminate use of drugs and chemicals thereby causing environmental contamination, drug residual effect and often occurrence of drug-resistant pathogens. Therefore, a specific, sensitive and rapid diagnostic tool is essential for any health management programme. Accordingly, disease diagnostic procedures in aquaculture have advanced from visual recognition and microscopic characterization of pathogens to molecular characterization and probe-based diagnosis. These advancements have helped aquaculture sector in a big way. These tools help not only in farm-level screening and monitoring of cultured animals, but also in disease surveillance, biosecurity measures and international trade. Further, development of efficient diagnostic assays for endemic as well as exotic pathogens is significant to achieve a kind of disease preparedness. Moreover, for international trade of frozen as well as live aquatic animals, diagnostic tools specified by international agency such as OIE is mandatory. Therefore, for successful health management, it is imperative that aquatic animal health laboratories have to focus

on developing, standardizing and refining the diagnostic tools for endemic, emerging and exotic diseases of aquaculture species.

2.2.1.2. Health monitoring

Constant monitoring of the health of cultured animals and the environment will help in detecting early stages of disease problem and responding before a disease manifest itself as uncontrollable outbreak. Further, since the changes in the health of cultured animals will be apparent only over a period of time, any change in the normal health of the animals would be obvious only when there is a combination of observation on the general appearance, feed consumption, growth, water quality etc. However, there would be difficulty in getting an accurate picture of the situation even after a constant monitoring, since in most disease cases the external signs are non-specific and it would be difficult to get enough information about the environmental problems that would predispose the animals to the disease as the environment in a pond would constantly fluctuate and it is not uniform. Nevertheless, it needs to be confirmed that conditions in the culture system are suitable for the animal's survival and healthy growth. Therefore, a stringent and constant health monitoring and accurate record keeping should be an integral component of a sound health management programme.

2.2.1.3. Health Management

As mentioned elsewhere, the major objective of an effective health management protocol is to ensure not only improved production, but also to reduce the risk associated with diseases. Further, an effective health management programme comprises steps and control measures that are carried out on a daily-basis. Overall, a science-based health management procedure includes seasonal factors and crop-planning, pond-preparation, post-larvae/fry selection and stocking process, water quality management, pond bottom management, feed management, fish health monitoring, farm record keeping, biosecurity measures, dealing with disease outbreaks, if any, and appropriate use of chemicals, if necessary. However, even the stringent health management procedure may not be able to eliminate the risk of diseases or mortality completely.

2.2.2. National and international level (Macro-level)

At a macro level, disease management strategies involve implementation of regulatory measures with respect to transboundary movements of aquatic animals and products such as import risk analysis, quarantine and certification. A number of strategies / approaches and efforts were made in the field of aquatic animal health management in India. These include the preparation of national strategic plan for aquatic exotics and quarantine, capacity building in disease diagnosis, harmonization of diagnostic tests and accreditation of PCR laboratories, developing, disseminating and implementing best management practices (BMPs) through

cluster-level approach. Further, disease information system and reporting are essential in dealing with diseases in the globalised era where transboundary movement of aquatic animals and products and culturing of exotic species are increasingly being undertaken. In this direction, for the first time, the country has implemented the National Surveillance Programme for Aquatic Animal Diseases recently. Moreover, post-*Penaeus vannamei* introduction, an Aquatic Quarantine Facility has been established for imported broodstock and strict biosecurity policy and guidelines are being implemented / followed.

2.3. Issues in aquatic animal health management

Despite a tremendous progress made in the field, there are several issues related to aquatic animal health management, which are vital to the sustainable development of aquaculture. These include lack of strong surveillance data on diseases of aquatic animals; lack of data on economic losses; limited information on chemicals and biologicals used in aquaculture and the environmental risks associated with that; continued reliance on imported broodstock; limitations in the effective implementation of biosecurity regulations etc. However, efforts are underway in addressing some of these issues. For the effective implementation of biosecurity, there is a need for increased awareness among the stakeholders. Further, application of biosecurity measures requires considerable research on diseases and epidemiology. In conclusion, it can be unequivocally stated that effective disease/health management for minimising the risk of disease-associated challenges in aquaculture can be achieved only through a coordinated efforts of all the stakeholders such as scientists, farmers, policymakers, developmental agencies and the industry. A science-based management strategy coupled with a coordinated effort and an effective implementation will be the way forward.

2.4. Summary

Aquaculture sector has undergone spectacular transformation through expansion, intensification and diversification. However, as a consequence, disease problems and the resultant production losses have been the major limiting factor in aquaculture. As sustainability of culturing native cultured shrimp, *P. monodon* had been severely affected by white spot disease, an alternative species, *P. vannamei* has been introduced to the country. As the diversification has taken place for augmenting production through introduction of non-native species, the transboundary movement of live aquatic animals has become a serious issue because of the threat posed by the alien species as a potential source of exotic pathogens and a threat to the native biodiversity. This necessitated the strengthening of aquatic animal quarantine and health management system in the country. Though many issues significant to health management in aquaculture prevail in the country, a concerted effort is underway in addressing these issues by

research and developmental organisations through research and technology development, capacity building and awareness. Further, formulation of effective policy and strict implementation of guidelines by regulatory authorities are also playing a crucial role in minimising the risk of diseases and thereby achieving sustainable aquaculture production.

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3

White Spot Disease: Prevention and Control

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3.1. Introduction

Aquaculture is a rapidly expanding industry, which augments cheap protein to a large portion of world population. In terms of value, shrimp is considered as the single largest seafood commodity because of its high export value. More than 75% of shrimp production is in the form of aquaculture and several modifications are being constantly carried out to increase the production. However, adoption of high stocking density, increase use of inputs and lack of proper biosecurity has resulted in the outbreak of diseases. All most all kinds of microorganisms have been found to be capable of producing some forms of disease to shrimp. However, the emergence of viral diseases since 1981 has made the situation more complicated and the industry is constantly facing huge loss due to mortality and crop failure. So far more than 20 viruses have been reported from shrimp and new viruses are frequently being added up to the existing list. Amongst all the viruses, white sot syndrome virus (WSSV) many times acts as a single most causative agent threatening the sustainability of shrimp aquaculture industry.

3.2. White spot syndrome virus (WSSV)

WSSV is a rapidly replicating and highly virulent shrimp virus that has wide spread presence throughout the world. Originating from Taiwan in 1992, it spread to Japan during 1993 and subsequently very quickly to all other Asian countries. By 1995, it had already spread to North America and further by 1999 to South America. The quick spread of this virus to different regions and simultaneous investigation by different scientists speculated the same agent to be different ones and thereby called them in different names such as Systemic Ectodermal and Mesodermal Baculovirus (SEMBV), rod shaped nuclear virus of *Penaeus Japonicus* (RV-Pj), Hypodermal and Haematopoietic Necrosis Baculovirus (HHNBV), third *Penaeus monodon* non-occluded virus (PmNOB III), penaeid rod shaped DNA virus or white spot baculovirus. On subsequent investigation and data analysis when it was known that all these names are for the same agent, it was unanimously called as white spot syndrome virus. Other than the penaeid shrimps which serve as host for this virus, a large number of other crustaceans serve as carrier and therefore it has been impossible to eradicate this virus from the culture system. The typical clinical symptoms of WSSV infection are the formation of circular whites spots on

the carapace. However, there can be many other reasons for this and therefore the infection should get confirmed by various diagnostic protocols.

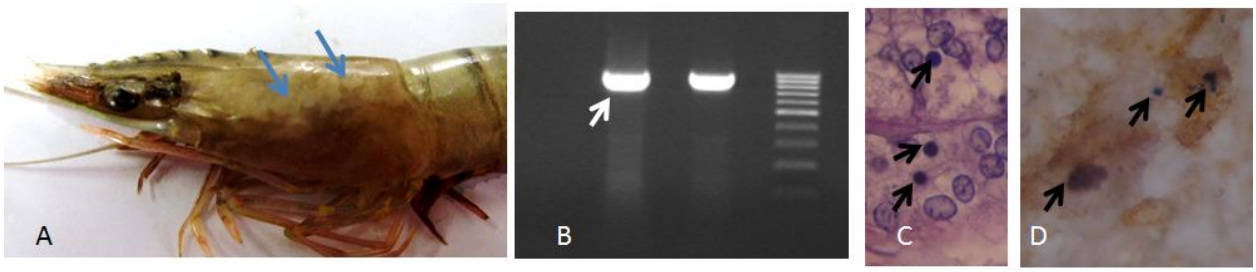

Fig. 1: Typical (circular white spot on the carapace) clinical sign of WSSV (A); The detection of the virus by PCR (B); Histopathology (C) and *in situ* hybridization (D)

WSSV is a double stranded DNA virus with approximately 300 kb genome size. Different genotypes of this virus with differential virulence properties have been reported. When compared with other viruses, genetic structure of WSSV is considered as unique in many aspects and therefore this is placed in an entirely new family, Nimaviridae and genus *Whispovirus*.

3.3. Prevention and control

3.3.1. Virus exclusion and preventing the entry

3.3.1.1. Genetic selection

Wherever possible, specific pathogen free (SPF) and specific pathogen resistant (SPR) animals should be stocked to avoid the disease occurrence. After determining the disease resistance markers, animals can be selected for the production of totally disease resistance progenies. Where complete resistance strain production is not possible, care can be taken to produce at least pathogen free stocks as has been tried for *Penaeus vannamei*. As in this case the seed will be free from major disease causing agents such as WSSV and subsequent better management practices can be followed to get a successful harvest.

3.3.1.2. Screening of larvae

Stress test should be carried out to choose the healthy larvae. Further, the larvae should be screened by PCR for the presence of virus. Early, accurate and sensitive detection of pathogens are important and at least a nested PCR protocol should be carried out to rule out the presence of WSSV in larvae.

3.3.1.3. Better management practices (BMPs)

Once the larvae are shifted from the hatchery to the pond, it is subjected to enormous stress. Stress is the main factor to initiate disease and this can be overcome to a large extent through BMP. Soil and water qualities are the important

aspects for health management. Ponds should be prepared adequately to ensure it as pathogen free. Sufficient gap should be there between the culture cycles to adequately prepare the ponds. WSSV can remain viable and infectious for considerable time period in the ponds. Therefore, ponds should be dried sufficiently before the real preparation.

3.3.1.4. Biosecurity

As has been already mentioned, WSSV is a highly virulent virus and has a wide host range. Utmost care should therefore be taken to prevent the spread of the causative agents. Source water, seed, equipment, workers and invasive organisms are the main source of virus spread. Step by step protocols and precautions should be taken from the beginning to avoid the entry of the pathogens to culture system. If strict biosecurity measures are adopted, disease can be avoided to a large extent. Adoption of proper sanitation protocols, development of a reservoir pond, bird fencing and crab fencing are some of the common biosecurity measures to avoid the spread of WSSV.

3.3.1.5. Development of recirculatory/zero water exchange culture system

Water can be an important source of infection as far as WSSV is considered. Care should be taken to avoid the virus from the intake water. Recirculatory/zero water exchange will ensure the prevention of pathogen entry into the system. Culturing the virus free larvae and adopting better management practice, it will then possible to prevent the entry of WSSV into the system.

3.3.2. Prevention of the spread

Once a disease outbreak occurs, it becomes a rich source of pathogen to contaminate the nearby brackishwater fishery resources. Therefore, it is necessary to confine the infected pond and do immediate treatment to ensure complete pathogen killing. Sufficient biosecurity and emergency planning should be in hand to prevent the spread through carrier aquatic organisms or birds.

3.3.3. Boosting the immunity and increasing disease resistance capacity

3.3.3.1. Immunostimulants

Non-specific immune system of shrimp is the primary defence against a wide range of pathogens. This system can be stimulated through various microbial and plant based products to provide better protection. Lipopolysaccharide (LPS) from gram negative bacteria and peptidoglycan/beta glucan from gram positive bacteria / yeast are widely used immunostimulants. Different plant based products with proven medicinal properties have been successfully used as immunostimulants to provide partial or full protection. In addition to providing protection against diseases, these immunostimulants have also been found useful to provide better

growth. Because of the lack of memory in shrimp, it is necessary to apply these immunostimulants very frequently during a culture period for successful protection. Similarly, the shrimp larvae can also be treated through immersion or feed to have better protection during the initial critical period of culture.

3.3.3.2. Adoption of Biofloc technology

This recently developed eco-friendly technology where beneficial microorganisms are multiplied in an ecosystem through the manipulation of carbon and nitrogen ratio has been found to be useful in boosting the immune system and thereby increasing the disease resistance capacity. Similar to probiotics, the microbes produced here maintain the water quality and at the same time stimulate the immune system of shrimp when taken as feed. Through this technology, the wastes generated are efficiently utilized and uses of many additional inputs are also avoided. Therefore, this technology has several added advantages to avoid disease problems, particularly from that of WSSV.

3.3.3.3. Use of probiotics

Probiotics are a group of 'good bacteria', which are proved to improve the host immune system and thereby provide good health when consumed. These bacteria go and colonize in the gut. In this way they occupy the space and do not allow the pathogenic bacteria to settle down. They also produce specific molecules which has ability to stimulate the host immune system. Based on its application, probiotics in aquatic system can be of two types. Gut probiotics does the usual function in replacing the pathogenic bacteria and stimulating the immune system. Whereas the water probiotics helps in increasing the diversity of good bacteria in water and thereby do not allow the multiplication of pathogenic bacteria. These bacteria are also known to secret extracellular products that has inhibitory effect against harmful bacteria.

A number of probiotics products, consisting of several bacteria species such as *Lactobacillus* spp., *Bacillus* spp., *Pseudomonas* spp., etc either as single species or as consortium, are available for the use both in fish and shellfish culture. Experimental evidences regarding precipitation of WSSV by secondary bacterial infection has been generated. Therefore, during this period, application of probiotics will prevent the multiplication of pathogenic bacteria and thereby prevent the quick precipitation of disease.

3.3.4. Control measures

3.3.4.1. Preventing the precipitation

There are evidences where farms can continue with WSSV and have successful harvest. This is mainly by preventing the precipitation. The virus can remain in a

latent/dormant stage and cannot cause disease. This is mainly through good water quality maintenance, BMP and other precautionary measures.

3.3.4.2. RNA interference (RNAi)

Experimentally, this technology has been proved to be very useful for the control of several pathogens including WSSV. It has been particularly very effective for the control of many viral diseases where treatments through medicines are not possible. Specific virulence genes of the pathogens are targeted to develop short RNA fragments (either single or double stranded) and this is either injected or supplied through oral route after modification. This brings degradation of the pathogen through post translational modification. The RNAi system has been found to be functional in shrimp. This method is particularly look promising for the treatment of viral diseases of shrimp where neither any treatment methods nor any vaccines are available. Several experiments have been carried out targeting some of the common virulent genes of WSSV such as VP28, VP19, VP15, rr1, rr2 etc. Unfortunately, many of these protections have been demonstrated through injection which is not a practical method during culture practice. However, the injection method to produce viral fee larvae will be helpful for some of the penaeid shrimps like Penaeus monodon where development of SPF stocks has not been possible.

3.3.4.3. Development of anti-WSSV medicine

A lot of medicinal plants have shown to have antiviral molecules and have been effectively used against a wide range of animal and human viral pathogens. Such types of screening from plants for the development of anti-WSSV molecules should also be tried seriously. Similarly many of the marine macro and microorganisms are also a good source of antiviral molecules and effort should be there for their screening. The newly developed concept, “synthetic biology” should also be applied to synthetically develop different molecules already proven to have anti-viral effect.

3.4. Conclusion

WSSV is a highly virulent pathogen that can bring mass mortality within a few days of time period. It can be a single most important factor in threatening the sustainability of shrimp aquaculture practice. Though, it has been more than two decades after its emergence, no solution to this problem has been possible to develop. In this context, general management practices to prevent the entry through viral exclusion will be a better mechanism. However, further research should be carried out to develop an effective medicine for control measure.

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4

Currently Prevailing Diseases of *Penaeus vannamei* Farming in India

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

The increasing trend in intensification and commercialization has exacerbated the epidemics of diseases and become a major constraint for the sustainability of this industry. Severe disease related mortality and thereby economic loss due to different viral agents such as monodon baculovirus (MBV) in Taiwan, infectious hypodermal and hematopoietic necrosis virus (IHHNV) in the Americas, yellow head virus (YHV) in Thailand and Taura syndrome virus (TSV) in the Americas during the different periods have been reported. In addition to all these, the major disease outbreak due to White Spot Syndrome Virus (WSSV) has also been reported from all parts of the world. During the recent past, the havoc created by Early Mortality Syndrome (EMS) in different South East Asian countries

has also been found to have severe economic impact on shrimp industry of these regions. Further, a number of other diseases by unidentified etiologies are constantly being associated with shrimp culture practice which has been responsible either for direct mortality or growth reduction and thereby bringing loss to farmers. Shrimp hatcheries are also prone to losses either due to disease outbreak or other unknown factors.

Some of the problems associated with current shrimp culture practice in India are discussed below. In addition to the already existing WSSV problems, the industry at present is going through a very critical phase due to several uncharacterized factors.

4.1. Currently prevailing diseases in hatcheries

4.1.1. Luminescent bacterial diseases

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

4.1.2. Zoea II syndrome

In recent years after the introduction of *P. vannamei* in India, Zoea II syndrome causing mass mortalities has been widely reported by the hatcheries. Due to the delayed moulting and mortality at zoea II stage, this syndrome was assigned with the name Zoea II syndrome. This was first reported in 1993 from the *P. vannamei* shrimp larvae of Ecuador, Mexico and the United states. *Penaeus stylirostris*

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

4.2. Currently prevailing diseases in Grow-out systems

4.2.1. White faeces syndrome (WFS)

White faeces syndrome reported since last decade, has recently been noted as serious problem for *P. vannamei* throughout the world. However, this disease has been reported from both cultured black tiger shrimp and pacific white shrimp. White faeces syndrome usually occurs after 60 days of culture (DOC) and it may be accompanied by high shrimp mortality. Ponds affected with white faeces syndrome show white faecal strings floating on the pond surface while the shrimps show white/golden brown intestine, reduced feed consumption, growth retardation and often associated with loose shell. The disease can cause moderate to severe economic loss by reducing the shrimp survival by 20–30 percent when compared to normal ponds. While investigating the aetiology of WFS this disease has been associated with presence of

vermiform like gregarine bodies, vibriosis, *Enterocytozoan hepatopenaei*, blue green algae and loose shell syndrome. Sriurairatana et al (2014) revealed that 96% of the ponds exhibiting WFS presented vermiform bodies resembling gregarines. When the contents of the gut or faecal strings were examined in squash mounts with the light microscope, they consisted of masses of vermiform bodies that superficially resembled gregarines. Bacteriological results showed that total bacteria and *Vibrio* spp. found in haemolymph and intestine were significantly higher in diseased shrimp than in healthy shrimp. Six species of fungi (*Aspergillus flavus, A. ochraceus, A. japonicus, Penicillium* spp., *Fusarium* spp., and *Cladosporium cladosporioides*) were isolated from shrimp naturally infected with white faeces syndrome. Histopathological examination revealed diffused haemocyte encapsulation and dilated hepatopancreatic tubules accompanied by necrosis. Tangprasittipap et al., 2013 revealed that the microsporidian newly found in *P. vannamei* is nonspecific with previously described *E. hepatopenaei* and it is not causally associated with WFS. Sriurairatana et al. (2014) concluded with Transmission electron microscopy (TEM) study that vermiform structures superficially resembling gregarines and commonly found now in the HP of Asian cultivated shrimp are not independent organisms but result from the transformation, sloughing and aggregation of microvilli from the HP tubule epithelial cells themselves and the denuded epithelial cells subsequently undergo lysis, can lead to the phenomenon called white faeces syndrome (WFS) and transformed microvilli (ATM) in very severe cases they may retard shrimp growth and may predispose shrimp to opportunistic pathogens. Furthermore it has been estimated that the Thai production losses due to WFS in 2010 were 10–15%. The cause of white faeces syndrome and treatment is uncertain. However reduced stocking density, proper water exchange together with better management practices will be helpful in evading White Faeces syndrome (WFS).

4.2.2. White muscle syndrome

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

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

4.2.3. Running Mortality Syndrome (RMS)

Since 2011, a new syndrome has brutally affected the shrimp industry and causing substantial mortality. The disease has been loosely termed as Running Mortality Syndrome (RMS) by the farming community. The affected ponds show different mortality patterns with unusual symptoms, no relation to any known diseases and a slow mortality rate (e.g. <1%/day), but the cumulative loss over phase will be high. Some farmers have lost up to four crops, with mortality percentage reaching 70% in most of the cases. Generally mortalities start after a month or 40 days of culture (DOC) but a portion of shrimp continue to survive and can grow to fully harvestable size. Investigations (conducted in ICAR-CIBA) revealed no association of Running Mortality Syndrome (RMS) with known shrimp viral infection. Further, bacteriological examination of haemolymph samples of RMS affected shrimp indicated predominance of *Vibrio* spp., such as *Vibrio parahaemolyticus* and *Vibrio azureus*. The population of anaerobic bacteria in the gut of RMS affected shrimp ranged from 72 - 252 x 10^{14} cfu mL^{-1} and were identified based on16S rRNA gene analysis as *Enterococcus faecium*, *E hirae*, and *Lactobacillus plantarum*. Bacterial diversity of RMS affected shrimp gut examined by Denaturing Gradient Gel Electrophoresis (DGGE) revealed a number of uncultured bacterial sequences. Histopathological examination of the hepatopancreas was largely normal. However, some samples showed karyomegaly and increased inter hepatopancreatic tubular space with haemolymph infiltration, muscle necrosis, loosened LO tubule cells and constricted lumen. Bioassay experiments carried out by feeding RMS affected shrimp tissue to healthy 13-14 g shrimp did not elicit any disease in the experimental shrimp. All the experimental animals were healthy and active even after 44 hrs of feeding RMS affected shrimp tissue like that of control animals. RMS affected shrimp showed recovery and appeared healthy and active after 155 hrs of transferring to wet lab in water with optimal parameters. Co-habitation experiment with healthy shrimp and the infected animals also failed to induce RMS. All shrimp appeared healthy and active. Relatively few studies done on Running Mortality syndrome, and still the causative agents or aetiology of RMS are unknown.

4.2.4. Size variation/ Growth retardation

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

4.2.5. White gut disease

This disease of *P. vannamei* is mostly caused by different species of *Vibrio* and is very much prevalent in Andhra Pradesh and Tamilnadu. Vibrios are normally present in water bodies. But sometimes stressed environmental factors such as sudden change of environment and salinity, low DO, mechanical injury, higher stocking density, etc. caused rapid multiplication of this organism in the gut and hepatopancreas. Vibriosis caused red discolouration and melanization of appendages (red disease), necrosis of tail, broken antennae, etc. Six species of *Vibrio* viz. *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus* and *V. splendidus* are generally associated with the diseased shrimp. The diagnosis of the disease can be done by isolation of organism by plating haemolymph on the TCBS agar followed by identification of the species of *Vibrio* by biochemical tests or 16S rRNA gene sequencing.

4.2.6. Muscle cramp syndrome (MCS)

This disease is mainly caused by environmental stresses, mainly low DO and sudden rise of environmental temperature and is especially common when the stocking density is high. MCS is very much prevalent in Tamilnadu, Andhra Pradesh and West Bengal. The body of the shrimp bends and stiffness of the muscle observed. The rate of mortalities varies. The increase in duration of aeration circumvents the problem to some extent.

4.2.7. Black gill disease

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

4.3. Conclusion

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

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5

Diseases of Brackishwater Finfishes

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Diseases in cultured brackishwater fishes are one of the major hurdles in successful and sustainable farming. Different bacterial diseases in brackishwater finfishes are very often severe and result in huge mortality and economic loss to the farmers. Bacterial diseases spread mostly horizontally through infected water and by the introduction of infected stock. Most of the diseases result in mortality or stunted growth or produce ulcers. Most of the diseases can be prevented by stocking diseases free stock and by following simple bio-security measures like, drying the pond before stocking, removing all wild fish in the pond, disinfecting nets, utensils etc., removing the dead fish immediately, frequent water exchange etc. Once a disease outbreak is seen quick diagnosis and control measures have to be adopted to reduce the mortality and spread of the disease. The following are some of the major diseases affecting brackishwater fishes.

5.1. Bacterial diseases

5.1.1. Aeromonad septicaemia

Aeromonad septicaemias are caused by *Aeromonas hydrophila* and to certain extend by *A. caviae*. The bacteria are ubiquitous and are commonly found in soil and water samples. It is also found in the gut and tissues of healthy freshwater and marine fishes. These organisms are Gram negative, motile rods measuring 0.3-1.0 to 1.0-3.5 µm in size. The bacteria are not fastidious and can be easily isolated in nutrient agar. The colonies appear within 24 hrs and are white, circular and convex. Selective media such as Rimler-Shotts agar containing novobiocin and *Aeromonas* isolation media containing ampicillin can be used to isolate the bacteria from samples containing other bacteria. The disease occurs when the fishes are stressed due to over-crowding, poor water quality, handling, high water temperature etc. Affected fishes exhibit darkening of the skin, haemorrhages on the skin and the base of fins. Shallow ulcers may develop at haemorrhagic sites. Congestion and haemorrhages can also be observed in the internal organs. The affected fishes may be treated with antibiotics like oxytetracyline and sulphonamides. Improving the water quality with frequent water exchange and removal of dead and moribund fish will reduce mortality.

5.1.2. Vibriosis

Vibriosis is caused by *Vibrio anguillarum*, *V. alginolyticus* and *V. parahaemolyticus*. Members of the family Vibrionaceae are Gram negative, straight or curved rods. They are commonly found in marine and brackishwater. The causative agents can be isolated in media containing seawater salts and common nutrient media containing 1-2% sodium chloride. Thiosulphate citrate bile salt sucrose (TCBS) agar is a selective medium for isolation of pathogenic *Vibrio* species. Colonies appear smooth, convex and white and develop within 48 hrs. *Vibrio* species can be differentiated form *Aeromonas* by their sensitivity to vibriostat 0/129. The bacteria are present in the alimentary tract of normal health fishes. The incubation period varies with temperature and virulence of the strain.

The disease occurs in late summer when water temperature is high. Mortality may reach 50% in young fish. The affected fish are anorectic with darkening of the skin. In acute infections deep necrotic skin ulcerations with blood coloured exudate is observed. Spleenomegaly is a common feature with petecheal haemorrhage on most of the internal organs. In chronic cases skin ulcers may become granulomatous. Gills become pale and corneal opacity is frequent. Although commercial vaccines are available in developed countries, they are not available in India. Antibiotic therapy with oxytetracyline and sulphonamides is the practical method of reducing mortality during outbreaks.

5.1.3. Columnaris disease

This disease is caused by a bacterial organism called *Flexibacter columnaris* and is important in different freshwater fishes. As per brackishwater fishes are concerned, Asian seabass (Bhetki / Barramundi) especially at juvenile and nursery stages are susceptible. The saddle-shaped lesion in the mid-body position near dorsal fin is seen and is mostly associated with over-stocking, poor hygiene and skin trauma. Treatment can be done by dipping affected fish with copper sulfate (2 mg/Litre) for 1-2 min or by application of potassium permanganate at the rate of 4-6 mg/Litre to the affected ponds.

5.1.4. Septicaemia and Organ rots

Gill rot in pearls spot (*Etroplus suratensis*) is caused by *Klebsiella pneumoniae,* a Gram negative bacterium under Family Enterobacteriaceae. The affected fishes exhibit isolated movement, anorexia, restlessness, orientation against current and gill tissue decay. Tail rot disease in pearl spot is characterized by loss of natural colour, swimming near water surface etc. This disease is caused by *Proteus vulgaris*, a Gram negative bacterium. *Pseudomonas aeruginosa* causes Haemorrhagic septicaemia in pearls spot. In this disease, the body of the affected fishes becomes reddened with swollen belly, septicaemia, inflamed anus, spleen, swim bladder and anaemia.

5.2. Viral diseases

5.2.1. Viral nervous necrosis (VNN)

Viral nervous necrosis (VNN) is one of the important diseases of brackishwater fishes affecting a wide range of fishes. The causative agent of the disease, viral nervous necrosis virus (VNNV), a betanodavirus has four genotypes viz, barfin flounder nervous necrosis virus (BFNNV), red spotted grouper necrosis virus (RGNNV), striped jack nervous necrosis virus (SJNNV) and tiger puffer necrosis virus (TPNNV). The disease affects early larval and juvenile stages of seabass causing upto 100% mortality. The virus is transmitted both horizontally and vertically. The virus also produces persistent infection especially in the adults resulting in asymptomatic carriers which act as a source of infection to larval and juvenile stages. Vaccination of juveniles and young adults appear promising in protecting the fish. Vaccination of brooders provides protection to larval stages through maternal transfer of immunity. Viral screening of broodstock should be done by Reverse-transcriptase PCR. Regular disinfection of the hatchery premises with chlorine (50 ppm) should be done. Fertilized eggs should be disinfected with different disinfectants like ozone (1 mg/L for 1 min). Separation of larvae/ juveniles from brooder will minimize the risk of disease transmission. An experienced fish health professional may be contacted for help.

5.2.2. Diseases caused by Iridoviruses

Iridovirus infection affects Asian seabass and farmed red sea bream (*Pagrus major*) with moderate to very high mortality, especially in the juvenile stages. The disease is generally transmitted by horizontal route through water. The incidence of the diseases is generally more during summer season. The viruses belonging to two genera *viz. Lymphocystivirus* and *Ranavirus* are considered as causal agents of this disease. Among these, the viruses under genus *Ranavirus* causes systemic disease leading to heavy economic losses, while the *Lymphocystivirus* causes localized infection and is usually not lethal.

The affected fish becomes lethargic and anaemic. The petecheal haemorrhage is seen in gills with enlargement of spleen. The mortality rate depends upon different environmental factors like age, water temperature, water quality and other culture conditions and it varies greatly from 0 to almost 100%. Diagnosis is mainly based on immunological detection of pathogen by IFAT (Indirect Fluorescent Antibody Test), and spleen and kidney tissue are the most suitable organ for this pathogen detection. On histopathology of liver and spleen with Giemsa staining, abnormally enlarged cells with very deep stain are observed. The viruses can be observed directly in the infected tissues by electron microscopy. ELISA has also been developed for detection of viral antigen. Different molecular methods like PCR

and real time PCR can also be employed for detection of this virus with high degree of specificity and sensitivity.

For control of Iridovirus infection, a good aquaculture practice is very helpful. These include stocking of pathogen free fish, maintenance of good water quality, avoidance of overcrowding and overfeeding, etc. However, the virus is sensitive to potassium permanganate, formalin and sodium hypochlorite. For red sea bream Iridovirus infection, a formalin-killed vaccine is commercially available.

5.3. Parasitic diseases

5.3.1. Argulosis

Argulosis is caused by a large ectoparasite, *Argulus*, commonly called as fish lice. It is the most important branchiurans parasite belonging to the family Argulidae. These parasites are dorsoventrally flattened measuring up to 1 cm in length. They are commonly found in the skin and fins of freshwater fishes and to a lesser extent in brackish water fishes. The trauma induced by the parasite due to the attachment and feeding method causes haemorrhagic ulcers and leads to secondary bacterial infection. Affected fish show lethargy, irritation and loss of appetite. It is practically difficult to eradicate *Argulus* in culture waters as the adults and larval stages are active swimmers. Infested fish can be treated with formalin or organophosphorus insecticides. Drying the ponds and tanks between cycles will reduce *Argulus* infestation.

5.3.2. Marine Ich

Marine Ich, caused by *Cryptocaryon irritans* is one of the common salt-water parasitic ciliate infestation. Some of the common signs of marine Ich are rubbing on the pond side or bottom, increased mucus secretion, breathing problems, loss of appetite, abnormal swimming behaviour, frayed fins, cloudy eyes, and white spots especially on the dorsal side. Due to presence of typical white spot on the body, this disease is also known as 'white spot disease'. The incidence is high with poor water quality and over-crowding. The parasite infests almost all marine and brackishwater teleosts. Fishes should be quarantined and only those free of any parasites have to be taken to the hatchery. Marine Ich can be diagnosed by microscopic examination of skin and gill scrapings. *Cryptocaryon irritans* can be observed as 0.3-0.5 mm structures with multi-lobed nucleus. The parasite can be kept away by maintaining good water quality. Infected fish can be treated with formalin @ 100 ppm (100 mg/Litre) for 1 hr for 3 days or copper sulphate @ 0.5 ppm (0.5. mg/Litre) for 7 days or by immersing the fish in freshwater for one hr daily for three days.

5.3.3. Trichodiniasis

Trichodiniasis in Asian seabass is caused by a ciliated protozoa called *Trichodina* spp. and the target organs of this parasite are skin and gills. In the affected fishes, heavy mucus production takes place around the gills leading to clogging of gills and respiratory distress. The disease can be controlled by acriflavin treatment and formalin bath.

5.3.4. Amyloodiniosis or Velvet disease

Amyloodiniosis caused by *Amyloodinium ocellatum,* a dinoflagellate is one of the most frequently encountered pathogens affecting tropical marine ornamental fishes. Amyloodiniosis is also called as 'marine velvet'. The symptoms of amyloodiniosis are difficulty in breathing, sluggishness, pale gills, excess mucus secretion, rubbing its surface against objects in the aquarium and anaemia. The parasite initially infects the gill and subsequently spreads throughout the body giving a velvety appearance and thus the name marine velvet. Affected fish appear dark in colour and emaciated. Amyloodiniosis can be easily diagnosed by microscopic examination of gill and skin scrapings. The condition can be treated with copper sulphate @ 0.5 ppm (0.5 mg/Litre) for 4-5 days or bath treatment with formalin @ 200 ppm (200 mg/Litre) for 1 hr. Good water quality is also advocated.

5.3.5. Monogenean infestation

These are ectoparasites that infest skin, gill and fins and are commonly known as gill or skin flukes. The common monogenean encountered in brackishwater fishes are *Dactylogyrus*, *Gyrodactylus*, *Diplectanum* and *Benedenia*. During heavy infections, these parasites can cause high mortality in fry and fingerlings. High stocking density combined with poor water quality will result in high incidence of monogenean infestation. Clinical signs include lethargy, high mucus production, rubbing of body against substrate, abnormal swimming behaviour and anorexia. Diagnosis is simple by observing the parasites from gill and skin scarping under microscope. Treatment includes formalin dip @ 100 ppm or dichlorvos @ 1 ppm for 1 hr.

5.3.6. Copepod infestation

Copepods are crustacean parasites having free living and parasitic stages. The important copepods infecting cultured brackishwater fishes are *Caligus* spp (sea lice), *Ergasilus* Spp. (gill maggots) and *Lernaea* spp. (anchor worm). Parasites are introduced into the culture system through water, live feed, wild fish and contaminated tools and equipment. Poor water quality and over crowding leads to heavy infestation with copepods. *Caligus* can cause serious damage if present in large numbers. The damage is caused by pre-adult and adult stages, which abrade the skin surface and feed on cutaneous and subcutaneous tissues. The parasite is

introduced into farmed stock though introduction of wild fish. Heavy infestation with copepods results in mechanical damage, impaired respiration, petecheal haemorrhage, anaemia and emaciation. These parasites also act as mechanical vectors for other bacterial and viral pathogens. Diagnosis can be done by simple microscopic examination of the gills and skin. Adult anchor worms are visible to the naked eye. Copepod infestation can be controlled using fresh water bath for 15 min or by using hydrogen peroxide @ 1000 ppm or formalin @ 100-200 ppm for 60 min. Complete draining, disinfecting and drying of the tank periodically help to break the life cycle of the parasite.

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6

Emerging Microbial Diseases and Issues in Brackishwater Aquaculture

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Shrimp is the largest single seafood commodity by value, accounting for 17% of all internationally traded fishery products. Approximately 75% of production is from aquaculture, which is now almost entirely dominated by two species – the black tiger shrimp (*Penaeus monodon)* and the Pacific white shrimp (*Penaeus vannamei*) that represent the two most important invertebrate food animals. Disease has had a major impact on the shrimp farming industry. Since 1981, a succession of new viral pathogens has emerged in Asia and the Americas, causing mass mortalities and threatening the economic sustainability of the industry.

6.1. Emerging viral pathogens of shrimp / prawn

Almost all shrimp pathogens are transmitted vertically and disease is the result of a massive viral amplification that follows exposure to various forms of environment or physiological stress. Stressors can include handling, spawning, poor water quality or abrupt changes in temperature or salinity. Shrimp viruses can also commonly be transmitted horizontally and once viral loads are high and disease is manifest, horizontal transmission of infection is accompanied by transmission of disease. Viruses listed by the OIE as causing notifiable diseases of marine and freshwater shrimp/prawn are as below.

6.1.1. White spot syndrome virus (WSSV)

White spot syndrome first emerged in Fujian Province of China in 1992. It was soon after reported in Taiwan and Japan and has since become panzootic throughout shrimp farming regions of Asia and the Americas. It is the most devastating disease of farmed shrimp with social and economic impacts over 15 years. Although first emerging in farmed kuruma shrimp (*P. japonicus*), WSSV has a very broad host range amongst decapod crustaceans, all of which appear to be susceptible to infection. However, susceptibility to disease varies and some crustacean species have been reported to develop very high viral loads in the absence of clinical signs. All farmed penaeid shrimp species are highly susceptible to white spot disease, with mass mortalities commonly reaching 80–100% in ponds within a period of 3-10 days. Persistent, low level infections in shrimp and other crustaceans occur commonly. The amplification of viral loads and onset of disease can be induced by environmental or physiological stress, or at ambient temperatures below 30°C.

6.1.2. Taura syndrome virus (TSV)

Taura syndrome virus first emerged in *L. vannamei* farms on the Taura River near Guayaquil in Ecuador in 1992, almost simultaneously with the emergence of WSSV in kuruma shrimp in China. The disease spread rapidly throughout most shrimp farming regions of Central and South America. In 1998, it was detected in Taiwan and has now spread throughout much of Asia. Acute, transitional (recovery) and chronic phases of TSV infection have been described. Mortalities in the acute phase can be as high as 95%, but surviving shrimp remain infected and a potential source of virus transmission. The susceptible host range of TSV is far more restricted than that of WSSV but includes most farmed marine shrimp species. However, susceptibility to disease varies and virulence varies for different strains of the virus. The rapid spread of TSV in the Americas and then to Asia has been attributed to the international trade in live shrimp.

6.1.3. Yellow head virus (YHV)

Yellow head virus is the most virulent of shrimp pathogens, causing total crop loss within several days of the first signs of disease in a pond. It first emerged in *P. monodon* in Central Thailand in 1990 and has since been reported in most major shrimp farming countries in Asia, including India, Indonesia, Malaysia, the Philippines, Sri Lanka, Vietnam and Taiwan. There is also a recent unconfirmed report that YHV is present in farmed *P. vannamei* and *P. stylirostris* in Mexico. Many other penaeid and palemonid shrimp species have been shown to be susceptible to experimental infection with YHV, but yellow-head-complex viruses are detected rarely in other penaeid shrimp species and *P. monodon* appears to be the natural host. A recent study has indicated that ~30% of yellow-head-complex viruses detected in *P. monodon* from across the Asia-Pacific region are recombinants. The prevalence and geographic distribution of these recombinant viruses suggests that aquaculture and the international trade in live shrimp are the source of rapidly increasing viral genetic diversity.

6.1.4. Infectious hypodermal and haematopoietic necrosis virus (IHHNV)

Infectious hypodermal and haematopoietic necrosis was first detected in Hawaii in 1981, causing mass mortalities in *P. stylirostris* farmed in super-intensive raceways. Following its initial detection in Hawaii, IHHNV was found to be widely distributed in both *P. stylirostris* and *P. vannamei* throughout farming regions of the Americas. Although it does not cause mortalities in *L. vannamei*, IHHNV has been shown to reduce growth by up to 30% and cause deformities of the rostrum and anterior appendages in a condition called “runt deformity syndrome”. In Asia, IHHNV is endemic and occurs commonly in *P. monodon,* which appears to be the

natural host and in which it does not cause disease and has no impact on growth or fecundity.

6.1.5. Infectious myonecrosis virus (IMNV)

Infectious myonecrosis is one of the most recent emerging viral diseases of shrimp. It first appeared in farmed *P. vannamei* at Pernambuco in Brazil in 2002 and has subsequently spread throughout coastal regions of north-east Brazil and to Indonesia, Thailand and Hainan Province in China. The original source of infection is unknown but the trans-continental spread has almost certainly been due to the voluminous trade in *P. vannamei* broodstock. Shrimp with the acute form of the disease display various degrees of skeletal muscle necrosis, visible as an opaque, whitish discolouration of the abdomen. Surviving shrimps progress to a chronic phase with persistent low-level mortalities. Several farmed shrimp species have been reported to be susceptible to infection but disease has only been reported in *P. vannamei*. The increasingly common practice in parts of Asia of co-cultivation of *P. vannamei* and *P. monodon* is likely to present opportunities for adaptation and further spread of the disease.

6.1.6. White tail disease (WTD)

White tail disease is an emerging infection of the giant freshwater prawn *Macrobrachium rosenbergii*. It was first reported in 1995 from the island of Guadeloupe and then nearby Martinique in the French West Indies and has since been reported from China, Taiwan, Thailand, India and Australia. The disease can affect larvae, post-larvae and early juvenile stages, causing up to 100% mortalities within 5–7 days of the first gross signs, which include a white or milky appearance of abdominal muscle. Adults are resistant to the disease but can be persistently infected and transmit the infection vertically. *Penaeus indicus, P. monodon* and *P. japonicas* have been shown to be susceptible to this infection, but did not develop disease. *Artemia* and some species of aquatic insects appear to be vectors. As the native endemic range of *M. rosenbergii* is restricted to south and south-east Asia, the wide geographic distribution of the disease most likely has been due to the movement of stock for aquaculture purposes.

6.2. Bacterial diseases

6.2.1. Vibriosis

Vibriosis is ubiquitous throughout the world and all marine crustaceans, including prawns, are susceptible. Epizootics occur in all life stages, but are more common in hatcheries. Major epizootics of vibriosis have been reported for *P. japonicas, P. monodon* and *P. vannamei*. Vibriosis is caused by a number of *Vibrio* species of bacteria, including *V. harveyi, V. vulnificus, V. parahaemolyticus, V. alginolyticus*, *V. penaeicida,* etc. *Vibrio* species are part of the natural microflora of wild and

cultured shrimp and become opportunistic pathogens when natural defence mechanisms are suppressed. They are usually associated with multiple aetiological agents. However, some *Vibrio* species, or strains of certain species, have been identified as primary pathogens. Pathogenic strains of *V. harveyi, V. vulnificus* and *V. parahaemolyticus* have caused massive epidemics. Mortalities due to vibriosis occur when shrimp are stressed by factors such as poor water quality, crowding, high water temperature and low water exchange. High mortalities usually occur in post-larvae and young juvenile shrimp. Shrimp suffering vibriosis may display localised lesions of the cuticle typical of bacterial shell disease, localised infections from puncture wounds, loss of limbs, cloudy musculature, localised infection of the gut or hepatopancreas and/or general septicaemia. Lesions of bacterial shell disease are brown or black and appear on the body cuticle, appendages or gills. Affected post-larvae may display cloudy hepatopancreas. Gills often appear brown. Septic hepatopancreatitis is characterised by atrophy of the hepatopancreas with multifocal necrosis and haemocytic inflammation.

6.2.2. Necrotizing hepatopancreatitis (NHP)

The clinical signs of NHP disease in an infected shrimp include lethargy, emaciation, soft shells, heavy fouling from external parasites, black gills and reduced growth. The digestive gland (hepatopancreas) degenerated - appears pale to white. NHP is caused by a species of alpha-proteobacterium that infects the hepatopancreas of shrimp, also referred to as NHP bacteria. Crustaceans known to be susceptible to the disease are *P. stylirostris, P. aztecus*, *P. setiferus, P. vannamei* and *P. californiensis*. 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). The disease is not transmitted either vertically (from parent to offspring) or through cannibalism.

6.2.3. Early mortality syndrome (EMS)

A new/emerging shrimp disease known as early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) has been reported to cause significant losses among shrimp farmers in South-east Asian countries. Since EMS was first reported in China in 2009, it has spread to Vietnam, Malaysia and Thailand, and now causes annual losses more than USD 1 billion. EMS outbreaks typically occur within the first 30 days after stocking a newly prepared shrimp pond, and mortality can exceed 70% (reaching up to 100% in some cases). The disease affects both *P. monodon* and *P. vannamei*. Clinical signs observed include slow growth, corkscrew swimming, loose shells, as well as pale coloration. Affected shrimp also consistently show an abnormal hepatopancreas (shrunken, small,

swollen or discoloured). The primary pathogen has been identified as a member of the *Vibrio harveyi* clade, most closely related to *V. parahaemolyticus*. The effects of EMS in both *P. monodon* and *P. vannamei* appear to be limited to the hepatopancreas (HP) and show lack of mitotic activity in generative E cells of the HP, dysfunction of central hepatopancreatic B, F and R cells, prominent karyomegaly and massive sloughing of central HP tubule epithelial cells and terminal stages including massive intertubular haemocytic aggregation followed by secondary bacterial infections.

6.3. Other emerging diseases of shrimp

6.3.1. White gut/faeces syndrome

The major observations reported on shrimp with white faeces syndrome are as below:

- Vermiform, gregarine-like bodies within hepatopancreas and midgut.
- Show no cellular or sub-cellular organelles, but the cause is unknown.
- No gregarine, other protozoan or metazoan involved.
- Microvilli found peeled away from HP tubule cells and aggregated in HP tubule lumen.
- Stripped of microvilli, the originating cells undergo lysis.
- Loss of microvilli and subsequent cell lysis indicate a pathological process.
- May retard shrimp growth and may predispose to opportunistic pathogens.

6.3.2. Enterocytozoon hepatopenaei (EHP)

EHP is a microsporidian parasite that has been widely found in Asia and other parts of the world, is impacting aquaculture production by severely retarding the growth of cultured shrimp. Although the most common pathology associated with microsporidians is a whitish discoloration in muscles due to spores that can stunt growth and cause other types of problems, EHP is different. It only infects the tubules of the hepatopancreas in shrimp, which damages the ability of these critical organs to gain nutrition from feed. It is widely understood that EHP does not cause mortality, but heavily limits growth. About 100 genera of microsporidians are known to infect crustaceans and fish. EHP is now endemic throughout China, Malaysia, Thailand, Indonesia and Vietnam, and likely present in India and possibly Mexico. It can likely be found anywhere that has imported live feeds from China and animals from areas where EHP is endemic. EHP is very difficult to eradicate.

6.4. Factors contributing to disease emergence in shrimp

The increasing rate of emergence of diseases of shrimp has been driven primarily by anthropogenic influences, the most profound of which have been associated with the global expansion of aquaculture. Farming of aquatic animals commonly

involves displacement from their natural habitat to an environment that is new and sometimes stressful, the use of feeds that are sometimes live and often unnatural or artificial, and culture in stocking densities that are much higher than occur naturally. This has provided opportunities for exposure to new pathogens and conditions that can compromise defensive responses and facilitate pathogen replication and disease transmission.

Emerging viral diseases of shrimp are usually caused either by (i) viruses that naturally infect the target species but are unobserved or not normally pathogenic in wild or unstressed populations, or (ii) the spill-over of viruses from other species that may not be encountered naturally. In shrimp, IHHNV, yellow-head-complex viruses and possibly MrNV appear to be naturally endemic in healthy wild populations and have emerged as significant pathogens only as a consequence of aquaculture practices. In the case of IHHNV, disease emergence has been due to the translocation of the natural host, *P. monodon*, from the Philippines to Hawaii and the Americas for use in aquaculture breeding programs, allowing spill-over into susceptible western hemisphere shrimp species. For viruses in the yellow head complex, the natural prevalence can approach 100% in some healthy wild *P. monodon* populations, but stressful culture conditions, in combination with yet uncharacterized virulence determinants, appear to trigger disease outbreaks. WSSV, TSV and IMNV each appear to have been initially introduced to shrimp populations from unidentified sources that could potentially include experimental live or frozen feeds or co-inhabitants of terrestrial pond environments such as insects or aquatic invertebrates. In the case of WSSV, aquaculture has also provided opportunities for spread of infection to a very wide range of new wild crustacean hosts in which the virus has now become endemic across a vast coastal area of Asia and the Americas. For shrimp, the prolific international trade in live broodstock has been the major driver of the explosive trans-boundary spread of emerging viral pathogens. The rapid spread of WSSV throughout Asia and then to the Americas has been attributed to the movement of live crustaceans. The magnitude of this problem is most clearly exemplified by the mass translocation of many hundreds of thousands of Pacific white shrimp broodstock from the Americas to Asia, accompanied by the introduction of TSV, IMNV and possibly other exotic pathogens. The international trade in frozen commodity shrimp and shrimp products has also been recognized as a potential mechanism of trans-boundary spread of disease. Other diseases have been spread by natural or unintentional movement of infected hosts or amplified by invasive species, while anthropogenic environmental pressures have caused changes in the severity of several endemic diseases.

6.5. Economic and social impacts of emerging diseases of shrimp

The impacts of emerging diseases of aquatic animals have been substantial. The most devastating economic and social impacts have been in shrimp aquaculture for which it was estimated in 1996 that the global direct and indirect costs of emerging diseases had reached USD 3 billion annually or 40% of the total production capacity of the industry. In many cases, impacts have continued for many years, particularly for small low-income farmers in developing countries who lack the proper knowledge, skill and resources to respond effectively. WSSV has been by far the most devastating of the shrimp pathogens. It has been estimated that the impact of WSSV in Asia alone during the 10 years after its emergence in 1992 was USD 4–6 billion. In the Americas, the emergence of WSSV in 1999–2000 resulted in immediate losses estimated at USD 1 billion. The combined impacts of TSV and IHHNV on aquaculture and wild shrimp fisheries in the Americas have been estimated at USD 1.5–3 billion. The consequences of disease emergence for some countries have been so severe that shrimp production has never fully recovered. Beyond the direct effects on production and profitability, disease impacts on the income and food-security of small-holder shrimp farmers and the job security of workers on larger farms and in feed mills and processing plants, with a flow-on effect to the sustaining local communities.

6.6. Diagnostic methods and control measures

An important aspect of any disease control program is the easy and convenient availability of rapid and reliable pathogen detection methods together with the ability to interpret results and apply them in a proper manner in health management programs. PCR and RT-PCR methods have been very important in helping to control the spread of major shrimp disease agents, but they have the disadvantage of requiring sophisticated equipment and highly trained personnel. Recently, lateral flow chromatographic immunodiagnostic strips similar to common drug-store pregnancy tests have begun to appear for some shrimp diseases. Using these, unskilled farm personnel can easily diagnose shrimp disease outbreaks at the pond side. The strips are relatively cheap and give an answer within 10 min. Other methods comparable to PCR and RT-PCR are now available or being developed for single and dual to multiple viral detection, but they too currently require advanced equipment and personnel. However, the impact of newly-emerging pathogens will be counteracted by the rapid response time for their characterization and for development of diagnostic tools and by the use of domesticated specific pathogen free (SPF) stocks in biosecure grow-out ponds.

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7

Biosecurity and Quarantine Measures for Aquaculture Health Management

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Aquaculture has rapidly grown during the last couple of decades, contributing significantly to the National economic development through export of fish and fishery products and providing food security to the country. Indian aquaculture sector has achieved remarkable growth during the past five years especially, with respect to the shrimp production through aquaculture after the introduction of Pacific white shrimp. During 2014-15, seafood exports crossed 10,51,243 tons valued at ₹ 33,441.61 crore (USD 5511.12 million). However, disease related losses in aquaculture have been a challenge to achieve higher productivity. Such setbacks have been experienced countries world over.

Aquatic animals are widely translocated across countries for enhancing aquaculture productions and species diversification. Such trans-boundary movement of live aquatic animals has the risk of introduction of new diseases. Responsible fisheries emphasizes the need to minimize the risk of disease transfer and adverse effects on wild and cultured stocks associated with the introduction of non-native species and transport of eggs, larvae, brood stock and other live organisms. Presently two amphibian, ten fish, seven molluscan and eight crustacean diseases have been listed by the World Organization for Animal Health (OIE). In mollusks, parasitic diseases are important, while in fish and crustaceans viral diseases are cause of concern. Whether a listed disease is due to a virus, fungus, bacterium or a parasite, the occurrence of the disease may adversely affect international trade among trading partners that have, or do not have, the listed disease. The purpose of this article is to emphasize the importance of quarantine measures and biosecurity of aquaculture in minimizing risks of disease and maximizing aquaculture productions.

7.1. Introduction of exotic species, import risk analysis and quarantine

Introduction of exotic species has both benefits and adverse impact. The exotics may be introduced with a view to enhancing growth, genetic up-gradation of local stocks, as biological control agents, disease resistance and for ornamental purpose.

The introduced species may escape into natural waters and establish in the wild. The adverse effect associated with exotics can be divided in to three main categories i.e. ecological, genetic and health. Ecological impacts may be due to habitat alteration, competition and predation. The genetic impact may result in the loss of native germplasm and genetic diversity, which has evolved through centuries to adapt to our conditions. The exotic may introduce pathogens of concern in the open water and destroy the native species. For the economic, social and trade development, movement of live aquatic organisms across national boundaries is necessary even though such activities may lead to the introduction of new pathogens and pose risks to the importing country. However, movement of live aquatic animals into our country should be considered only after critically examining the options for utilizing the natural biodiversity of India. The import of live aquatic organisms has to be considered taking into consideration the Convention on Biodiversity (CBD) as well as the Biodiversity Act of India.

As the movement of live aquatic animals involves certain degree of risk to the importing country, an import risk analysis (IRA) to assess the possible risk associated with the import is imperative. The main components of IRA are hazard identification, risk assessment, risk management and risk communication. Though various countries use different methods, they should be science-based and transparent with detailed documentation. The importing country has an obligation to ensure that IRA is scientifically sound, adequately documented and critically evaluated as per international obligations. The claims about its own aquatic animal health status should be accurate and based on scientific data and rigorous surveillance as demanded by the exporting country. The exporting country should also ensure that the information provided on its health status is accurate and based on international standards. It also has an obligation to report any significant change in the health status to all trading partners and international conventions. In India, *Penaeus vannamei*, a non-native species of shrimp, has been introduced to brackishwater aquaculture system as an alternative to *P. monodon*, culture, recently. The introduction was mainly driven by the negative impact of white spot disease (WSD) caused by white spot virus (WSV) on the sustainability of *P. monodon* culture. The introduction of this exotic species was facilitated by the Coastal Aquaculture Authority and ICAR-CIBA and NBFGR carried out import risk analysis prior to the introduction of the species in the country and recommended for establishment and maintenance of quarantine facility by Government.

The strategy of the aquatic quarantine and health management system, primarily involves the protection of a country's aquatic biodiversity from exotic organisms, pathogens and containment of the diseases. The guiding principles in establishing a quarantine policy for responsible movement of aquatic organism and products have been formulated by consensus among various countries. According

to FAO (2006), *"Quarantine means maintaining a group of aquatic animals in isolation with no direct or indirect contact with other aquatic animals, in order to undergo observation for a specified length of time and, if appropriate, testing and treatment, including proper treatment of the effluent waters*." Detailed aquatic animal quarantine guidelines are provided in Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals and the Beijing Consensus and Implementation Strategy (FAO/NACA, 2000). For the success in formulating and implementing aquatic quarantine, health management and certification system, it is necessary to identify pathogens of concern. Viruses such as white spot virus and bacteria such as vibrios are of great concern. International Aquatic Animal Health code requires that all the member countries make available through OIE whatever information is necessary to minimize the spread of important pathogens and their etiological agents to assist in achieving better worldwide control of these diseases. The OIE has two lists of Diseases of Aquatic animals i.e. diseases notifiable to OIE' and 'other significant diseases'. These diseases, especially the notifiable ones, are of significance in international trade. At present, the OIE notifiable and other significant diseases are only considered for quarantine purposes. But there are other diseases, which are infectious and capable of causing economic, and biodiversity loss. To comply with the OIE guidelines to deal with the diseases, it is essential that a country has to develop disease information and reporting system built on a disease surveillance programme. In this direction, Aquatic Animal Disease Surveillance programme has been implemented in India since 2013, and diseases of concern are being listed. India also established its Aquatic Quarantine Facility (AQF) in the year 2009 at Chennai at the behest of Ministry of Agriculture for quarantining the *P. vannamei* broodstock imported to India. Establishment of this facility has boosted vannamei farming in India.

7.2. Aquaculture biosecurity

Biosecurity is a broad concept and the application of biosecurity concepts to shrimp aquaculture will contribute significantly to reduce losses due to diseases and make this sector more sustainable and environmentally responsible. Biosecurity means 'life protecting', but its use appears to be restricted to issues related to preventing the introduction, establishment or spread of unwanted biological organisms or agents. The principles of biosecurity should be considered to keep the pathogen not only out of the culture environment but also out of the country and the region. Implementation of biosecurity practices is an increasingly pressing issue for fisheries and aquaculture managers, considering the importance of this sector in terms of economic development of the people. Resource protection, food security, trade, production and development issues are driving this change (Beers, et al, 2005). Developing biosecurity programme includes identification of the

species at-risk that is required to be protected by the programme, threats, pathways of hazards; assessment of the level of harm that would result; measures that could be used to mitigate the risk; documentation of the programme, its performance and auditing of the programme, preparation of contingency plans, and finally provision of adequate resources to implement the programme. The sanitary and phyto-sanitary measures (SPS Agreement) provide an internationally enforceable set of rights and obligations on the use of biosecurity measures by governments.

A biosecurity programme is developed after scientific analysis of information with the aim of adopting procedures to manage risks to an acceptably low level. The use of sound epidemiological principles and a logical, structured approach will help in achieving the biosecurity. At farm level, implementing biosecurity plan requires modifying existing farms and management routines. In this instance, white spot disease (WSD) is taken as a case considering its devastating nature and biosecurity measures required for minimizing risks associated with this disease in aquaculture are outlined. Basic husbandry practices have to be expanded to include elements of disease prevention and control. Biosecurity measures implemented appropriately can be a cost- effective way of managing disease risks.

Among the viruses causing diseases in shrimp, the white spot syndrome virus (WSSV) is an extremely virulent pathogen. It is one of the most prevalent and widespread viruses in shrimp aquaculture systems around the globe. Transmission of viral diseases in aquaculture occurs through two pathways, viz., horizontally (transmitted by direct contact, or indirectly, by ingestion of infected organisms, and water), and vertically (virus is passed from an infected female parent to her progeny). A number of vectors such as rotifers, marine molluscs, polychaete worms and non-decapod crustaceans including *Artemia salina* and the copepods, as well as non-crustacean aquatic arthropods such as sea Slaters (Isopoda) and Euphydradae insect larvae can serve as host to this virus and transmit the disease in cultured stock. To date, more than 93 species of arthropods have been reported as hosts or carriers of WSSV either from culture facilities, the wild or experimental infection. Birds also can serve as potential sources of disease transmission. At present there is no treatment available to interfere with the unrestrained occurrence and spread of the disease, while biosecurity and better management practices involving rigorous sanitation practices on shrimp farms have been helpful in prevention and control of the disease.

Farm level of biosecurity measures have to be implemented by the farmers. Achieving biosecurity in hatcheries and farms requires preventing the entry of WSV into hatcheries and farms, monitoring the health status of the shrimp population, adoption of better management practices including recommended protocols from using pathogen-free stock, pond preparation and management measures.

Preventing the entry of WSSV in hatcheries and grow-out phase of aquaculture can be achieved primarily using specific pathogen-free (SPF) or specific pathogen-resistant (SPR) and genetically improved (selective breeding method) stock. Specific pathogen-free history comes only from a long-term captive breeding and disease surveillance programme at a facility that has a fully functional and effective biosecurity plan. Pathogen carriers such as vectors, intermediate hosts, reservoir hosts, non-host biological carriers such as birds, insects, other predators, human beings and, fomites such as water, vehicles, buckets, shoes, nets, clothing also pose serious threat and management measures need to be incorporated to prevent entry of pathogens from these sources.

Adopting quarantine measures for broodstock prior to their use, adopting better management practices (BMPs) and standard operating protocols (SOPs) by implementing good sanitary practices, treating water before use, optimizing stocking density of larvae and maintaining good water quality, treating hatchery effluent during seed production in hatchery will help in achieving biosecurity in hatcheries.

Main preventive measures at pond / farm level include proper pond preparation to eliminate pathogens and their carriers, treatment of water in reservoirs to inactivate free viruses and kill virus carriers, water filtration using fine filters to keep carriers out, closed systems to avoid contamination from source water, reduced water exchange to minimize the entry from source water and even changing the water source. Transmission of virus can be prevented by providing crab fencing, fencing, foot baths, wheel baths, and disinfection protocols. Improved husbandry practices have been successfully employed for the control of diseases. 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 such farms.

Pond preparation is essential to reduce the risks of shrimp disease outbreaks. Removal of bottom sludge, especially in ponds with high stocking densities, ploughing of soil when wet, use of lime during pond preparation will help in minimizing disease risks. Farms with poor bottom soil quality such as presence of a black soil layer, will suffer crop failures. Hence, the sludge must be removed and disposed away from the pond site. Extra precaution should be taken while disposing sludge from farms affected by disease outbreak during the last crop. Sludge removal should pay attention to areas of the pond where there is a high accumulation of organic matter from pervious crop, such as feeding areas. Ponds must be ploughed to expose the black soil layer underneath bottom soil to sunlight and atmospheric oxygen. By this process, the organic waste (sludge) will be oxidized. Ploughing on wet soil is particularly recommended for ponds if the sludge cannot be removed properly by manual or mechanical methods. After ploughing,

ponds must be dried for 2-3 weeks and even more when pond had a history of WSD outbreak since WSSV can be viable for three weeks despite sun-drying. In case a heavy tractor is used for ploughing, then plough the dry soil and then fill the pond with water to wet the soil and then again dry. Ploughed pond bottom leads to turbid water conditions during culture period. Therefore, compaction of the bottom using heavy rollers after the whole process of pond preparation, i.e., before water intake, helps avoid the turbid water condition. Liming during pond preparation optimizes pH and alkalinity conditions of soil and water. The type and amount of lime to be added depends mainly on the soil pH and also on pond water pH.

7.3. Some important biosecurity measures to be adopted in the shrimp farm:

Potassium permanganate foot bath

- Stocking should be done with disease-free SPF post-larvae.
- The post-larvae should only be procured from registered hatchery.
- The stocking density should not be very high.
- The crab fencing and bird scare lines should be in place.
- Movement of people should be restricted inside the farm.
- There should be proper education and awareness among the farmers on biosecurity.
- Environmental cleanliness should be maintained.
- Proper pond preparation measures should be followed.
- Foot bath and hand disinfection should be there at the entrance.
- If possible, the separate feeding trays, boats, refractometer and sampling materials should be used for each pond.

7.4. Summary

Aquaculture has been contributing significantly to the National economic development in India. However, diseases have become the major limiting factor determining aquaculture production. Further, as translocation of aquatic animals for diversification has emerged as a potential threat and source of new diseases, quarantine and health management have become integral components of aquaculture. The strategy of the aquatic quarantine and health management system, primarily involves the protection of our country's aquatic biodiversity from exotic organisms, pathogens and containment of the diseases. In the process of introducing *P. vannamei* in India, ICAR-CIBA and NBFGR carried out import risk analysis and recommended introduction of the species and this introduction has

resulted in substantial increase in shrimp production. This is also facilitated by the establishment of Aquatic Quarantine Facility (AQF) in the year 2009 at Chennai for *P. vannamei* broodstock imported to India. In this situation, application of biosecurity concepts will contribute significantly to reduce losses due to diseases and make shrimp aquaculture sustainable and environmentally responsible.

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8

Sampling of Fish and Shellfish for Bacteriological and Histopathological Examination

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Proper sampling procedure is very much important for appropriate diagnosis of fish and shrimp diseases. Improper sampling may result in wrong disease diagnosis leading to incorrect interpretation. Sampling should be carried out in such a way as to provide the best possible likelihood that the sample will be representative for the population. For proper sampling, the actual objective of the surveillance programme should be considered. The principal objective of sampling from a population is to select the subsets units from the population, which is representative of that particular population. The simple random selection of sample is called as probability sampling and is one of the most common sampling techniques. When this is not possible, the sampling should provide the best practical generating optimal inferences about disease patterns in the target population. Usually, the disease survey is done with the objective of exhibiting presence or absence of a certain factor (e.g. Disease) or to estimate the prevalence of any disease.

8.1. Sampling methods for disease diagnosis of shrimps

The target organs in case of crustaceans depend upon the disease or pathogen to be tested, age and size of the animals and objectives of testing i.e. diagnosis of overt disease, detection of sub-clinical pathogens or carriers or sampling for targeted surveillance to show absence of specific diseases. For collection of shrimp samples, the proper record of sample is very much important and the sample should be properly labeled preferably with a sample code and proper record should be noted against the sample code. The record should include name and address of the farm, contact information, cultured species along with stocking density, seed source, water exchange information, days of culture (DOC), date and time of commencement of disease symptom, details of samples (organs, collection media, etc.), water source, feed used, number of ponds, sampling pond number, water salinity, time of collection of samples and date of submission to the laboratories. When any specific disease is suspected based on symptoms and history, special consideration should be given targeting that particular disease.

8.1.1. Sampling media based on analysis procedure

Sampling media may vary according to the investigation procedure to be followed:

8.1.1.1. For molecular diagnosis by PCR

The collected samples may be transported in 95 % ethyl alcohol. If the samples are intended for detection of RNA viruses, then it can be preserved in any RNA preservation media (e.g. RNA later). The samples can be kept at refrigerated temperature, if delay is expected to reach the competent laboratory. For PCR analysis, the sample can also be transported in frozen or chilled condition preferably in dry ice or even ordinary ice, if dry ice is not available.

8.1.1.2. For bacteriological analysis

Live shrimp is the most suitable sample for bacteriological analysis. The moribund shrimp samples (preferably minimum three in number) can be collected in double polypack with oxygen. The bags should be kept in styrofoam or thermocol boxes to keep the temperature cool. While sending to long distance, either ice pack or gel pack should be kept inside the boxes. The equal number of normal shrimp may also be collected and sent to the laboratory in similar condition for comparison study. However, if live shrimps are not available, the dead shrimp samples for bacteriological analysis can be dispatched on ice.

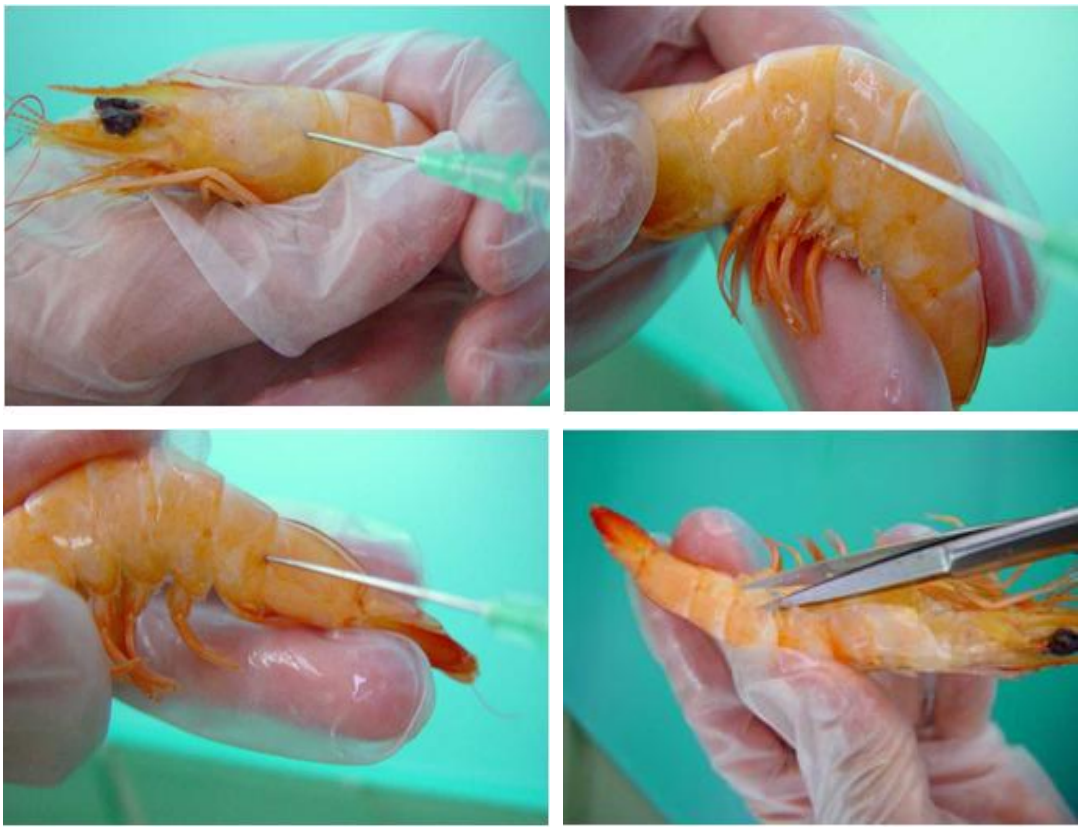
Alternatively, the haemolymph from the moribund shrimp may be drawn with the help of syringe and 1-2 drops of haemolymph may be spread on pre-dried Thiosulfate citrate bile salt sucrose (TCBS) agar or Zobell marine agar (ZMA). After inoculation, the plates should be incubated at 30 °C for 18-24 hrs. When the sample has to be collected from parts with necrosis or blisters, the area should be cleaned thoroughly with sterile normal saline. Then the sample can be collected from infected area with the help of a sterile swab.

For collection of sample from internal organs: After carefully dissecting the shrimp aseptically, the sample can be collected from hepatopancreas with an inoculating loop or swab. Similarly, other organs can also be collected, homogenized in 0.1 % peptone water and inoculated into culture broth or solid culture plate. The particular species of bacteria can be identified by Gram's staining, different biochemical tests or different molecular methods such as PCR, Real time PCR, hybridization with DNA probe, 16S rRNA gene sequencing, etc.

8.1.1.3. For histopathological analysis

For histopathological analysis, the moribund shrimp should be selected. The shrimp should have preferable the clinical symptom and is about to die. Davidson's fixative is the most suitable media for preservation of shrimp for histopathology. The shrimp should be injected with Davidson's fixative at the rate of 10 % of the body weight. If the weight of the shrimp is 20 g, then 2 ml of Davidson's fixative is

required. At first, the fixative should be directly injected into hepatopancreas to ensure rapid fixation. Then the remaining portion of the fixative should be injected at different parts of body of the shrimp.



Fixing of shrimp with Davidson's fixative

Then the cuticle of the shrimp should be silted along the midline to ensure penetration of fixative. Then the sample should be placed in a container with 10 volumes of fixative. If the size of shrimp is 20 g, then requirement of fixative will be 200 ml. Now, the sample in this condition is ready to be transported to laboratory.

Davidson's fixative:

Formalin	: 220 ml
Ethanol (95 %)	: 330 ml
Distilled water	: 335 ml
Glacial acetic acid	: 115 ml

8.2. Sampling methods for disease diagnosis of finfish:

8.2.1. For molecular diagnosis by PCR

The sample (preferred organs depending upon the species of the fish and the disease to be diagnosed) should be collected in 95 % ethyl alcohol as in case of shrimp.

8.2.2. For bacteriological analysis

Spleen, kidney, heart and brain are the most suitable organs for isolation of pathogenic bacteria from diseased fish. Spleen is most suitable organ when bacterial septicaemia is suspected.

8.2.2.1. General internal organ culture: The fish should be de-scaled. The surface of the fish should be wiped with 80 % ethyl alcohol. The abdomen should be dissected and different organ may be collected aseptically and separately with immersion of instruments (scissor, forceps, etc.) in alcohol followed by flaming before collecting each organ. Then each organ should be placed on a sterile petridish before microbial culture. For collection of kidney, the gastrointestinal tract should be removed to expose the swim bladder and underlying kidney. Then the swim bladder is peeled away from the kidney with a sterile forceps. Then the kidney along with blood from anterior portion of the kidney can be collected.

8.2.3. For histopathological analysis

Only live or moribund fishes are suitable for histopathological analysis. For better fixation, euthanasia of fish should be done before fixation. For preservation of finfish for histopathological examination, 10 % neutral buffered formalin (NBF) is the preservative of choice. However, when rapid penetration is required, then Davidson's fixative or Bouin's fixative may be used. Both of them are acidic fixatives with slight decalcifying action. Bouin's fixative is suitable for fixing very small fish and also for preservation of skin of large fish. For easy penetration of fixative, the scales may be removed. The total volume of the fixative should be 10 times of volume of the tissue. Autolysis may occur due to less amount of fixative. Davidson's fixative is suitable for preservation of gill with suspected parasite. If fixed with Davidson's fixative, the same should be replaced with 70 % alcohol after 48-72 hrs. Long storage of finfish tissues with Davidson's fixative may result in excessive hardening of tissue. Very small fish (Length < 5 cm) can be fixed as a whole. But fish larger than 5 cm should not be fixed as whole until internal organs are well-exposed. The selection of the organ depends upon the site of suspected lesion and diseases suspected. Table 1 shows the preferable organs to be preserved for different diseases.

10 % Neutral buffered formalin

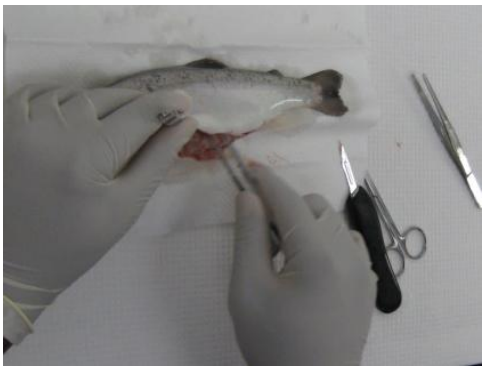
40 % Formaldehyde	: 100 ml
Distilled water	: 900 ml
NaH_2PO_4	: 4 g
Na_2HPO_4	: 6 g

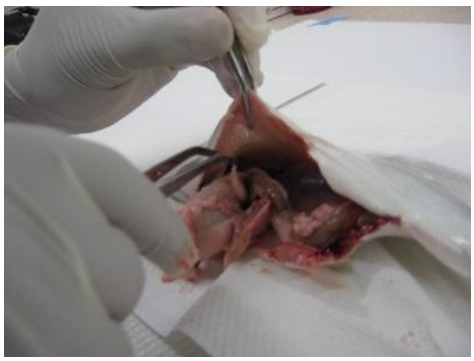
Bouin's fixative

Picric acid (Saturated aquatic solution) : 75 ml

Formalin (40 % w/v) : 25 ml

Glacial acetic acid : 5 ml





8.3. Sampling for diagnosis of specific suspected disease

When diagnosis has to be done for specific disease, the target sample will vary depending upon the disease suspected. The appropriate sample to be collected for specific disease has been summarized in Table 1.

Table 1: Samples to be collected for different diseases

Disease	**Causative agent**	**Preferred sample**
Shrimp diseases		
Early mortality syndrome	Specific strain of *Vibrio parahaemolyticus*	1. For bacteriology: Live / moribund shrimp 2. For histopathology: Shrimp fixed in Davidson's fixative.
Vibriosis in shrimp	Different species of Vibrio	1. Haemolymph aspirated directly from heart on TCBS or ZMA media for isolation and identification of *Vibrio* colony 2. Gut and hepatopancreas for isolation of bacteria 3. Finding of luminescence in case of luminescent *Vibrio* (e.g. *V. harveyi*) under dark
White spot disease	White spot syndrome virus (*Whispovirus*)	1. Gills and cuticular epithelium for microscopy and PCR analysis 2. Haemolymph: For demonstration of aggregates of WSSV virions

Infectious hypodermal and haematopoietic necrosis (IHHN) disease	Viral infection caused by *Brevidensovirus* (Parvoviridae family)	For histopathology: Ectodermal and mesodermal tissues including gill, cuticular epithelium, connective tissue, haematopoietic tissue, lymphoid organs, antennal gland, etc.
Black gill disease	Bacteria (*Flavobacterium* spp., *Cytophaga* spp.) and parasite (e.g. *Zoothamnium* spp.)	Gills for observing parasites directly under the microscope and also for isolation of specific bacteria.
Hepatopancreatic parvovirus (HPV) infection	*Brevidensovirus* (Parvoviridae family)	Hepatopancreas of the affected shrimp
Yellow Head Disease	Yellow head virus (*Okavirus* under Roniviridae family)	For histopathology: Cephalothorax tissue of moribund shrimp for haematoxylin-eosin staining. Light microscopy of tissues of ectodermal and mesodermal origin for finding of cytoplasmic inclusion bodies.
Taura syndrome	Taura syndrome virus (*Aparavirus* under family Dicistroviridae)	Cuticular epithelium, appendages, gill, hindgut and subcuticular connective tissue
Loose shell syndrome	Unknown aetiology	For histopathology: Hepatopancreas, lymphoid organs and muscle.
Finfish diseases		
Vibriosis in brackishwater finfish (e.g. Asian seabass)	Different species of *Vibrio* (e.g. *Vibrio anguillarum*)	Bacteriology: Isolation and identification of species of *Vibrio* from kidney and brain
Viral Nervous Necrosis (VNN) or Viral encephalopathy and retinopathy (VER)	Betanodavirus	Histopathology: Brain, spinal cord and retina for finding of vacuolation and intra-nuclear inclusion bodies
Iridovirus infection	Viral infection (*Lymphocystivirus* and *Ranavirus*)	Histopathology: Liver and spleen for Giemsa staining
Aeromonas infection	Different species of *Aeromonas* (*A. hydrophila*, *A. caviae* and *A. punctata*)	Spleen and other affected areas including tail, fin, etc. for bacterial isolation

Septicaemia in pearl spot	*Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*.	Gill tissue, spleen and lymphoid organs for isolation of bacteria
Epizootic Ulcerative syndrome	*Aphanomyces invadans* (An oomycetous fungus)	For isolation of *Aphanomyces*: Muscle tissue underneath the ulcer. For histopathology: Muscle tissue at the edge of the ulcer

8.4. Conclusion

Proper sampling is one of the pre-requisites for disease diagnosis. The aquatic health experts should take care at each and every steps of processing of samples for correct diagnosis of every disease. Every aspects of the sampling such as sample size, objective of the surveillance programme, choice of proper fixatives (as in case of histopathology), choice of proper media (as in case of microbiological analysis), proper incubation temperature should be taken into consideration for sampling procedure of both finfish and shrimp.

8.5. Further reading

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9

Molecular Tools for Rapid Diagnosis of Shrimp and Brackishwater Finfish Diseases

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9.1. Introduction

Molecular methods are routinely used to detect pathogens in tissue samples and confirm the identity of pathogenic agent. Nucleic acid-based techniques for the detection of pathogens gained colossal importance due to their high sensitivity and specificity. For rapid wide-scale testing, robust easy-to-use molecular test systems that can be applied in the field provide an immense advantage, as they allow rapid on-site nucleic acid detection at the point of need. The reports on use of these techniques for the detection of microbial pathogens of fish and shrimp are extensive. This topic gives an idea on molecular approaches applicable in brackish water aquaculture, their merits and demerits.

9.2. Molecular biology techniques and their application in brackishwater aquaculture

9.2.1. Polymerase Chain Reaction (PCR)

One of the significant achievements in the field of molecular biology is development of diagnostic Polymerase Chain Reaction technique. PCR is a method of enzymatic synthesis and *in vitro* amplification of specific DNA fragments. The basic protocol of PCR involves generation of million copies of specific DNA sequences, in a thermocyclic process consisting of repetitive cycles of DNA denaturation, primer annealing, and elongation using a thermostable DNA polymerase and specific PCR primers. Reverse Transcription PCR (RT-PCR) is used to reverse-transcribe and amplify RNA to cDNA. PCR is preceded by a reaction using reverse transcriptase, enzyme that converts RNA into cDNA. The products produced that are generally of 50-10,000 bp size, can be analyzed by gel electrophoresis or DNA sequencing. PCR is routinely used for screening of major OIE listed pathogens like White spot syndrome virus (WSSV), Infectious hypodermal and hematopoietic necrosis virus (IHHNV), Yellow head virus (YHV), Infectious myonecrosis virus (IMNV) and Taura syndrome virus (TSV) in shrimp. PCR has been widely applied for the detection of Viral nervous necrosis (VNN) and its genotyping. PCR is used for screening of broodstock, larvae and post larvae in the hatchery and before stocking, identifying carriers, checking water and sediment for viral contamination and monitoring

health of shrimp in grow-out ponds to reduce the risk of disease. PCR protocols using non lethal method of PCR testing is added advantage for screening of valuable broodstock. CIBA was first institute in India to commercialize PCR based WSSV diagnostic kit for detection of this shrimp virus. At present, there are commercial kits available for the detection of many shrimp virus. PCR is specific, rapid and sensitive method for detection of pathogens. However conventional PCR is prone to contamination from previously amplified products, but this can be overcome by good laboratory practice and separating the extraction, amplification and electrophoresis processes. PCR cannot detect very low numbers and quantification of the pathogens.

9.2.2.1. Variations of Polymerase Chain Reaction

Several variations of PCR assay exist such as nested PCR or nested reverse transcriptase- PCR (RT-PCR), and Variable Number of Tandem Repeats (VNTR) PCR

- Nested PCR (nPCR): Nested PCR is used to enhance the specificity of PCR amplification of DNA. In nPCR, two sets of primers are used in two successive reactions. In the first PCR, one pair of primers is used to generate DNA products and then the products from the first PCR are used as template in a second PCR, using primers whose binding sites are located within the first set. Nested PCR is routinely used for screening of most of pathogens of aquatic importance. Nested PCR is more sensitive and often more successful in specifically amplifying long DNA products than conventional PCR. But it requires more detailed knowledge of the sequence of the target, more prone for false positives due to contamination.
- Variable Number of Tandem Repeats (VNTR-PCR): VNTR PCR targets areas of the genome that exhibit length variation. The analysis of the genotypes of the sample usually involves sizing of the amplification products by gel electrophoresis. VNTR-PCR is found to be the most useful tool for molecular epizootiological studies in aquaculture and various studies have been used as markers to identify variations and distinguish WSSV genotypes associated with disease outbreaks, genotypes infecting farmed shrimp, wild crustaceans and comparison of geographical isolates and to compare WSSV transmission routes.

9.2.2.2. Improvements in Polymerase Chain Reaction

- Quantitative Real-Time PCR (qPCR): Real-time PCR assays employing either SYBR-green or TaqMan probe fluorescence detection systems have been developed to detect and quantify several pathogens. Due to elimination of post-PCR manipulation of amplified products, faster analysis, reduced risk of amplicon contamination high throughput and the robustness of the technology real-time PCR is preferred over conventional PCR in clinical laboratories. The real-time PCR method had been used to detect and

quantify shrimp viruses such as WSSV, IHHNV, MBV, Hepatopancreatic parvovirus (HPV), TSV and IMNV. Using this technique, WSSV less than 10 copies could be detected in the post-larvae with very low infection. Thus, it is useful tool to pre-screen broodstock or larvae for selective breeding, stocking in production systems and wild broodstock with the late chronic phase of infection. Real-time RT-PCR method was shown to detect TSV in chronically infected *Penaeus vannamei* that had survived up to 236 days after exposure to TSV. Multiplex qPCR was shown to be a feasible diagnostic tool for the simultaneous detection of the WSSV, MBV, HPV and Decapod penstyldensovirus1 (PstDV1) in allowing the identification of double or triple co-infections in diseased shrimp.

- Multiplex-PCR: The technique uses several pairs of primers annealing to different target sequences. That permits the simultaneous analysis of multiple targets in a single PCR assay. Since its introduction, multiplex PCR has been shown to be a valuable method for identification of viruses, bacteria, fungi and parasites. Specific primers have been developed and multiplex Reverse transcriptase-PCR was demonstrated for simultaneously detection of the major shrimp viruses such as WSSV and TSV in pacific white shrimp *P. vannamei*; IHHNV, TSV and WSSV in penaeid shrimp and WSSV, IHHNV, MBV in shrimp post-larvae. The tool was also applied for differential detection of different species of *Vibro viz. Vibrio harveyi*, *V. campbellii* and a variant strain of *Vibrio* pathogenic to shrimp and differentiation of gill-associated virus and yellow head virus in *P. monodon*. Multiplex-PCR methods are becoming more popular due to cost efficiency and savings of time as a sample can be screened for a variety of pathogens within a single reaction. However standardization of the assay is very difficult and time-consuming. Multiplex reverse transcription-polymerase chain reaction was developed for simultaneously detection of six major shrimp viruses including YHV, WSSV, TSV, HPV, IHHNV and MBV. Multiplex RT-nested PCR used for differentiation of gill associated virus from yellow-head virus of *P. monodon* with enhanced sensitivity.

9.2.3. Diagnosis of shrimp diseases by isothermal amplification

The isothermal amplification refers to amplification of DNA segment in isothermal condition at a constant temperature. Unlike PCR, no sophisticated thermal cycler is required for these tools. The important tools for isothermal amplification are Loop mediated isothermal amplification (LAMP) and Nucleic acid sequence based amplification (NASBA).

9.2.3.1. Loop-mediated isothermal amplification (LAMP)

Like PCR, LAMP also amplifies a specific region of DNA within a target region of the template, but the reaction is carried out at a constant temperature i.e. usually at

65 ºC. The detection of amplified product can be done either by running agarose gel or by addition of a reagent i.e. calcein, which produces a bright fluorescence in case of positive reaction. For LAMP reaction, a minimum of four specially designed primers are required: a pair of inner primers of over 50 nucleotides in length and another pair of outer primers of around 25 nucleotides in length. Two inner primers are called as forward inner primer (FIP) and backward inner primer (BIP). These four primers recognize six distinct nucleotide sequences within target region. In addition to these, two loop primers are incorporated to accelerate the reactions to finish the reaction within a very short period of time. Like *Taq* DNA polymerase in case of PCR, the DNA polymerase used in LAMP is *Bst* DNA polymerase, which is obtained from *Bacillus subtilis*. In LAMP reaction, one set of primers anneal to the target region one after another on the same strand and the primer annealed at later stage displaces the strand formed by previous primers. This strand displacement is aided by *Bst* DNA polymerase. When both the strands are targeted by the primers, there will be formation of loop. This cycling reaction will go on and there will be production of 10^9 copies of target DNA sequence within 1 hr time in isothermal condition. The final products are a series of stem loop DNA of various lengths. The main advantage of LAMP over PCR is its more specificity due to presence of six distinct regions within the target sequence and less-time consuming as the reaction is generally over by one hr. The real time detection of products is also possible by addition of calcein.

LAMP has been successfully employed for detection of many pathogens of brackishwater aquaculture systems including *Vibrio anguillarum, V. nigripulchritudo*, *V. harveyi*, *V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, Iridovirus, White spot syndrome virus (WSSV), Yellow head virus (YHV), Infectious hypodermal and haematopoietic necrosis virus (IHHNV), Taura syndrome virus (TSV), Infectious myonecrosis virus (IMNV), etc. Like PCR technique reverse transcriptase LAMP can also be employed for RNA viruses. Use of LAMP combined with a lateral flow dipstick (LAMP-LED) is described for detection of WSSV, TSV and *P. monodon* densovirus (PmDNV), to improve assay specificity. However the tool is vulnerable to contamination.

For quantitative detection, Real Time quantitative LAMP (Q-LAMP) has been successfully employed by some scientists as in case of real time PCR (qPCR). During LAMP reaction, the precipitation of magnesium pyrophosphate is produced in an amount, which is generally proportional to the amount of amplified target DNA. By measuring the turbidity of magnesium pyrophosphate, the quantification of amplified DNA can be done. A multiplex LAMP (mLAMP) assay for simultaneous detection of WSSV and IHHNV in Penaeid shrimps has been reported.

9.2.3.2. Nucleic acid sequence-based amplification (NASBA)

NASBA is another method of isothermal amplification and it is a RNA-based method to amplify RNA target sequence. This method was invented by J. Compton in 1991. The NASBA reaction is generally carried out at a constant temperature of 41 ºC. But a single melting step is required at higher temperature prior to amplification step. The advantage of NASBA over PCR is that it is less time-consuming and more sensitive. Unlike PCR and LAMP, in case of NASBA, three different types of enzymes are required namely reverse transcriptase, RNaseH and T7 RNA polymerase. In NASBA reaction, the first primer attaches to the complimentary site at the 3` side of the template. Then the reverse transcriptase enzyme will synthesize the complimentary DNA strand and DNA-RNA hybrid will form. Then the RNA part is destroyed by an enzyme called RNaseH. Then another primer will bind at 5` end of the DNA strand and another DNA strand is being synthesized by reverse transcriptase enzyme resulting in double stranded DNA strand. T7 RNA polymerase continuously produces complimentary RNA strand and this cycle will go on. The product of NASBA is generally mainly single-stranded RNA and detection is generally by usual procedure of ethidium bromide stained agarose gel electrophoresis. The detection can also be done by electro-chemiluminescence using a specific captured probe. As the target sequence is RNA, the NASBA can effectively used for detection of RNA viruses. But the major disadvantage of NASBA reaction is that all the three enzymes used in NASBA are thermolabile and can only be added after melting step and this method is more expensive than PCR and LAMP due to involvement of three enzymes. NASBA has been successfully employed for detection of Taura syndrome virus (TSV) from *P. vannamei*.

9.2.4. DNA microarrays

DNA microarray based analysis is gaining popularity due to high-throughput capacity, accuracy and in creation of profile of many pathogens in a single diagnostic assay. The method involves hybridizing samples of DNA fragments, amplified by PCR, onto specific DNA detector fragments spotted onto a solid support. As DNA microarray assays utilize similar-sized products amplified by PCR before hybridization, PCR template bias is reduced. Fluorescence is the most common method of detection for microarrays. The advantage of microarrays technology is the method allows multiplexing for different pathogens with high degree of sensitivity and specificity. The high throughput reduces the cost and increase the speed of a comprehensive disease screen. However, the initial set up cost for the use of DNA microarrays is very high. DNA microarray has been successfully employed for identification of different aquatic pathogens. By microarray screening, three WSSV latency related genes in Specific-Pathogen-Free asymptomatic carrier shrimps and novel white spot syndrome virus-induced gene *PmERP15*, an ER stress-induced, ER resident protein in *P. monodon* were identified**.**

Recently, microarray assays have been used for the detection of multiple pathogens of aquatic importance.

9.2.5. Metagenomics

The term “metagenomics” was first used by Handelsman in 1998 to describe the study of the collective genetic material from all microbes in a specific environment. Metagenomics is "the application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and laboratory cultivation of individual species. Essentially, a metagenomic analysis involves three main steps: 1. Sampling and nucleic acids extraction, 2. Library construction and 3. Analysis of metagenomics libraries. The technology is applied in the study of an array of microbial diversities like deep sea aquatic microflora, soil microbes and gastrointestinal ecosystems of human and animals. Recently a nodavirus *(Farfantepenaeus duorarum* nodavirus, *Fd*NV) and a new DNA virus possessing a circular genome designated shrimp hepatopancreas-associated circular DNA virus (Shrimp CDV) were identified using metagenomics and it highlights the potential of metagenomic approaches in aquaculture to identify new latent pathogens in asymptomatic carriers, uncharacterized pathogens causing a new disease or multiple pathogens associated with disease syndromes. Limited information on the gastrointestinal microbiome of fish and tiger shrimp using metagenomics is available. Metagenomics allows the study of microorganisms in their natural environment and therefore the total genetic diversity of microorganisms can be studied. However, the potential risk of sample contamination, significant investment in sequencing and computation challenges in huge bioinformatics data analysis are some of the limitations of this tool.

9.2.6. Microfluidics

The microfluidics technology combines engineering, physics, chemistry, biology and computing to control the devices. Recently use of microfluidics in development of techniques like DNA chips, lab-on-a-chip and micro-thermal technologies offer a promising tool for gene analysis and disease diagnostics. Many improvements of microfluidic chips led to technical advantages like reduced time of DNA amplification, no need for the additional thermal cycling steps, simplified reaction system increased portability, compactness, durable materials allow use of low cost disposable chips which effectively prevents the contamination, high sensitivity, fast diagnosis, low reagent and sample consumption, portability, low power consumption and potential for automation of analyses. Attempts have been made to use integrated microfluidic chips as a diagnostic tool in aquaculture. The detection limit of the microfluidic-based system using reverse transcription polymerase chain reactions is found to be 10^1 copies/ µL for detection of nervous

necrosis virus (NNV), Iridovirus, *Vibrio anguillarum* and the grouper *Mx* protein gene. Microfluidic chips integrated with LAMP for the detection of grouper Nervous necrosis virus (NNV) and Integrated microfluidic chip for simultaneous detection of microbial pathogens in ornamental fish and waterborne pathogens are reported.

9.3. Conclusion

Disease outbreaks continue to constrain aquaculture productivity and sustainability. Rapid efficient, specific and sensitive diagnostics are required for detection of important diseases affecting aquaculture. Early detection of disease is very important to take rational decisions for animal health management and control. For field diagnosis, the optimal detection system should be specific, sensitive, cheap, quick, and easy to operate. Molecular biology techniques have been applied in aquaculture to prevent the spread of infectious pathogens for diagnosis and disease monitoring in the form of surveillance.

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10

Farm Level Measures for Prevention and Control of Diseases in Brackishwater Aquaculture Systems

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10.1. Introduction

Increased intensification and commercialization of aquatic production is the current trend in aquaculture development all over the globe and has evolved as fastest growing food producing sector. Like other farming sectors, chance of occurrence of major disease problem increases as aquaculture activities intensify and expand. In line with those farming sectors, the aquaculture industry has been overwhelmed with its share of diseases and problems. Causative agent of those diseases may be viruses, bacteria, fungi, parasites or other undiagnosed and emerging pathogens. Disease is now a primary constraint to the culture of many aquatic species, impeding both economic and social development in many countries including India. In India, brackishwater aquaculture plays an important role in export earnings, employment generation and socioeconomic development. Success in aquaculture in terms of growth and economic viability primarily depend upon successful prevention and control of disease outbreaks.

Production of maximum possible biomass per culture unit area without disease problem in a sustainable manner is the ultimate goal of most aquaculture operations regardless of the type of operation and the species cultured. Intensification in farming systems lead to higher stocking density and increasing stress to the farmed organisms. Diseases often occur when cultured animals remain in stress as disease outbreak is an end result of negative interaction between pathogen, host and the environment. Management of disease problems must be aimed towards broader ecosystem management and measures against the introduction of pathogens into the aquaculture system. Clear understanding about the environment, biology of the target species along with the in depth knowledge on the pathogen, disease development, diagnostics and control measures are essential factors in management of a disease problem. Fish health management requires a multidimensional approach considering all aspects that contribute to the development of disease.

Control of disease in aquaculture is different from land-based animal rearing systems. In terrestrial animal rearing system, diseased animals can be identified and treated individually. Isolation of the diseased animals from the normal

population in aquaculture system and subsequent treatment is extremely difficult and not practically possible in most cases. The traditional pathogen focused approach is impractical in aquaculture disease management and should be replaced by more holistic approach focusing the whole ecosystem and integration of fish quarantine, disease monitoring, animal nutrition, environmental health and principles of hazard analysis and critical control point (HACCP). Successful integration of farm health management and fish heath management though best management practices (BMPs) and strict biosecurity measures is the key to success in aquaculture.

10.2. BMPs (Better management practices) in farm health management

Farm health management in brackishwater aquaculture systems comprises of maintenance of soil and water quality, feed management and proper farm quarantine. Farm health management is carried out through adoption of better management practices (BMPs) which starts with site selection before establishment of the farm and ends at harvest and marketing. Important steps in BMPs for farm health management are:

10.2.1. Site selection

Selection of suitable site always plays a vital role in shrimp farming. Criteria are herein presented that could serve as guidelines in judging site suitability for brackishwater aquaculture.

- Availability of pollution free water having desired salinity and pH.
- Water must not be too turbid.
- Moderate tidal fluctuations preferably 2–3 meters.
- Clay-loam or silty-clay loam soil is preferred. Acid-sulphate soil should be avoided.
- Coastal sites where the slopes run gently towards the sea are preferred.
- Close proximity of the site to the hatchery is advantageous.
- The farm should be accessible in all seasons.
- Availability of technical assistance, labor, electricity and marketing channels.

10.2.2. Farm design and construction

Proper designing and construction of farms are essential for their efficient management and for promoting environmental protection. Since site characteristics

vary greatly from place to place, a site specific approach to design and construction is necessary. The following aspects should be given importance:

- The peripheral dyke should be strong enough to protect the farm from flood, tidal thrust and cyclone.
- Slope of the dyke may range from 1:1 for clayey soil and 3:1 for sandy soil.
- Outlet should be located diagonally opposite to the inlet.
- Bed of the drainage canal should be at least 30 cm below the pond bed level.
- Rectangular or square ponds are appropriate for most of the systems.
- Pond bottom should have a slope of 1:2000 towards the outlet.
- Reservoir pond is required to act as settlement pond and water storage.

10.2.3. Pond preparation

Before a pond can be stocked for a new crop, the excessive wastes accumulated in the pond during the previous crop must be removed. Good pond preparation is the key to reduce disease risks. Following steps are important in good pond preparation.

- The pond bottom should be checked for the presence of black layer when it is wet. Black soil can be removed paying special attention to feeding areas and corners. After removing, the black soil should be disposed away from the pond.
- Average soil pH should be 6.5-7.5, lower soil pH should be corrected applying lime.
- Water during intake should be screened through at least 2 layers of fine nets.
- Aerators should be positioned properly to achieve maximum water circulation.
- Pond is disinfected by applying bleaching powder @ 60 ppm (20 ppm effective chlorine).
- After 7 days of bleaching application, fertilization is done to stimulate plankton bloom.
- Pond water depth should be 120-140 cm prior to stocking.
- Daily fluctuation of pH should be below 0.5 and other parameters in optimum range. The alkalinity of the water should be in between 90-220 mg of $CaCO_3$ per litre.

10.2.4. Stocking

Selection of good quality seeds for stocking into a pond is an important step in shellfish and finfish grow-out management. The farmer must ensure that they get healthy and pathogen free seeds from reliable hatcheries. Following are the criteria for seed selection and stocking.

- Reputed hatcheries should be selected within 6-8 hrs distance.
- Good quality seeds are of uniform size and show good activity.
- Stocking within 2 weeks and from the same batch in neighboring ponds is beneficial.
- On-farm nursery (5 to 10% of the total pond area) improves post-stocking survival.
- Stocking should be done at recommended rearing density for the species.
- Stocking should be done during early morning or late night after proper acclimatization.

10.2.5. Feed management

Among total operational expenditure, feed only accounts for about 60%. Both over feeding and under feeding may result crop loss. Following are the most important criteria for feed management.

- Appropriate crumble or pellet size as per size of the cultured organisms.
- Feed should contain appropriate nutrients for the particular species.
- Feed should be freshly manufactured, not older than 2 months.
- Daily ration should be calculated based on estimated biomass through sampling.
- Strict feed monitoring as per recommended procedure for the species.

10.2.6. Water and soil quality management

Maintenance of good water and soil quality is very important to reduce disease risks and to achieve better production. Following are the criteria for better water and soil quality management.

- Water depth in the shallowest part of the pond should be at least 120 cm.
- If there are benthic or floating algae in the pond, remove them manually.
- Liming should be done at regular intervals to maintain optimum pH.
- Optimum pH range is 7.5-8.5 with daily fluctuation below 0.05.

- The free ammonia, nitrite nitrogen and nitrate nitrogen should always be below critical level.
- Rainwater should be drained as soon as possible through overflow system.
- Appropriate feeding is the key to better water quality management.
- Biomass should be estimated weekly by sampling to maintain proper feeding.
- The dissolved oxygen level should be maintained in between 3-10 mg/L (ppm).

10.2.7. Biosecurity measures

Biosecurity measures to prevent entry of pathogens into the farm are of immense importance in brackishwater aquaculture, especially shrimp farming to protect the stock from diseases. Following are the farm level biosecurity measures to be adopted for successful shrimp farming.

Bird fencing lines

Crab fencing

- Farm should be protected with bird fencing and crab fencing.
- Every pond should have separate utilities.
- Ponds with signs of disease should be sealed and bleached immediately.
- Proper hand and foot wash in potassium permanganate solution is mandatory before entering the farm.
- A reservoir should be there for water exchanges and maintenance of depth.
- Depth more than 120 cm reduces stress and risks of diseases.
- Farming in closed ponds i.e 'zero exchange' restricts entry of pathogen and carriers of diseases.
- There should not be any seepage through dykes.
- All utilities should be washed in chlorinated water before first use. biomass should be estimated weekly by sampling to maintain proper feeding.

- Proper understanding of personals about biosecurity is most important.

10.2.8. Fish health management

Fish health management is generally practiced with implementation of proper animal quarantine, screening of broodstock, larvae, fingerlings and crop health monitoring which depends on the early and accurate diagnosis of the disease causing factor. Timely and early diagnostics can be used as an effective tool for health care management. Different types of diagnostic methods used in aquaculture are, history of disease at the farm or in region, behavior of finfish or shellfish in culture, gross, clinical signs, direct microscopy, histology and histopathology, electron microscopy, culture and biochemical identification, bioassay, serological methods, tissue culture, PCR, gene probes and DNA chips etc. It is required draw a diagnostic procedure to identify the root cause of the problem when infection or disease is suspected. Selected diagnostic tests are performed in routine pathogen watch or health monitoring. As brackishwater aquaculture is mostly a rural activity, availability of the diagnostic facility and expertise is limited. Diseases escalate rapidly and do not always allow much time to carryout diagnostic procedures at a distant place. Visual observations on the behavior of the stock and gross clinical signs sometimes helps farmer to fix the problem and take necessary action.

10.2.9. Visual inspection of shellfish and finfish for disease diagnosis

Gross observations of clinical signs in shrimp can be easily made at the farm or pond side. Although, in most cases, such observations are insufficient for a definite diagnosis, such information is essential for preliminary compilation of a strong "case description" (or case history). Accurate and detailed gross observations also help with initiation of an action plan which can effectively reduce losses or spread of the disease, *e.g.*, destruction or isolation of affected stocks, treatments or alterations to husbandry practices (*i.e.*, feeding regimes, stocking densities, pond fertilization, *etc.*). These can all be started while waiting for more conclusive diagnostic results.

10.2.9.1. Behavior

Farmers or farm workers, through daily contact with their stocks, rapidly develop a subconscious sense of when "something is wrong". This may be noticeable changes in feeding behavior, swimming movement or unusual aggregations. It is important that farmers and workers on the farm, as well as field support staff, get to know the "normal" (healthy) behavior of their stocks.

10.2.9.2. Mortalities

Mortalities that reach levels of concern to a farmer should be examined for any patterns in losses. Relatively uniform mortalities throughout a system should be

examined immediately and environmental factors determined. Apparently random or sporadic mortalities may indicate a within-system or stock problems. Mortalities that spread suggest an infectious cause and should be sampled immediately.

10.2.9.3. Feeding

Abnormalities in feeding behavior and lack of feed in the gut are good indicators of potential problems. If these are empty, especially just after providing feed, it may indicate underfeeding, or onset of cessation of feeding (anorexia).

10.2.9.4. Colonization and Erosion

The presence of numerous surface organisms (*e.g.* "parasites" - which damage their host; or "commensals" - that do not adversely impact their host) suggests sub-optimal holding conditions or a possible disease problem. Apparent wearing away of the cuticle or appendages (legs, tail, antennae, rostrum) or loss of appendages with or without blackening (melanization) are also highly indicative of a disease problem.

10.2.9.5. Cuticle Softening, Spots and Damage

Softening of the shell other than during moulting may also indicate the presence of infection. Damage or wounds to the shell provide an opportunity for opportunistic infections (mainly bacterial and fungal) to invade the soft-tissues and proliferate, which can seriously impact the health of the shrimp. Certain diseases, such as White Spot Disease, directly affect the appearance of the shell; however, few changes are specific to a particular infection.

10.2.9.6. Colour

Many crustaceans become more reddish in color when infected by a wide range of organisms, or when exposed to toxic conditions especially those that affect the hepatopancreas. Yellowish coloration of the cephalothorax is associated with yellow head disease and overall reddening can be associated with gill associated virus infections. Under certain conditions, some crustaceans may turn a distinct blue colour. This has been shown to be due to low levels of a carotenoid pigment in the hepatopancreas (and other tissues), which may be induced by environmental or toxic conditions.

10.2.9.7. Soft-Tissue Surfaces

A readily observable change to soft tissues is fouling of the gill area, sometimes accompanied by brown discoloration. This can be due to disease and should trigger action since it reduces the shrimp's ability to take up oxygen and survive. In some conditions, the hepatopancreas may appear discoloured (*i.e.*, yellowish, pale, red), swollen or shrunken, compared with healthy shrimp. If the hepatopancreas is gently teased out of the shell, the mid-gut will become visible and permit direct examination of colour (dark - feeding; light/white/yellow - mucoid, empty or not

feeding). This information is useful for determining the health of the shrimp and if infectious disease agents are present.

10.3. Environmental Parameters

Water temperature, salinity, turbidity, fouling and plankton blooms are all important factors. Rapid changes in conditions, rather than gradual changes, are particularly important as potential triggers for disease. Therefore, the farm manager and workers, should attempt to keep pond rearing conditions within the optimum range for the species and as constant as possible within that range. High stocking rates are common in aquaculture but predispose individuals to stress so that even minor changes in environmental conditions may precipitate disease. In addition, many small changes do not on affect shrimp health. However, when several of these small changes occur simultaneously, results can be far more severe.

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11

Pond Preparation and Management Practices with Special Reference to Prevention of Diseases in Brackishwater Aquaculture

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The water and soil quality variables affecting shrimp/fish survival and growth are determining factors for disease outbreaks. Disease is an expression of a complex interaction between host (shrimp/fish), pathogen (bacteria/virus) and environment (pond soil and water quality). Severe alterations in the culture environment deviated from the optimum pose stress on the system leading to reduced immune status of the animal to fight infections. Generally disease does not occur when the culture environment (water and soil parameters) is maintained at optimum and balanced condition. Adverse water quality conditions compromise management and increase stress level thus, making them more susceptible to diseases. Even if the site is good with optimum soil and water characteristics, problems may still crop up by the large quantity of inputs like feed and fertilizers, which lead to excessive phytoplankton production, low dissolved oxygen, high ammonia, poor bottom soil condition and other problems. Most of these problems can be avoided by proper management practices during pond preparation and culture period.

11.1. Pond preparation

The main objectives of pond preparation are to provide the animal with a clean pond base and appropriate stable water quality. Pond preparation is generally dealt in two categories viz., newly constructed ponds and existing culture ponds. In newly dug out ponds, the characteristics of the soil have to be understood first, and soil deficiencies should be identified and treated in new ponds instead of waiting until poor bottom soil quality develops later. For example, if soil in a new pond is acidic, it should be limed before initiation of aquaculture. The pond preparation after harvest before initiating next crop is entirely different from that of a newly dug-out pond and comprises of removal of waste accumulated during the previous crop by draining and drying of the pond bottom.

11.1.1. Drying

The pond bottom should be dried for at least 7-10 days for mineralization of organic matter and release of nutrients. Exposure of the pond bottom to sunlight until it dries and cracks, 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. Pond drying certainly enhances the mineralization of organic nitrogen and phosphorus.

11.1.2. Tilling

Tilling bottom soils can enhance drying to increase aeration and accelerate organic matter decomposition and oxidation of reduced compounds. Soil amendments such as agricultural limestone or burnt lime can be mixed into soil by tilling. Accumulation of organic matter and other substances in the surface layer of soil also can be mixed with deeper soils to reduce concentrations of the substances in the surface layer. Pond bottom should not be tilled when they are too wet to support tillage machinery. Ruts caused by machinery will fill with soft sediment and be likely sites for anaerobic conditions. Depth of tillage usually should be 5 to 10 cm, mould board plow often called turning plow, can be used to turn soil over.

11.1.3. Liming

The reason for liming aquaculture ponds is to neutralize soil acidity and increase total alkalinity and total hardness concentrations in water. This can enhance availability of nutrients in the pond water and improves the conditions for productivity of food organisms and increase aquatic animal production. Either total alkalinity or soil pH may be used to estimate the liming dose. If both are available but values are not in agreement, use the variable that gives the greatest liming dose. Brackishwater ponds with total alkalinity below 60 mg l^{-1} and any pond with soil pH below 7 usually will benefit from liming. Agricultural limestone will not react with dry soil, so when applying over the bottom of empty ponds, it should be applied while soils are still visibly moist but dry enough to walk on. In ponds with highly acidic soil (pH < 6), liming can increase phosphorus availability by increasing the soil pH. The amount of different lime materials required to raise the pH to7 is given in Table 1.

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

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

Agricultural limestone will not react with dry soil, hence when applying over the bottom of empty ponds, it should be applied while soils are still visibly moist but dry enough to walk on. Generally lime is applied after slight turning over of bottom soil.

11.1.4. Fertilization and nutrients transformation

The two most important nutrients in pond aquaculture are nitrogen and phosphorus, because these two nutrients often are present in short supply and limit phytoplankton growth. These two nutrients are added to ponds in fertilizers, manures, and feeds. Fertilizer nitrogen usually is in the form of urea or ammonium, and urea quickly hydrolyses to ammonium in pond water. Ammonium may be absorbed by phytoplankton, converted to organic nitrogen, and eventually transformed into nitrogen of fish protein via the food web. Ammonium may be oxidized to nitrate by nitrifying bacteria, and nitrate may be used by phytoplankton or denitrified by anaerobic microorganisms in the sediment. Nitrogen gas formed by denitrification diffuses from sediment to pond water to the atmosphere. Ammonium is in equilibrium with ammonia, and ammonia also can diffuse from pond waters to the atmosphere. A small amount of ammonium may be adsorbed on cation exchange sites in pond bottom soils. Organic nitrogen in plankton and in aquatic animal faeces may settle to the bottom to become soil organic nitrogen. Nitrogen in soil organic matter may be mineralized to ammonia and recycled to the pond water, but the rate is slow.

Soils that are near neutral in pH have less capacity to adsorb phosphorus and a greater tendency to release phosphorus than do acidic or alkaline soils. Phosphate is released from iron and aluminium combination when reducing conditions develop from oxygen depletion. A dynamic equilibrium exists between sediment and overlaying water so that a small amount of phosphorus is maintained in solution. Phosphorus exchange between soil and water can conceivably be influenced by pond management procedures which influence dissolved oxygen concentrations in bottom water, disturb the bottom soil surface, suspend soil particles into the water, mix interstitial water into pond water, influence pH, or alter concentrations of iron, aluminium, and calcium.

11.1.5. Management of pond bottom soil during culture period

All aquaculture ponds soil bottom 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. The sediment – water interface is an intricate system where complex chemical and microbial changes occur and plays important role in brackish water aquaculture. In the optimum conditions, organic matter present in the soil will be mineralized by using different microorganisms such as

autotrophic, heterotrophic microorganisms and it will release the nutrients in the available form.

11.1.6. Monitoring of soil parameters during culture period

Monitoring of soil quality can be valuable in fish 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 characterize the soils based on soil type, a pond core sampler (Fig.1) fabricated by the Environment Section of CIBA can be used for the depth-wise collection of cores.

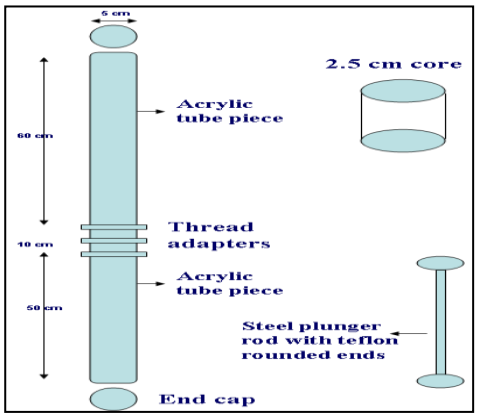

Fig.1. Pond soil core sampler

The low pH of bottom sediment indicates unhygienic condition needs regular check-up. The change in the bottom in terms of increasing organic load should be recorded regularly for the management of the pond bottom. Anaerobic condition can be developed in pond, when input of organic matter exceeds the supply of oxygen needed for decomposition of organic matter. This reducing condition can be measured as the redox potential (E_h). E_h indicates whether the water or soil is in reduced (E_h with `-ve' value) or oxidized ($E_h$ with `+' ve value) condition. 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, sulfate, and carbon dioxide to decompose organic matter, but they release nitrogen gas, ammonia, ferrous iron, manganous manganese, hydrogen sulphide, and methane as metabolites (Fig. 2). Some of these metabolites hydrogen sulfide, ammonia and nitrite can enter the water and be potentially toxic to fishes. The redox potential (E_h) of mud should not exceed -

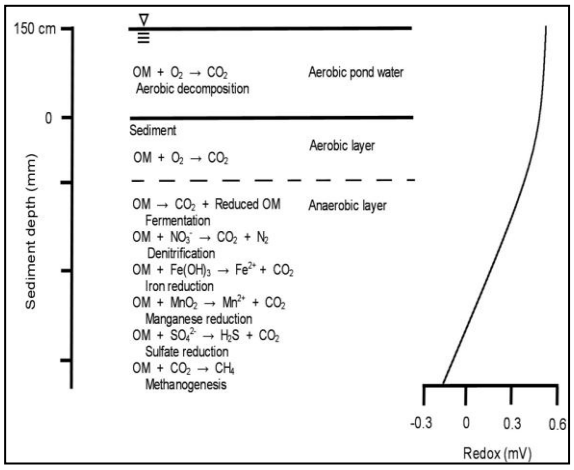

Fig.2 Reactions at the pond bottom soil during aerobic and anaerobic conditions

200 mV. The oxidized layer at the sediment surface is highly beneficial and should be maintained throughout the culture period. Ponds should be managed to prevent large accumulations of fresh organic matter at the soil surface, or in the upper few mm of soil. Hence, it is extremely important to maintain the oxidized layer at the sediment surface in culture ponds.

11.2. Water management

Maintenance of good water quality is essential for both survival and optimum growth of fish. Water treatment is an important step during pond preparation for the maintenance of good water quality at later stage.

11.2.1. Culture of brackishwater species under varying source waters

Shrimp species *P. monodon* and *P. vannamei* and, finfishes Seabass, Mullet and Milk fish are being cultured by farmers in sea, brackish and fresh waters. Though high salinity and clear water with less plankton always causes shrimp stunt, but this high salinity water affects shrimp only at juvenile stage when they mainly consume zooplankton. Bacterial infection and pond bottom deterioration generally caused by over blooming of phytoplankton as in brackishwater ponds are not observed in seawater based culture ponds. Culture in freshwater requires closed system to avoid viral diseases as virus carriers grow very fast in fresh water. Groundwater may differ significantly in terms of its relative ionic composition compared to seawater. Most saline groundwater is deficient in potassium although other key ions such as sodium, chloride, calcium and magnesium can also vary considerably depending on the aquifer. Low salinity water can also react with bottom soils, significantly affecting the ionic composition of water held in open ponds. 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.

11.2.2. Intake water treatment

Polluted or self-polluted source water through aquaculture causes slow growth, disease outbreak and accelerated mortalities of cultured animals. Direct use of creek or sea water carries the risk of introducing the virus through aquatic crustaceans. There is a need to eliminate these from water before use in culture ponds. Reservoir has to be integral component and should be attached to grow-out ponds for sedimentation to settle organic loads and silt and chlorination treatment. Adding treated water from reservoir (approximately 30%) throughout the crop is essential to prevent excess salinity which may gradually increase through

evaporation. Following points should be considered for management of water quality.

- Water from the source is filtered through filter nets of 60 micron mesh/cm^2 to prevent the entry of parasites and crustaceans that are carriers of diseases.
- Inorganic turbidity (suspended solids) should be removed by providing sedimentation/ reservoir pond before water to be taken into production ponds.
- Chlorination as a means to sterilize the water is practiced by many farmers. To achieve this enough chlorine should be applied so as to overcome the chlorine demand of organic matter and other substances in the water. Water should be taken in reservoir ponds and treated with calcium hypochlorite @ 30 ppm. Permissible level of chlorine residuals in treated water for use in grow-out ponds should be less than 0.001 ppm.

11.2.3. Monitoring of water stress parameters during culture period

11.2.3.1. Salinity

Optimal salinity range of 10 to 35 ppt is considered optimum for growth and proper metabolic processes of tiger shrimp. Researchers indicated that a population of *P. vannamei* juveniles infected by IHHNV (Infectious Hypodermal and Hematopoietic Necrosis Virus) grew at a slower rate when reared in a high salinity (49 ppt) than in lower salinities (5-15 or 25 ppt).

11.2.3.2. Temperature

Temperature is one factor controlling the speed 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 shrimp making it more susceptible to diseases due to *Vibrio*. If shrimp are infected, either as PL or older shrimp, they can survive reasonably well as long as the temperature remains above 30^{0} C. However, if the temperature drops below around 27^{0}C, mortality rates increases. Studies show that that the rate of mortality in shrimp infected with some virus diseases such as WSSV and TSV is affected by water temperature and had total crop failures unlike those who stocked later when the temperature was high and stable.

11.2.3.3. pH

The pH stress could trigger the disease outbreaks by reducing the immune defense mechanisms of the host. pH in pond waters should be maintained in the range of 7.5-8.5. The influence of pH is harmful to the shrimp are usually caused by the mechanism of increasing the concentration of toxic or poisonous substances, such as an increase in anionic ammonia (NH_3) at pH above 7. Whereas in waters with

low pH will cause an increase in the fraction of anionic sulphide (H_2S) and the toxicity of nitrite, as well as physiological disorders in shrimp.

11.2.3.4. DO (Dissolved oxygen)

In intensive aquaculture practices, dissolved oxygen (DO) is a major limiting factor especially in the bottom layers of shrimp culture ponds. Decomposition of accumulated feed and the animal faeces lead to hypoxic and sometime anoxic conditions particularly at night time. DO levels were above 4 mg/L during day time with aeration, whereas the levels may go less than 2 to 3 mg/L during night time/early morning. DO less than 2.8 mg/l is considered hypoxic condition and it is known to influence growth, survival, feeding, moulting, behavior, osmoregulatory capacity and immune response of Penaeid shrimps.

11.2.3.5. Transparency and water colour

It reflects the type and density of plankton. The more intense colour of water signifies the more number of existing plankton. Too high plankton density may affect fluctuations in dissolved oxygen and pH in the pond. On a sunny day, the amount of dissolved oxygen will be very high and the pH tends to lower, while the evening will be very high pH and DO can decrease to less than 2 ppm. Transparency must be maintained at a level of 30-40 cm.

11.2.3.6. Metabolites

Unfortunately a single metabolite may not be responsible for retarded growth or mortality of shrimp in ponds. It is essential to study at what level of toxicity shrimp can tolerate under combinations of two or more metabolites (ammonia, nitrite, sulphide).

11.2.3.7. TAN (Total Ammonia Nitrogen)

The concentration of total ammonia nitrogen (TAN) in intensive grow-out ponds increases as culture progress and levels of more than 1.0 ppm are toxic. The percentage of the toxic form increases as pH and temperature rise during the day and can reach critical levels. In addition to immune response, elevated concentration of TAN affects the growth, moulting, oxygen consumption and ammonia excretion. Increased concentration of TAN decreases the activity of superoxide dismutase responsible for the scavenging of reactive oxygen species (ROS) leading to increase in superoxide anion. Reduced phagocytic activity and clearance efficiency lead to increased susceptibility to bacterial infections.

11.2.3.8. 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. The higher concentration of the nitrite is known to decrease the levels of total haemocyte counts to the reduced

prophenoloxidase and phagocytic activities. Nitrite is more toxic in low saline conditions compared to brackish and seawater based culture ponds.

11.2.3.9. Hydrogen sulphide

Sulphide 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 0.05 mg/Litre of H_2S may be lethal to aquatic organisms and any detectable concentration is undesirable. Presence of sulphide affects the immune parameters like total haemocyte count, hyaline cells, phenol oxidase activity, phagocytic activity and clearance efficiency thereby making the shrimp more susceptible to pathogenic infections like, vibriosis.

11.2.3.10. Water exchange

Traditionally the management of water quality is through water exchange to reduce organic and to flush excess nutrients and plankton (cyanobacteria) out of the pond. Periodic partial removal of cyanobacteria and algal blooms by flushing or scooping out the scum facilitates optimum density and prevents sudden die-off of the bloom. Minimization of water exchange will prevent viruses and carriers / bacterial pathogens from entering the ponds and reduce the possibility of disease transmission into culture ponds. But the reduction of water exchange requires closer control of water quality parameter such as pH and ammonia, effective sediment management, careful control of feeding and reduction of stocking density. However, improperly managed closed system increases the risk of stressful rearing conditions, bad water quality and diseases in ponds. Hence, the best water management option available to farmers is limited water exchange from treated reservoir, which enables good water quality conditions in ponds, while reducing the potential of disease introduction to the farms through intake water.

11.2.3.11. Aeration

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

11.2.4. Feed management and water quality

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

11.3. Conclusion

Sustainability of aquaculture depends on the maintenance of a good environment. The two-pronged approach of combining pond management and health monitoring is the key for successful shrimp/finfish production. It is important to know how much biomass can be supported by the pond environment (carrying capacity of pond). Although the ideal carrying capacity can be low, higher production volumes can be achieved by partial harvesting more than once. The well-designed management practices pertaining to soil and water quality should increase the efficiency and productivity by reducing the risk of shrimp / fish health problems, and reduce or mitigate the impacts of farming on the environment. Regular monitoring of environmental parameters and timely mitigation using appropriate biological agents is the key to protect potential losses due to stress and opportunistic bacterial infections. The understanding on ecological process occurring in culture ponds through regular monitoring will help to solve some of the disease issues faced by the farmers.

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12

Role of Zero Water Exchange-based Technologies in Shrimp Culture with Special Reference to Prevention of Diseases

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12.1. Introduction

Shrimp aquaculture is an important commercial activity in coastal ecosystem of many Asian and American countries including India. Traditional aquaculture has evolved through centuries to create agricultural systems adapted to local environmental and cultural conditions. Under these scenarios, pond water is frequently exchanged to maintain the desirable water quality for shrimp growth. Nutrient laden effluent discharge causes environmental degradation and eutrophication of the nearby ecosystem. Growing attention of people, media and government over the environmental issues demand better culture practices which could have least negative impact over the environment. Further, over the last two decades, the global shrimp aquaculture is plagued with large number of devastating viral diseases such as white spot disease, IHHNV etc. Shrimp pathogens appear to be transmitted horizontally from effluent water. As no therapeutics or vaccines is available to control the viral disease problem in shrimp culture, prevention becomes the ultimate remedies. Under these circumstances, recent development of zero water exchange, biofloc and periphyton based farming system will provide the necessary boost in disease control.

12.2. Zero water exchange

Global shrimp aquaculture is presently plagued with large number of diseases. In such scenario water exchange become a risky management practice. Central Institute of Brackishwater Aquaculture has developed a Biosecured Zero Water Exchange System Technology (BZEST). This system relies upon zero or minimal water exchange. The monsoon precipitations mostly take the care of evaporation loss. Zero or minimal water exchange coupled with stocking of disease free shrimp post-larvae and quality feeds help to realize the disease free shrimp culture.

12.2.1. Principle

Unlike the open system where water replacement is done as per the level of intensification, the zero water exchange shrimp farming system is a closed system which provides a means to achieve higher degree of biosecurity. The biosecured system ensures the prevention of bacterial/viral contamination, which is the major

bottleneck to have sustainable shrimp farming. Once the disinfected (with 60 ppm of chlorine) water is taken and cultured for optimum bloom, no further water is taken from the source and the evaporation loss etc. is compensated by treated water or the crop is so scheduled to take the advantage of rainwater for that purpose.

12.2.2. Role of zero water exchange in biosecurity

Biosecurity is the protection of living organisms by the Exclusion of Pathogens and Other Undesirables. The adoption of biosecurity protocols in shrimp aquaculture has resulted in the shrimp overall production increase. Success of establishment and implementation of biosecurity program in shrimp aquaculture system demands the pivotal role of cluster farming in which each individual farmer plays crucial role. Thus Zero water exchange system provides a means to achieve higher degree of biosecurity in shrimp culture system.

12.2.3. Methods adapted for disease control under zero water exchange culture system

Following culture procedure should be ensured for disease free rearing of shrimp;

- Preparation of pond: After harvesting pond bottom contains high load of organic matter, toxic compounds, bacteria, parasites, virus particles as well as many WSD virus carriers. Effective pond preparation include black material removal by washing with high pressure water or scraping, drying for at least two weeks to kill all disease causing organism such as fungi, protozoa, bacteria and viruses by oxidation. Longer exposure to sunlight should be ensured in a pond which has recorded disease.
- Remove virus carriers such as wild shrimp, crabs, copepods and other crustaceans using nylon screen of 60-80 meshes/cm^2. These meshes should be placed as three tier filtration at the inlet of the reservoir. Many animals like mudskippers, snakes, frogs could be kept out of farm by installing a fine net enclosure.
- Stocking of Specific Pathogen Free (SPF) post-larvae from well recognized and coastal aquaculture authority approved hatchery is the fundamental requirement to get disease free shrimp culture under zero water exchange. Insure that hatchery has supplied post-larvae to your farm after properly testing the major viral infections such as WSSV, IHHNV, TSV, YHV, etc.
- Avoid very high density. Acclimatize the PL properly before release in pond at dawn or dusk.
- Avoid excess feeding and old feeds. Use check tray and monitor feeding as per the standing biomass and physiological condition of shrimp. Restricted

feeding should be done during moulting. Never feed live crustaceans or trash fish and its frozen product. Use pellet feed with balanced nutrients.

- Oral probiotics and effective immunostimulants help to improve the host immunity by eliciting the non-specific immune response. This can be tried at regular interval.
- Avoid stressful conditions such as low water depth, overcrowding, poor water quality.
- Water quality monitoring and management with application of lime, gypsum.
- High temperature, no aeration, algal bloom or crash, and disturbing pH may increase the susceptibility of WSSV infection.
- Regular health monitoring and PCR testing of white spot virus and other infection should be done.
- Virus can be inactivated by halogenous disinfectants including sodium hypochlorite or formalin - 0.25%, 0.5 ppm chlorine and 0.3 ppm Iodine.
- Treat pond effluents as per the norms set by aquaculture authority of India. Treatment of effluent is mandatory for bigger farms and collectively for smaller farms. This includes disinfection or biological filtration through cultivation of algae, sea weeds, clams, and filter feeders or omnivorous fishes to reduce the excess organic matter and pathogenic microorganisms.
- Every drop of intake water must be disinfected with 30 ppm calcium hypochlorite and left for 3-4 days. Minimize the water exchange.

12.3. Biofloc technology

Biofloc is a heterogeneous mixture of bacteria, algae, protozoa, zooplankton, food particles, organic polymer and dead cells. But generally, it is a bacterial dominated system, which often reaches in the order of 10^7 CFU/ml. This provides enough time for shrimp to engulf the floc particle.

12.3.1. Principle

Biofloc technology is based on the manipulation of carbon nitrogen ratio (C: N ratio) in aquaculture system. It has been suggested that C: N ratio of 10:1 is the best for biofloc production (Avnimelech, 1999). The C: N ratio can be manipulated by application of various carbohydrate sources such as molasses, rice flour, tapioca powder etc. or by changing the protein level in the feed. As a thumb rule, for each 1 g of nitrogen, 20 g carbohydrate should be added. Otherwise, 30% protein level in the feed also works best for biofloc system. At high C: N ratio, heterotrophic bacteria immobilize the ammonium ions for production of biofloc. This helps to reduce toxic ammonia– N in aquatic system (Avnimelech 1999). At CIBA, our work

suggests that biofloc improved the growth rate in juvenile and adult by 29.0 and 12.6%, respectively. Work conducted at Belize farm in Central America suggests that biofloc system in zero water exchange lead to 29% more nitrogen retention in *Penaeus vannamei* (Burford *et al.*, 2004). Thus apart from faster growth rate, biofloc based system could help to reduce the feed quantity intake which will lead to reduced cost of production. Most of the farmers rely upon commercial probiotics application to improve the digestive performance of shrimp. The work conducted at CIBA suggests that biofloc improve the digestive enzymes and assimilative capacity of cultured shrimp (Anand *et al.,* 2015). Similar results have come from the work conducted at many organizations worldwide. This shows the true potential of biofloc as probiotics to stimulate growth rate in cultured shrimp.

12.3.2. Role of biofloc in disease control

Many recent studies including work at CIBA suggests that biofloc has the capacity to prevent the disease occurrences. Crab *et al.* (2010) reported that provision of biofloc improved the disease resistance ability of shrimp naupli by 3 fold. Numerous studies have shown that shrimp grows better in presence of bacteria, algae and other microbiota. This may be due to inherent immunostimulant properties of many bacterial strains in shrimp culture pond. Shrimp is solely dependent on innate arm of immune system for disease resistance. Therefore, enhancement of innate immunity of cultured shrimp by biofloc will provide the resistance against broad range of pathogens.

12.3.3. Challenges in biofloc system

Though biofloc based system provide many opportunities over conventional zero water exchange system. But the practice is full of limitation and challenges. The biggest challenge a biofloc based system provide is excessive turbidity accompanied with high level of bacterial growth. This also leads to deposition of large amount of sludge at the bottom of the pond which needs regular removal. In *P. vannamei* ponds at Belize farm in Central America, sludge removal progressed from weekly interval in the mid phase of culture to everyday at the end phase of culture. This definitely limits its utility in semi-intensive farm, which most often exists in Indian condition. Another big challenge lies in maintaining the dissolved oxygen level. In routine, oxygenation in biofloc system should be done 24 hrs a day. The yard trial study conducted at CIBA indicates that oxygen drop is up to 1 ppm/hr in biofloc based system (Kumar *et al.,* 2015a). This could be still higher in pond condition seeing the more depth. Thus more than one hr without oxygenation could be disastrous in biofloc system. Therefore, we suggest keeping generator back up 24 hrs in ready condition. Further, regular checkup of biofloc volume should be done using Imhoff cone. Once floc volume crosses the critical level, addition of carbohydrate should be stopped and sludge removal should be initiated.

12.4. Periphyton based farming system

Periphyton refers to the entire complex of attached aquatic biota on submerged substrates. It comprises phytoplankton, zooplankton, benthic organisms and detritus. Natural biota associated with submerged substrates forms an excellent quality natural food for the cultured shrimps. Various kinds of substrates are used in periphyton based system. This includes bamboo, kanchi, jute stick, paddy straw, sugarcane bagasse, nylon, velon, etc.

12.4.1. Role of periphyton in shrimp health management

It has been reported that provision of substrate in the early growth stages of penaeid shrimp improves the survival even at high stocking density. The consumption of microbes and algal community present over submerged substrates enhances the growth of penaeid shrimp by providing quality natural food. The trials conducted at Kakdwip Research Centre of CIBA, Kakdwip yielded 17.9% gain in production and 22.3 % reduction in FCR compared to conventional culture practice (Kumar *et al.,* 2015b).

Algal products and their cell wall components are widely used to elicit non-specific defence mechanism in fishes and shrimp. Periphyton has been reported to enhance immune responses in shrimps like *P. vannamei* (Zhang *et al.,* 2010). Recently, work conducted at CIBA showed that periphyton powder as dietary supplement enhances immune response and disease resistance in *P. monodon* (Anand *et al.,* 2015). Apart from improving productivity, periphyton based farming practice could also help in improving the shrimp immunity and health management.

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13

Application of Probiotics and Immunostimulants in Aquaculture and their Role in Aquaculture Disease Management

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Export of marine products has become one of the major foreign exchange earners in recent years. India exported 10.51 lakh tons of marine products worth ₹ 33,441.61 crores (USD 5.51 billion) during the year 2014-15. Shrimp constitutes major portions with 3.57 tons worth ₹ 22,468.12 crores (USD 3.71 billion), while fresh finfish constitutes 3.09 lakh tons worth ₹ 3,778.50 crores (USD 0.61 billion) (www.mpeda.com). For almost one decade the wild catch has been stagnant and contribution from the aquaculture is growing. Ever increasing population, awareness about the health benefits and the higher demand for aqua food products in international markets has made aquaculture as one of the profitable businesses in developing countries especially India. During the last two decades aquaculture in India has grown many folds, however health management of is one of the major area of concern for the economical sustainability of the industry. Diseases like, white spot syndrome, vibriosis and emerging diseases like Early mortality syndrome, white gut, white muscle, white faeces has necessitated the development of strategies to combat the issues related to disease which are safe for host and environment.

Recent trend of intensification in aquaculture after introduction of *Penaeus vannamei* has posed several challenges both for safety of host and maintaining the quality of environment. Similar trend is observed in almost all the major shrimp producing countries in the world. Recent intensification in aquaculture in India has resulted in deterioration in pond environment exposing the animals to stressful conditions. Such animals are more susceptible to opportunistic pathogens especially those leading to vibriosis, resulting in severe economic loss. Extensive use of antibiotics for prevention of bacterial infections leads to the development of antibiotic resistance and export restrictions.

In an effort to achieve higher production targets various chemicals and biological products are used in hatchery and grow-out culture without clear understanding of the scientific basis. Probiotics and immunostimulants are one such group of products, which are used and abused extensively in Indian aquaculture. Probiotics are single or mixed culture of live microorganisms, which has the beneficial effect on host and the environment. On the other hand,

immunostimulants are microbial, animal, plant, chemical or synthetic substances effectively stimulate the immune system to enable the host to fight stress and infections. Though probiotics and immunostimulants are effectively used in human and veterinary medicine, their efficacy and mode of application needs intensive study in aquaculture.

In a recent estimate aqua farmers spend about ₹15-20 worth probiotics and immunostimulants per kg of shrimp produced which is about 10% of the production cost. Clear scientific understanding at the level of scientific community and proper advise to the farming sector will help in effective utilize the beneficial effect of these products in Indian finfish and shellfish culture operations. There is an urgent need to set standards for quality of the probiotic and immune stimulating agents so that genuine products are made available in the market.

13.1. Probiotics in shrimp aquaculture

Generally probiotics for aquaculture applications can be classified as gut probiotics, water probiotics and soil probiotics. However, some bacteria have demonstrated beneficial effects in both gut and soil. Several mechanisms have been attributed to the beneficial effects of probiotics which include competitive exclusion of pathogenic bacteria, helps digestion and provide nutrients, utilize organic matter, enhance immunity and improved water quality. While immune stimulating agents of microbial or plant origin are most commonly used in aquaculture practice.

13.1.1. Gut probiotics

Several bacterial species have been reported to have beneficial effect in gut of aquatic animals. However, only few species are extensively used in commercial probiotic products, like, *Bacillus pumilus*, *B. megaterum*, *B. subtilis*, *B. polymyxa*, *B. licheniformis*, *Cellolomonas* spp., etc. Gut probiotics generally colonize the gut and competitively inhibit the pathogenic bacteria in addition to possible release of some bactericidal molecules. These probiotics are applied regularly throughout culture period as feed top dressing.

13.1.2. Water probiotics

It has been estimated that almost 70% of the feed in shrimp pond is left unconsumed and accumulates in pond bottom. Build-up of uneaten feed and practice of zero water exchange deteriorates the pond environment causing stress to animals. It is important to note that in line with human medicine, most of the emerging diseases in recent times are due to stress due to pollution. Over a period of culture cycle toxins stockpile in the environment causing stress to animals which leads to reduce immune system making animals susceptible for opportunistic pathogens. Stressed animals also face metabolic malfunction and

show reduced growth and mortality. *Nitrosomonas* spp. and *Nitrobacter* spp. are the autotrophic bacteria extensively used as water probiotics in aquaculture. These bacteria are associated with nitrogen recycling and help in mitigating the toxic effects of ammonia, nitrite and nitrate in pond water.

13.1.3. Soil probiotics

Accumulation of uneaten feed deteriorates the pond bottom leading to black soil due to hydrogen sulphide and other sulphur metabolites. This condition needs immediate attention otherwise leading to releases of toxic gases into water column. Bacteria commonly used in soil probiotics are *Paracoccus* spp., *Rhodococcus* spp., *Rhodobacter* spp. and *Thiobacillus denitrificans*. These are mostly sulphur recycling bacteria establish in the pond bottom and help in converting toxic hydrogen sulphide.

13.2. Immunostimulants in shrimp aquaculture

The stress imposed due to intensive aquaculture practices by the high-density, supress the immune system of animals. Immune compromised animals are susceptible to pathogens ultimately leading to retarded growth, mortality and economic loss. Environmental hazards of using chemicals in aquaculture have prompted the scientists to search for alternative strategies that improve the immune competence of aquatic animals. For development of effective immune stimulatory compounds, it is essential to understand the basic mechanisms of host physiology and the defence system.

Depending on the origin, immunostimulants used in aquaculture can be classified as bacterial, algae-derived, animal-derived, nutritional factors as immunostimulants, and hormones/cytokines. Immunostimulating agents are defined as 'naturally occurring compound that modulates the immune system by increasing the host's resistance against diseases that in most circumstances are caused by pathogens'. Unlike vertebrates, shrimp do not have adaptive immune system and depend mostly on innate immune mechanism. Recent interest in immunostimulants as an alternative to the drugs, chemicals and antibiotics for health management in aquaculture is mainly due to the ability of immunostimulating agents to enhance the innate (or non-specific) immune response. The major advantages of immunostimulants are that it can be administered by bathing or orally as feed top-dressing to shrimp. Live and killed bacteria, bacterial cell wall, lipopolysaccharides, peptidoglycan, glucans and chitin/chitosan are some of the extensively studied immune stimulating agents for aquatic species. However, synthetic compounds, polysaccharides, vitamins and animal and plant extracts are also reported to enhance the non-specific immune response in finfish and shellfish.

13.3 Logic in method of probiotics and immunostimulant application

For probiotics bacteria to colonize the environment and exhibiting the beneficial functions, application of these products in optimum dosage and schedule is very important. Suitable conditions required for multiplication of the probiotic bacteria depends on the concentration and schedule of application, combination of probiotics bacteria and mode of application. It is essential to understand that bacteria in the probiotic products are very sensitive to water parameters like pH, temperature, salinity, DO, nitrite, ammonia etc. Hence it is not necessary that a probiotic product works similarly in all seasons, regions and stage of grow-out culture. Similarly immune stimulating agents reach specific organs in the host and enhance the secretary and differentiating functions for effective mitigation of stress and to encounter the pathogens effectively. Application of these products in appropriate dose and schedule play very important role in expression of beneficial effects on health and growth of animals.

13.4. Labelling of products

Medicinal products including probiotics and immunostimulants used in human veterinary and aquaculture practice need to be labelled with contents, concentration, dose and indications. However, most of the products do not follow such regulations in Indian aquaculture. Thus the market is full of products without proper labelling and some manufacturers claim effectiveness of the product in removing ammonia, cleaning the pond bottom and improve the growth and survival of shrimp. So, farmers should be aware of such products and avoid their applications without scientific backing.

13.5. Conclusion

Though the mechanism behind the beneficial effects of probiotic and immunostimulant application in aquaculture is not unequivocally established, it is definitely an important activity in Better Management Practices (BMPs), both in hatchery and grow-out cultures. It is essential to understand the composition of products and mode of application, suitable for the use in different types of culture systems. Since these products are prophylactic in nature and not therapeutic agents, hence need regular application. It is essential to understand that, beneficial effects of these products could be realized only when quality products are applied in prescribed schedule and dose. Farmers need to understand the scientific basis behind the application of these products to obtain their intended prophylactic effects and reduce the cost of production.

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Introduction to Crustacean Culture Activities Kakdwip Research Centre of ICAR-CIBA

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14.1. Introduction

Among all the brackishwater crustacean species, the major candidate species for culture in India includes Tiger shrimp (*Penaeus monodon*), Indian white shrimp (*P. indicus*), White leg shrimp (*P. vannamei*), Kuruma shrimp (*P. japonicas*), Red tail shrimp (*P. penicillatus*), Banana shrimp (*P. merguiensis*) and Mud crab (*Scylla serrata* and *S. olivacea*). Shrimp aquaculture in India in its predominant form was essentially farming of *P. monodon*; the decline production of which is gradually compensated by *P. vannamei* introduced in 2009 through SPF broodstock imported from other countries. Of the total shrimp produced in India during 2014-15, production of *P. vannamei* increased by 41% to 3,53,413 tons where as black tiger shrimp (*P. monodon*) production remained stagnant at 71,400 tons. Unlike shrimp, there is presently no organized aquaculture of mud crab in India due to the inconsistent availability of crab seed for farming. ICAR- Central Institute of Brackishwater Aquaculture, Chennai has standardized the hatchery seed production cycle of *S. serrata* in late 1990s. A package of technology for mud crab culture as well as production of seed in hatchery available for commercialization envisages the scope for large scale development of crab farming in the country. *Scylla serrata* attains a maximum carapace size of 280 mm and 3.5 kg recording even 14–28 g/week weight gain. In the light of virus affected coastal ponds, mud crab farming forms an excellent mean of diversification of brackishwater aquaculture. Scientific crab culture is getting momentum due to its high value in the live export market (mainly South East Asian countries) and minimum disease risk noticed during the culture period

14.2. Shrimp culture activities

For conducting any shrimp culture operation, it is important to follow strict biosecurity measures to prevent transmission of diseases in shrimp farm. Farm biosecurity requirements like reservoir pond, crab fencing, bird fencing, disinfection facilities and allocation of separate implements for each pond should be incorporated in the system. Good pond management in shrimp culture includes the following practices:

14.2.1. Pond bottom soil removal

The upper 25 to 75 cm layer of soil should be removed after complete draining and drying of pond. This top layer contains high organic content resulting from deposition of uneaten feed and faecal matter during the culture period. High concentration of organic matter can lead to anaerobic sediment that can have adverse effect on shrimp growth and survival.

2.2.2. Installation of bird fencing and crab fencing

Farms must establish adequate bio-security measures including fencing, reservoirs, bird-scare lines, separate implements for each of the ponds etc. The farms should be managed by the personnel who are trained and/or experienced in management of bio-security measures.

14.2.3. Water intake

Intake water must be filtered at the main sluice and at each pond feeder pipe with fine mesh screen filter bag (60 mesh/inch) to prevent entry of vectors and pathogens that maybe present in the source water. The water must be disinfected with 60 ppm of active chlorine and left for at least a week.

14.2.4. Seed Stocking

Only quality seed should be procured for culture and PCR testing should be done to ensure virus free seed. *Penaeus vannamei* seed should be procured only from hatcheries authorized for import of broodstock and/or production of seed. Before stocking at pond, PL should be treated with formalin at 100 ppm concentration for 30 min in well aerated tanks and acclimatized gradually to pond salinity, temperature and pH. Stocking density should not exceed 60 nos./m^2 for *P. vannamei* and 10–12 nos./m^2 for *P. monodon.*

14.2.5. Soil and water quality management

Degradation of water quality is detrimental to shrimp growth and survival. Regular monitoring of soil and water quality parameters like temperature, salinity, pH, dissolved oxygen, TAN, nitrate, nitrite, total suspended solids, etc. should be done.

14.2.6. Pond aeration

Aeration of ponds should be carried out to increase the dissolve oxygen and remove stratification in ponds. *Penaeus vannamei* in particular is sensitive to oxygen stress

and since a higher stocking density is maintained all along, aeration is very critical aspects for this species.

14.2.7. Feeding management

Optimal feeding rate and frequency are essential in maximizing conversion rate of feed to shrimp biomass. Check tray should be monitor at interval to avoid overfeeding or underfeeding of shrimp and feed requirements must be calculated as per the standing biomass. Feeding is usually done @ 5% of total biomass at the beginning which is gradually reduced to 3.5% at the end of culture period.

14.2.8. Health management

Shrimp should be sampled once a week and should be checked for their general health condition like external appearance (body colour, missing appendages, external/ gill fouling, black gills or gill choking, etc.), gut condition, and growth in terms of weight or length. Shrimp behavior and feeding trends should also be monitored. The gut content colour is a good indicator of the probable health status and corrective action to be taken. A black/ brown/ green gut implies under feeding whereas a red or pink gut indicate disease manifestation, whereas a pale whitish gut showed gut infection. A normal gut will have a light or golden brown colour.

14.2.9. Effluent treatment system (ETS)

Pond effluents should be treated and should conform to the standards prescribed under the guidelines issued by the Coastal Aquaculture Authority (CAA), Govt. of India. Waste water should be retained in the ETS for a minimum period of two days. Treatments may include disinfector or biological filtration through cultivation of algae, seaweeds, clams and filter feeders or omnivorous fishes to reduce the excess organic matter and pathogen. In case of disease outbreak, water should be chlorinated and dechlorinated before discharge into the drainage system.

14.2.10. Farm record maintenance

Maintenance of record is necessary to identify problems in the pond environment and shrimp health and to rectify these problems at the earliest during the production cycle. Farm records ideally should contain details on pond preparation, seed and its stocking, feed management, water quality parameters and its management, pond bottom management, shrimp health and harvest.

14.3. Culture system

14.3.1. Closed System

This system involves filling up the ponds with cleaned brackishwater which is disinfected using chemicals. The shrimps are stocked up to 12 and 60 PL/m^2 in case of *P. monodon* and *P. vannamei*, respectively and cultured for a period of 100–120 days. Water loss due to evaporation and seepage is replenished with water

from reservoir by pumping. The disadvantages of this system are that it requires low stocking density and high efficient water and waste management. However, it can be operated anywhere, even in the inland area where seawater is not easily accessible.

14.3.2. Periphyton based system

Microalgae are important dietary source during larval and post larval stages for penaeid and non penaeid shrimps. Development of periphytic algae and its distribution is dependent upon the nutrient level in the water column. Application of organic and inorganic fertilizers stimulates the growth of periphytic algae. The recommended levels are - organic fertilizer @ 500-1000 kg ha^{-1} and inorganic fertilizers like urea and single super phosphate (SSP) @ 25-100 kg ha^{-1}. Fertilized ponds develop a considerable amount of periphyton over a period of 2–4 weeks. About 10% of the original dose of inorganic fertilizers i.e. urea and SSP at 10-20 kg ha^{-1} are applied periodically (monthly) if sufficient primary productivity of pond is not maintained. The periphytic algae must be grazed constantly and kept in an exponential growth in order to stimulate periphyton production and maintain high productivity. Increased standing biomass in the absence of grazers may lead to self-shading and death of algae, with consequent sloughing and dislodgement of the community.

14.3.3. Biofloc based system

In aquatic ecosystems, heterotrophic community mainly bacteria, protozoa, fungi and associated detritus form a major contributor to the total production of cultured species. Microbial protein is generated in aquaculture ponds when organic matter added as manure or feed is decomposed by microorganisms under aerobic condition. Microbial breakdown of organic matter leads to the production of new bacterial cell with the direct assimilation of dissolved nitrogenous matters. Studies reveals that incorporation of dried biofloc at 4- 20% inclusion level in shrimp diet significantly improved growth rate in penaeid shrimps. About 10-20% potential feed gain is estimated by application of biofloc technology which can reduce the production costs considerably since feed represents 40-50% of the total production costs.

14.3.4. Organic shrimp farming technology

The pond based organic farming of *P. monodon* was taken up at Kakdwip Research Centre with inputs like biocompost / vermicompost, yeast based organic preparations, and low fish meal feed. Effective utilization and exploration of natural productivity through organic manuring, zero tolerance to artificial fertilizer, pesticides, chemotherapeutants, medicines including antibiotics and integration of mangroves and other plants in the organic ponds were among some of the salient features of this farming practice. Application of yeast based organic preparations

and vermicompost prepared from different substrates with inocula of vermin *Eisoenia foetida*, which were developed and standardized through yard trials could ensure higher (275–350 mgC/m3/h) natural productivity in organic ponds compared to that in conventional ponds (200–240 mg C/m3/hr). Low fish meal based organic feed prepared from different plant protein sources were tested in different combinations to arrive at a low cost feed with 15% fish meal (protein contribution from different sources- fish meal: other marine protein sources: plant protein sources- is 38:35:27 in control feed and 23:24:53 in low fish meal feed). The overall growth performance was better in organically managed ponds with a productivity in the range of 1200–1400 kg/ha. The success of this farming technology is marked with improvement in production level (14–21%), size at harvest (10–19%) with better FCR (lowered by 4–18%) in the organic ponds.

14.4. Mud crab farming activities

Mud crabs belongs to genus *Scylla* spp., are commercially important and fetches high value in export market. In India most widely distributed species are green mud crab, *S. serrata* and orange mud crab *S. olivacea*. Mud crab farming includes nursery rearing, grow-out culture and crab fattening.

14.4.1. Nursery rearing

Nursery rearing involves rearing of megalopa to crab instar in two phases *i.e.,* up to 3 g in hapa and 3-25 g in nursery ponds. In nursery rearing first phase, megalopa or early crablets can be stocked in nylon net hapa (3x 2x1) fixed in open brackishwater ponds or nursery earthen ponds. In order to reduce cannibalism seaweed bunches or nylon threads or nets bundles can be provided in nursery ponds for refuge. The average expected survival in the nursery rearing system is up to 60%. In nursery rearing phase II, the crablets of 3 g sizes are reared up to crab juveniles at 2-20 nos./m^2 in fenced nursery ponds using fresh feed at 10% of body weight.

14.4.2. Grow-out culture techniques

Grow-out crab culture can be broadly divided in to two major techniques like crab fattening and grow-out farming. In grow-out techniques, nursery reared crab juvenile (30-50 g) are reared for a period of 6-7 month period to attain marketable size of 500 g whereas fattening refers to the holding of water crabs for short duration to acquire maximum marketable traits to obtain better economic returns.

14.4.2.1. Grow-out ponds

Mud crab grow-out culture can be carried out in any coastal ponds or abandon shrimp ponds with little modification. Since mud crabs can crawl out of the ponds, it is imperative to provide crab fencing around the ponds to provide the escape of the crabs. As mud crabs are highly cannibalistic in nature, hideouts like PVC pipes need to be provided to protect the crabs during moulting and to increases the survival. Construction of dry raised feeding platforms or mounds within the ponds are also appreciable as it can mimic their periodic exposure that occurs in the natural system. These platforms can also act as the feeding zones for the crabs. In grow-out system, mud crabs are generally stocked 0.5–1.5 nos./m^2. Mud crabs can be fed with formulated feed or trash fish or locally available molluscan meat at 2-8% of body weight. A scientifically managed grow-out rearing ponds can yield up to 2 tons per ha with an estimated survival of around 60%.

14.4.2.2. Polyculture

As mud crabs cannot catch fast moving preys like shrimps or finfishes, polyculture of mud crab with finfishes or shrimp has tremendous scope to increase the economic return of the culture. Polyculture with milk fishes or mullets and tiger shrimps or seaweeds can yield up to 2.5-3 tons per ha in six month culture period.

14.4.2.3. Monosex culture

Monosex culture are getting momentum now a days as crabs are sexually differentiated and stocking with monosex minimize post-harvest processing measure and minimize aggressive behavior between crabs associated with sexual maturity. Studies reveal that monosex culture of female or male increases the survival rate compared to mixed sex culture.

14.4.3. Crab fattening

Crab fattening is the technique where water crabs or newly molted are held for a period of few weeks until they are full of meat and ready to market. Generally floating cages or tanks cans be used for fattening. Crabs are fed with bivalve meat / trash fish with a daily ration at about 5-10% of body weight. Carb fattening can be carried out at high densities provided with good quality of water, optimum feed management and health management.

14.5. Further reading

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15

Introduction to Brackishwater Finfish Culture Activities of Kakdwip Research Centre of ICAR-CIBA

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15.1. Introduction

The estimated potential area under coastal aquaculture is 1.2 million ha, of which only around 13% area has been brought under the culture. Considering the production potential of the sector, its production is projected to grow by four-fold by 2020 from the present at 0.45 million tons. Development of Indian coastal aquaculture in the country was driven by the technologies for seed production of tiger shrimp, *Penaeus monodon* and the white shrimp *P. indicus*. Disease outbreaks due to WSSV during the last two decades have acutely affected shrimp farming in the country and in other continents. With the diagnostic kits developed for detecting WSSV, PCR-tested seed is available all over the country. For the sustainable and eco-friendly aquaculture practice, diversification to other species is considered as one of the important steps. Fishes like Asian seabass (*Lates calcarifer)*, grouper (*Epinephelus tauvina)*, snappers (*Lutjanus* spp.), which are high value carnivorous fishes and grey mullet (*Mugil cephalus)*, milk fish (*Chanos chanos)*, pearl spot (*Etroplus suratensis)*, rabbit fish (*Siganus* spp.), orange chromide (*Etroplus maculatus*) which are herbivorous/omnivorous are suitable for farming in the coastal eco-system. The species like cobia (*Rachycentron canadum*), silver pomfret (*Pampus argenteus*) and pampano (*Trachinotus carolinus*) are being considered as candidate species for farming. Efforts have been made to develop comprehensive technology packages for seed production under controlled conditions and farming of these candidate species. Technologies have been developed elsewhere in the world for several brackishwater and marine finfishes. In Indian scenario, the successful technology has been developed for the year round seed production of Asian seabass, *L. calcarifer* under controlled conditions and farming by the Central Institute of Brackishwater Aquaculture. The institute has also accomplished controlled breeding of grouper, *E. tauvina*, milkfish, *C. chanos* and pearl spot, *E. suratensis*. In addition, a new avenue has been made by the successful breeding and seed production of ornamental fishes, spotted scat, *Scatophagus argus*, crescent perch (*Terapon jarbua*) and orange chromide, *E. maculatus.* Successful demonstration of seabass farming has been conducted in all the coastal states.

15.2. Seed rearing of finfishes

15.2.1. Seabass nursery rearing in grow-out site

Rearing of fry to stockable size seed in the hatchery itself has some problems. All hatcheries may not have such facilities since the requirement of space will be 5-6 times more than larval rearing space. Maintenance requires additional man power, energy etc. Above all, transportation of large sized seed to culture site would be expensive. To avoid these problems nursery rearing in grow-out site itself can be done wherever possible.

15.2.1.1. Seabass nursery rearing in ponds

Nursery ponds can be around 200-500 m^2 area with provision to retain at least 70-80 cm water level. Adequate provision for water inlet and water drainage should be provided. Towards drainage side there should be slope. Suitable sized (normally 1 mm) mesh screen nets should be provided in the inlet side and outlet side to avoid entry of unwanted fishes and escape of the stocked fish, respectively. The pond is prepared before stocking. If there are any predator/pest fishes, they have to be removed. In case where complete draining is not possible, water level is reduced to the extent possible and treated with Derris root powder @ 20 kg/ha or mohua oil cake @ 2000-3000 kg/ha-m to eradicate unwanted fishes. Use of other inorganic chemicals or pesticides is avoided because these may have residual effects. After checking the pond bottom quality, water is filled. If the pond bottom is acidic, neutralization is done with lime application. In order to make the natural food abundant, the pond is fertilized with chicken manure @ 500 kg/ha keeping the pond water level 40-50 cm. The water level is gradually increased. After 2-3 weeks period when the natural algal food is more, freshly hatched *Artemia* nauplii are introduced. Normally 1 kg of cyst is used for 1 ha pond. These stocked nauplii grow and become biomass in the pond forming food for the seabass fry.

Seabass fry is stocked @ 20-30 nos./m^2. Stocking should be done in the early hrs of the day. Fry should be acclimatized to the pond condition. Acclimatization for the pond condition is done as follows: the fry in the transport container are emptied into another tank and the pond water is gradually added into the container. This process is continued for a day or two depending upon the difference in the parameters. When the water temperature and salinity in the pond and tank water reach same, fry can be released into the pond. Water is changed @ 30% daily. Supplementary feeding is done with chopped, cooked fish/shrimp meat. The larvae can be weaned to artificial feed at this stage. The feeding rate can be as mentioned earlier. Excessive feeding should be avoided since it would deteriorate the pond condition and also promote filamentous algal growth. The excessive algal growth would deplete dissolved oxygen level in the early hrs of the day leading to fish mortality. Hence, excessive algae if any should be removed.

15.2.1.2. Seabass nursery rearing in cages/*hapas*

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

Floating net cages / *hapas* can be in the size of 2×1×1 to 2×2×1 m depending upon necessity. Cages are made with nylon/polyethylene webbings with mesh size of <1 mm. Fry can be stocked @ 400-500/m^3. The net cages have to be checked daily for damages, those may be caused by other animals like crabs. The net cages will be clogged by the adherence of suspended and detritus materials and siltation or due to fouler resulting in the restriction of water flow. This would create confinement in the cages and unhealthy conditions. To avoid this, cages/*hapas* should be cleaned once in one or two days. Regular grading should be done to avoid cannibalism and increase the survival rate. Even in higher stocking density @ 500/m^3, farmer could get survival of 80% in the farm site when the fry were reared in *hapas* adopting the trash fish feeding and other management strategies mentioned above.

15.2.2. Nursery rearing of grey mullet

Nursery rearing of wild collected stripped grey mullet fry can be conducted in brackishwater tide-fed ponds for production of advanced fingerlings. Grey mullet fry (0.17g/ 23.77mm) were stocked in ponds at 7500 and 15,000 nos./ha and reared for 6 months. In feed system, low cost feed prepared from locally available ingredients was provided in powder form for initial 4 months @ 20-5% and in pellet form for the rest 2 months @ 5-3.5% body weight daily in feed trays. In fertilization system, ponds were fertilized with cattle dung, urea and single super phosphate @ 500, 30 and 30 kg/ha, respectively at fortnightly applications. After 180 days of rearing, fish in fertilization system, achieved higher growth and survival than feed system.

In the previous trial a stocking density of 15,000 nos./ha was found as the optimum density for advanced grey mullet fingerlings rearing. With this density, the effect of fertilization and feeding was evaluated. (i) With only fertilization: Fertilization of the ponds with cattle dung, urea and single super phosphate. Initial application was done seven days prior to fish stocking and intermittent application was continued at 15 days intervals; (ii) Feed alone: Low cost formulated feed in powder form for first 1 month, then as pellet form for next 4 months given in feed

trays. Feed was composed of rice bran, mustard oil cake, wheat flour, fish meal and vitamin-mineral mixture with 27% CP and 6% lipid; (iii) Feed and fertilization: Combination of both as given under (i) and (ii). The rearing duration was 150 days and initial larval size of 0.55 g (36.03 mm). Feed and fertilization was found to be the best rearing system in term of final bodyweight. Though the survival was higher in the treatment with feed and fertilization, the difference with other treatments was not statistically significant. For the rearing systems of fertilization alone, feed alone, and feed and fertilization, the cost of production averaged ₹ 92300, 106250 and 128300/ha, respectively with the highest net return of ₹ 93400/ha from the later method.

15.2.3. Tank based breeding and seed production of pearl spot, *E. suratensis*

15.2.3.1. Breeding tank set up

Captive breeding of this fish was undertaken in specially designed circular cement cistern under simulated natural conditions. The flat bottom circular cistern of 10 tons capacity had an outlet pipe. Artificial nesting materials comprising of fire clay tiles (9 pieces) were hanged 1.0-1.2 m apart from the top of the tank keeping 50-60 cm inside from the tank wall and 45-50 cm above the bottom. Nine circular plastic tubs (55 cm dia × 35 cm deep) were filled up to 30 cm with bottom soils collected from a pearl spot broodstock pond. The tubs were placed at the tank bottom just under the tiles to facilitate the parental care. The tank was filled to a depth of 1.2 m, with dechlorinated filtered brackishwater (10-12 ppt). Three feeding pots made of fire clay were placed hanging in the tank at 50 cm water depth. Oxygen supply in the tank was maintained using compressed air.

15.2.3.2. Selection and stocking of brood fish

The pearl spot is heterosexual and females outnumber males in sex ratio in the natural population. The fish becomes sexually mature within a year of age with over 10 cm size. Pearl spot breeds in both brackish and freshwater throughout the year with two peaks in February- April and June- October. Pearl spot brood fish were raised in separate broodstock ponds. Mature males and females were selected based on their secondary sexual characteristics developed during breeding season. In males, the colour bands on body surfaces become darker and conspicuous, the greenish-blue iridescence and the pearly white spots are very prominent, ventral part of the body is covered with numerous dark pigmentations and overall the male turns gorgeously darker. The female is generally smaller in size with muddy yellowish darker colour. The mature male and female can also be differentiated by the presence of a projected genital papilla. In mature male, it is slender and pointed, whereas in female it is broader, swollen with tip blunt and reddish.

Altogether 12 pairs of selected brood fish of size 14-18 cm (80-120 g) were released in the breeding tank with 1:1 sex ratio.

15.2.3.3. Post-stocking breeding tank management

Pellet feed with 30% protein content was provided in the feeding pots at 2% of body weight twice daily. Fifty per cent of water in the breeding tank was replaced at weekly intervals with fresh treated brackishwater (10-12 ppt) and care was taken to keep the substrate tiles submerged during water exchange. Mostly the bottom water was exchanged to remove the accumulated feed wastage and faecal matter in order to prevent water quality deterioration. Aeration for 2 hrs was alternated with 2 hrs non-aeration in the tank.

15.2.3.4. Reproductive behavior, spawning and larvae production

In the breeding tank, captive seed production was facilitated by promoting parental care of the paired spawners. During the breeding season, after pair formation both male and female participate in nest making with the male contributing more to this activity. In nature, for nest making they utilize submerged stationary solid objects such as coconut leaves, coconut husk, wooden logs and stones placed 11 to 45 cm above the tank bottom. Within 2-3 days after release in the breeding tank, breeding pairs were formed. Then the pair started making nest by cleaning the tile surfaces and was seen to indulge in spawning acts within a month. The female was observed to lay flat on the spawning nest and gently move from one side to the other, attaching their eggs carefully on to the substratum with the help of its tubular and fleshy ovipositor and ventral fins. The male fish with a slow and quicker movement fertilized the deposited eggs instantly by releasing a spray of milt. This process of repeated egg laying and fertilization was continued for 2-3 hrs and the eggs were placed closely in a patch without touching each other in a single layer. A distinguished parental care of eggs and hatchlings was seen after the eggs were laid, the female brooded with their rhythmic fanning and mouthing activity and the male guarded the territory preventing entry of intruders. Formation of pits (6-8 cm dia and 2-3 cm deep) for larval brooding on the mud of plastic tubs was also found. The eggs hatched out in 80-90 hrs and the hatchlings were picked up by the brooding mother in her mouth and transferred to the pits. During this period also, fanning and mouthing of the female continued. After absorption of yolk sack in a week, the hatchlings became free swimming and gradually moved out of the pits to the open waters led by the parents. Natural food in the form of zooplanktons collected from brackishwater culture ponds was supplied in the tank as preferred food for larvae. Initially the larvae were devoid of body pigmentation and as they grew, they became free swimming and body pigmentation developed.

15.2.3.5. Collection of seeds

Within a month the seed resembled adult form with a size of about 2.0-2.2 cm. Seeds were collected by opening the outlet fitted with a net cage in a sump filled with brackishwater. Thus, in a breeding season of 4 months from this tank breeding 5000-10,000 pearl spot seeds could be produced. Therefore, 3 cycles could be accomplished in a year with a lucrative benefit-cost ratio (BCR) of 2.02.

15.3. Pond based grow-out culture

15.3.1. Traditional grow-out practices of seabass

Seabass is cultured in ponds traditionally as an extensive type culture throughout the areas in the Indo-pacific region where seabass is distributed. Low-lying excavated ponds are stocked whenever the seabass juveniles are available in the wild seed collection centers (For e.g. April-June in West Bengal, May-August in Andhra Pradesh, Sept-Nov in Tamil Nadu, May-July in Kerala and June-July in Maharashtra). Juveniles of assorted size seabass are collected and introduced into the traditional ponds which will be already present with some species of fish, shrimps and prawns. These ponds have the water source from adjoining brackishwater or freshwater canals, or from monsoon flood. The juvenile seabass introduced in the pond will prey upon the available fish or shrimp juveniles as much as available and grow. Since seabass by nature is a species with differential growth, on introduction into the pond at times of food scarcity, the larger may resort to feed upon the smaller ones reducing the number. Seabass are allowed to grow for 6-7 months of culture period till such time water level is available in these ponds and then harvested. At the time of harvesting there will be large fish of 4 to 5 kg as well as very small fish. In this manner, production up to 2 tons/ha/7-8 months has been obtained depending upon the number and size of the fish entered/introduced into the pond and the feed available in the pond.

However, this practice is highly unorganized and without any guarantee on production or return to the aquaculturists. With advances in the technology in the production of seed under captivity assuring the supply of uniform sized seed for stocking and quality feed for feeding, the seabass culture is done in South East Asian countries and Australia in more organized manner. The major problem in the development of seabass aquaculture in India is the unavailability of seed in adequate quantity and in time and quality feed for nursery rearing and grow-out culture. The former has been overcome and the technology package for the seed production of seabass under controlled conditions is available. The suitable feed for the culture of seabass has been developed. The seed production technology developed by CIBA has already been commercialized and the feed technology (CIBA Bhetki AHAAR) is ready for commercialization. These technological improvements in the seabass culture have motivated the farmers to select seabass as a candidate

species for aquaculture. Farmers have been adopting improved farming practices in seabass culture.

15.3.2. Improved seabass grow-out practices

The traditional culture method is improved with stocking of uniform sized seed at specific density and fed with low cost trash fishes/formulated feed of required quantity. Water quality is maintained with exchange periodically. Fish are allowed to grow to marketable size, harvested and marketed for high unit price. Seabass culture can be done in a more organized manner as a small-scale/large scale aquaculture in brackishwater and freshwater pond cages. This practice was further demonstrated in Public Private Partnership mode in three different costal states of India. Successful crops have been demonstrated in Andhra Pradesh, Tamil Nadu and Maharashtra with average production of 3-4 tons/ha.

15.3.3. Monoculture of grey mullet, *M. cephalus*

Grey mullet can be farmed in monoculture ponds. The pond for monoculture is prepared first, following eradication of unwanted organisms and application of manures and fertilizers. Advanced fingerlings of >50 g size are stocked at 10,000 nos./ha. Fish are fed with supplementary feed. In an 8-month culture, fish become 500-800 g with total production of 3-4 tons/ha.

15.3.4. Monoculture of milk fish, *C. chanos*

Milk fish can be farmed in monoculture and polyculture ponds. The wild seeds are collected in organized manner in Tamil Nadu and seeds are stocked in costal ponds. The milkfish farming follows a protocol of nursery rearing and farmed with farm made feed and floating pellet available in the market for other species. The scientific water quality management and supplementary feeding have given a production of 2 to 2.5 tons/ha in West Bengal and a higher production has been achieved in Andhra Pradesh.

15.3.5. Polyculture of fishes and shrimps

Polyculture is a farming practice where two or more species of fishes are reared together. The concept of polyculture is based on the fact that rearing of two or more compatible aquatic species together will result in higher production compared to monoculture. The underlying goal of polyculture involves increasing productivity by more efficiently utilizing ecological resources within an aquatic environment. Sometimes, one species enhances food availability to other species and thus increases total fish production per unit area. It is commonly believed that polyculture gives higher production than monoculture in extensive and semi-intensive systems and is considered more ecologically sound than monoculture. Before stocking of seeds, pond is prepared well following eradication of pest and predatory fishes, removal of bottom mud and liming, fertilization etc. The preferred

species among fishes are: mullets- *Mugil cephalus* (striped grey mullet), *Liza tade* (tade grey mullet), *L. parsia* (gold spot mullet), *Chanos chanos* (Milk fish), *Etroplus suratensis* (pearl spot) and tiger shrimp- *P. monodon*. The ready ponds are stocked with seeds of fish species at 8000-15,000 nos./ha along with tiger shrimp seeds of 15,000-30,000 nos./ha. The stocking density varies with the quantum of seed availability. Natural pond productivity is maintained by fertilization. In addition, supplementary feed prepared from locally available ingredients can be used at 2-5% body weight daily. This kind of system can yield a total production of 1.5-3.0 tons/ha in 6-10 months. In an out-station demonstration, three ponds of 1 ha each were stocked with grey mullet @ 10000/ha, pearl spot @ 1000/ha and tiger shrimp @ 10000/ha. After 10 months culture, total production of 3 tons/ha was achieved with net return of ₹ 1.7 lakh/ha.

15.4. Conclusion

Major constraint in brackishwater fish culture is inadequate availability of seeds. The natural seed availability has become uncertain and sporadic now-a-days. Breeding and seed production of Asian seabass, milkfish and pearl spot have been achieved. However, breeding of mullets which are important cultivable species is yet to be achieved in India. A complete package of practice comprising seed production, seed rearing and grow-out culture for each species is made to be available for sustainable development of brackishwater fish culture.

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16

Introduction to Hilsa, *Tenualosa ilisha* (Hamilton, 1822) Culture Activities of Kakdwip Research Centre

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16.1. Introduction

Five species of tropical shads *viz. Tenualosa ilisha*, *T. toli, T. macrura, T. reevesii and T. thibaudeaui* live in the coastal waters, estuaries and rivers of tropical Asia. *Tenualosa ilisha* is a widely distributed clupeid species inhabiting Bangladesh, Myanmar, South Vietnam to Sumatra and ascending the estuaries, rivers and brackish waters of Indo-Pacific faunistic region. In India, it is most abundant in the Ganga-Brahmaputra river system.

16.2. Taxonomic position

Hilsa belongs to order Clupeiformes, family clupeidae, genus *Hilsa* and species *Tenualosa ilisha* (Hamilton, 1822).

16.3. Behaviour

The normal habitat of hilsa is the lower region of the estuaries and the foreshore areas of the sea. Hilsa can tolerate a wide range of salinity from freshwater to coastal waters. Some stock of hilsa remains permanently in freshwater e.g. Vallabasagar (Ukai) reservoir in Gujarat. The species is known to be fast swimmer. In the foreshore region, hilsa moves on the surface; as they swim upstream, the fish moves near the bottom of the river. They form large schools in the coastal region, but during upstream migration, they congregate instead of forming big shoals.

16.4. Food and Feeding

Hilsa is mainly a sight feeder. It possesses well developed optic lobes since both olfactory lobes and vegal lobes are reduced. The young hilsa mainly feeds on zooplankton in surface and column water bodies. On the contrary, the adults are microphagus planktivore and feed at bottom and column water during upstream migration. The qualitative and quantitative analyses of food observed in the stomach of fry, juveniles and adult hilsa obtained from freshwater stretch of Hooghly estuary reveal that copepods are the most important food items consumed by the fish of all sizes throughout the year. The only basic difference is that the fry and juvenile hilsa feed mainly on copepods, while adults on organic matter and copepod. De et al. (2013) studied feeding preference of different size groups of hilsa

and reported that it prefers copepod in their early stages and shift their preference towards diatom when they grew beyond 50 g size. Other food items were diatoms (*Melosira, Synedra, Navicula, Pinularia*), rotifer (*Trichocerca, Brachjionus*), green algae (*Spirogyra Pediastrum, Closterium, Eudorina, Scenedesmus*) and blue green algae (*Lyngbya, Oscillatoria*) in the stomach of adult during feeding activity. Cessation of feeding activity during peak spawning seasons is noticed by De (1986). Most of the workers are of the opinion that adult hilsa do not feed during upstream migration.

16.5. Migration

The anadromous migration of hilsa towards estuaries, rivers, lakes and backwaters from the foreshore areas of oceanic region is mainly for breeding. After spawning, the spent fish as well as their progeny, migrate towards coastal areas. Thus, a correlation between the season of upstream migration and the spawning season of the species is noticed. De (1986) studied the migratory trend of juvenile hilsa in the Hooghly estuarine system. Since upper freshwater zone of the estuary is considered as the breeding ground of hilsa, the post larvae, fry and fingerlings of hilsa were available here from October-November to May-June with a peak during November and December. The peak spawning season of this species is usually between September and November and stray spawning activity continued up to February or March. As a result, fry and fingerlings appeared for the first time in the freshwater zone of the estuary from October-November and the availability was noticed up to May or June. The freshwater zone of the estuary acts as the nursery ground for few progeny until they attain an average size of 108 mm after a period of seven months. It was also observed by De (1986) that the juvenile hilsa, generally after attainment of size ranging from 80 mm to 110 mm, started their downstream migration towards coast (saline regions), which commenced from February and continue up to June.

16.6. Reproductive Biology

Many researchers have observed that majority of females and males mature when they are 240 mm to 270 mm and 230 mm to 250 mm in length, respectively. Fully ripe ovary may attain 211 mm covering 40% of the total body length (De, 1986). Ovary and testis ducts open just behind the anal opening through the urinogenital pore. Gonad of female hilsa has been classified by De (1986) into seven stages of maturity following ICES scale. Gonado-somatic index (GSI) is indicative of breeding season. The values of GSI in Hooghly hilsa (female) were observed by De (1986). The maximum values of GSI were found during a prolonged period from July to March with one major peak in September to November and another minor peak in February to March. Maximum average values of GSI were observed in the months of September, October, November, February and March. During April and June,

GSI was minimum. De (1986) observed that late maturing and mature ovaries of stage VI occur in September to November and February to March indicating that the species has an extended spawning season. Since the maximum number of ripe specimens is observed in the months of September to November and February to March, these periods appear to be the peak spawning seasons of the fish. De (1986) successfully bred the fish and described the embryonic and larval development of hilsa up to 5th day of fertilization. Breeding was done through wet stripping method at Baniagram-Nimtita stretch down to Farakka Barrage during September to early November, 1985. Fertilized eggs were light greenish yellow and transparent. Diameter of eggs was 1.95 to 2.10 mm and they were non-adhesive. Yolk mass were 0.88 mm in diameter. In developing embryo, the egg membrane had a single layer. Yolk mass contained numerous oil globules. Fertilized eggs were easily buoyed up and get drifted with slight water current. An about 17 hrs after fertilization, embryo hatched out. The newly hatched larva was comma shaped, almost transparent, devoid of any pigmentation and had a large yolk mass with prominent oil globules consisting of one large and 5 to 6 small ones. Total length of newly hatched larvae varies from 2. 37 to 2.44 mm.

16.7. Breeding behaviour

Hilsa is a prolific breeder. The favourable time for fertilization is found in the afternoon and evening. The fertilized eggs are demersal, soft, smooth, non-adhesive and almost spherical in shape.

16.8. Artificial breeding

Wilson (1909) was first to achieve success in artificial fertilization of hilsa eggs at the lower anicut on Coleroon, a branch of river Cauvery. The breakthrough was registered when Malhotra et al. (1969, 1970 and 1973) successfully bred the fish by stripping collected from the Ganga River at Sirsha, 50 Km downstream of Allahabad. Artificial breeding through stripping was also achieved by De (1986), De and Sinha (1987) and Sen et al. (1990).

16.9. Growth of Hilsa in pond culture system

Malhotra et al. (1969, 1970) reared the hilsa hatchlings in freshwater pond for a period of 2 years and 4 months and the stock grew to an average length of 34.5 cm. The stock attained an average length of 15.5 cm and 32.5 cm, respectively at the end of one and two years. An experiment on hilsa culture was conducted by Bhanot and De (1984) for the first time in a freshwater pond of 0.1 ha size at Central Inland Fisheries Research Institute, Barrackpore for 2 year and 8 months. The stock attained an average length/weight of 180 mm/125 g and 310 mm/300 g at the end of the 1st and 2nd year with the survival of 30% and 11%, respectively.

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17

Feed Management in Brackishwater Aquaculture with Special Reference to Aquatic Animal Health

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Feed management means optimum utilization of feed with minimum wastage by achieving best feed conversion ratio and maximum growth. A very good quality feed can produce poor result if the feed management is poor whereas, a moderate feed can produce very good results under good feed management. The foremost critical factor in feed management is selection of appropriate feeds and planning of optimal feeding regimens. Suitable feed should fulfill the nutritional requirements of species under culture. Proteins, lipids, carbohydrates, vitamins, minerals and water are the six major classes of nutrients, which are used for building, maintenance of tissues and supply of energy. The requirement for these nutrients varies depending on the species according to their feeding habit, habitat in which they live in and the stage in their life cycle. Our aim should therefore be to produce nutritionally balanced feed with optimum protein energy ratio. It should also ensure that nutrients are not lost in water during the feeding process. Therefore, aquaculture feeds of different formulations are processed using the special technologies to ensure the diet remains intact in water before ingestion, and nutrients are prevented from dissolving. These general categories of feeds used in aquaculture are wet feeds with moisture contents of 50-70 percent, semi moist formulated feed with moisture contents of 20-40 percent and dry pelleted feeds with moisture contents of less than 10 percents. Since problems are associated with the distribution, handling, utilization, storage and quality of wet feeds and moist feeds, more and more dry feeds are manufactured either by steam pelleting or by extrusion pelleting.

17.1. Feeding management in fish culture systems

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

- Pond biomass should be assessed regularly and ration should be offered as per biomass of the pond.
- Time, frequency and method of feeding should be proper.

17.1.1. Ration size

The size of daily food ration, the frequency and timing of meals are the key factors influencing the growth and feed conversion. Hence, the optimal feeding regimens must be determined as per the feeding behavior, appetite and functioning of the digestive systems. Fish lose weight when their feed intake falls below that required for maintenance. When ration size increases, the growth rate increases. Generally the method of calculating the daily ration is based on the body weight of fish.

$$Ration\ size\ (Kg) = \frac{ABW\ (g) \times Stocked\ nos.\ (\%) \times Rate\ of\ feeding\ (\%) \times 1kg}{1000\ \times 100}$$

The quantity of ration varies from 100% of body weight for larvae and fry and gradually reduced to 50, 20, 10, 5 and 2-3 % as the fish/shrimp grows to the marketable size. Ration size is also estimated by various methods using the feeding charts, feed equations, growth prediction and check tray etc. Besides the ration size, the optimal feed particle size also affects the growth and feed conversion efficiency. Large fish can ingest small particles, but it requires more energy to capture the required equivalent weight or smaller food particles. This results in measurable reduction in food conversion efficiency. Attention should also be given to the influences of feed shapes, colors and textures of pellets on ingestion rates.

17.1.2. Feeding methods

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

17.1.3. Schedule and frequency of feeding

The total feed required in a day should not be fed at a time. Scheduling and frequency of feeding greatly help in successful feed management. Time schedule for feeding the fish may be fixed in such a way that larger ration may be given when the fish is expected to be most hungry. Most of the Brackishwater fishes are fed 3-4 times a day. High feeding frequencies reduces starvation & stunting thereby resulting in uniform in size. There should be a minimum of three time schedules of

feeding in a day- morning, noon and evening. Frequent feeding of small portion of ration help in better utilization of the feed and thereby lead to efficient FCR. There must also be a mechanism in each case to monitor the feed consumption and offering of next dose of feed should be regulated on basis of consumption from the previous feed offered.

17.2. Feeding management in shrimp culture system

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

- Proper feeding guidelines should be followed to fix ration size for shrimp culture pond
- Check tray should be monitored daily
- Time and frequency of feeding should be proper.

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

17.2.1. Ration size

Generally the method of calculating the daily ration is based on the body weight of shrimp (Table 1 and 2). Daily ration should be divided and given 2 to 5 times a day (Table 1, 2 and 3). The feeding activity and quantity of feed consumed may be checked by keeping feed in check trays (size: 80 cm x 80 cm) @ 6 nos./ha in different places in pond. After one month of stocking, consumption of feed should be checked by using check trays. Besides the ration size, the optimal feed particle size also affects the feed intake and growth of shrimp. Feed particle size should vary as per body weight of shrimp (Table 4). Feed should be broadcasted evenly in a periphery of about 2 meters from dyke in all sides of the pond.

Table 1: Feeding schedule for first fifty days of shrimp farming

Age (Days)	Feed increment per day (g)	No. of meals per day	Feed per day per lakh PL-20 (Kg)
1	-	2	2.0
2-10	400	2	2.4-5.6
11-30	600	3	6.2-17.6
31-50	500	4	18.1-27.6

Table 2: Feeding schedule after 50 days of culture based on check tray performance

Days of culture	Expected ABW (g)	% of ABW to be used as feed	Feed % in Check tray	No. of meals per day
51-55	6-7	5.0-4.8	2.0	4
56-60	7-8	4.8-4.6	2.2	4
61-65	8-9	4.6-4.4	2.2	4
66-70	9-10	4.4-4.2	2.4	4
71-77	10-12	4.2-4.0	2.6	4
78-83	12-14	4.0-3.7	2.7	4
84-90	14-16	3.7-3.5	2.8	4
91-97	16-18	3.5-3.2	2.9	4
98-104	18-21	3.2-2.9	3.0	4
105-110	21-24	2.9-2.7	3.2	4
111-117	24-27	2.7-2.5	3.3	5
118-124	27-30	2.5-2.2	3.5	5
125-131	30-33	2.2-2.0	3.6	5
131-133	33-36	2.0-1.8	3.7	5

Table 3: Feeding Schedule for shrimp

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

Table 4: Recommended shrimp pellet size

Feed type	Size of shrimp (g)	Pellet size
Starter	0-4.0	0.5-1.0 mm crumble
Grower	4.0-25.0	2 - 2.3 mm x 4 - 5 mm
Finisher	>25	2-2.5 mm x 6 – 8 mm

17.2.2. Check tray monitoring:

Quantity of feed to be kept in check tray depends upon pond size and average body weight of shrimp and can be determined using the following formula:

$$Quantity\ of\ feed\ in\ each\ check\ tray\ (g) = \frac{1600}{Area\ of\ pond\ (m^2)} \times \frac{Feed\ \%\ in\ check\ tray}{100} \times 1000$$

The check trays should be observed after 2 hrs of feeding. Depending on the quantity of feed consumed in the check tray, the next dose should be increased or decreased. Special care should be taken during moulting, low dissolved oxygen and stressed condition due to heavy rain, high temperature, unfavourable pond bottom and water quality.

Success of feed management depends on the farmer's experience and observation on the feeding behaviour and feed intake of shrimp. Following a strict feed management, survivability up to 80 % and average weight of 30 g can be achieved in culture duration of 120 days.

17.2.3. Water quality

The interrelationships between feeding and water quality in aquaculture is complex. By providing optimal species-specific requirements such as temperature, dissolved oxygen, pH and salinity, adequate feeding to satiation, improved growth and high survival can be ensured. When the water quality parameters fall below optimal levels, the species under culture will be stressed and feeding and growth will be impaired. Accumulation of left over feed together with excretory products is associated with high BOD, NH_3, H_2S, CH_4 and harmful effects of eutrophication. This is a critical issue in management since effluent quality can be linked directly to feeds and feeding practices and is regulated under water pollution control laws in many countries. Thus, feeding regimes should be designed to minimize the nutrient loss and faecal output and to maximize the nutrient retention and health status of the cultured fishes/shrimps.

17.2.4. Handling and storage of feeds

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

17.3. Disease preventive measures

- Always use dry pellet feed, in which the bacteria cannot grow due to low water activity and moisture.
- Supply nutritionally balanced diet for respective species to avoid nutrition deficiency.

- Avoid dumping more feed in culture pond without monitoring the feed intake as uneaten fed will accumulate and deteriorate the soil and water quality of pond.
- Feed should be offered in split doses instead of a single dose for better utilization by fish/shrimp and less accumulation of uneaten feed in pond bottom.
- Feed should be stored properly to avoid pest and disease infestation in feed. Freshly prepared good quality feed proven with best potential FCR, could reduce feed waste and disease in fish/shrimp.
- Feed with poor water stability, which have lost their nutritional potency and are poorly accepted by the fish or shrimp should be rejected.
- Appropriate particle size of the feed should be designed for a particular stage of life cycle.
- The ration size and feeding Schedules should be regulated with reference to feeding guides, response of fish and environmental conditions.
- At poor feed management, bloom may be formed in pond which causes pH and D.O. fluctuation and cause stress to the fish/shrimp. Water exchange, liming and aeration may be required to reduce the stress of fish/shrimp.
- Judicious feed management is important factor in achieving good feed efficiency and reducing feed wastage.

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18

Live Feeds and its Role in Health Management in the Larviculture of Brackishwater Finfish and Shellfishes

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18.1. Introduction

Aquaculture is one of the fastest growing food producing sectors in the world, accounting for almost half of the total food fish supply. The aquaculture industry prefers the hatchery produced larvae of finfish and shellfish as seed for the farming rather than the wild collected ones. In larviculture of any aquatic species, providing ideal starter feed for the larvae is the challenging task. Many of these larvae are with small mouth gape and therefore the larval feed should be sufficiently small enough to be consumed by the tiny larvae. Suitability of larval feeds are not only valued based on their particle size, but also on their nutritional composition and their ability to preserve the quality of rearing water. Till today, no replacement for tiny plant and animal water creatures commonly called as live feeds has been found to match their range of particle sizes and nutritional quality. Several species of micro algae and plankton crustaceans have been used as classical larval feeds in hatchery production finfishes and shellfishes larvae.

The development of mariculture and brackishwater aquaculture around the world can be attributed to the development of standard techniques for mass production of live feed. The hatchery production of penaeid shrimp post-larvae depends on the use of live diatoms (*Chaetoceros* spp., *Skeletonema* spp. and *Thalassiosira* spp.) for the early stages and *Artemia* for later stages. Globally the hatchery production of juveniles of marine and brackishwater finfish is achieved by the use of rotifers and *Artemia* (Fig. 1). Microalgae are also routinely used in the '*green water technique*' employed in larval rearing especially for marine finfish and crab larviculture.

Importance of live feed is due to several factors such as presence of essential nutrients, broad spectrum composition of food, better intake due to the movement, auto-digestion characteristics, better nutrient assimilation in larvae, stimulation of feeding behavior due to soft texture and attractability and ample scope for enrichment. Live feed organisms are able to swim / disperse in the water column and thus they are constantly available to the larvae. The movement of live feed in water stimulates larval feeding responses. Live feed organisms with a thin exoskeleton and high water content may be more palatable to the larvae when

compared to the hard formulated diets. This is why live feed organisms are commonly called living capsules of nutrition.

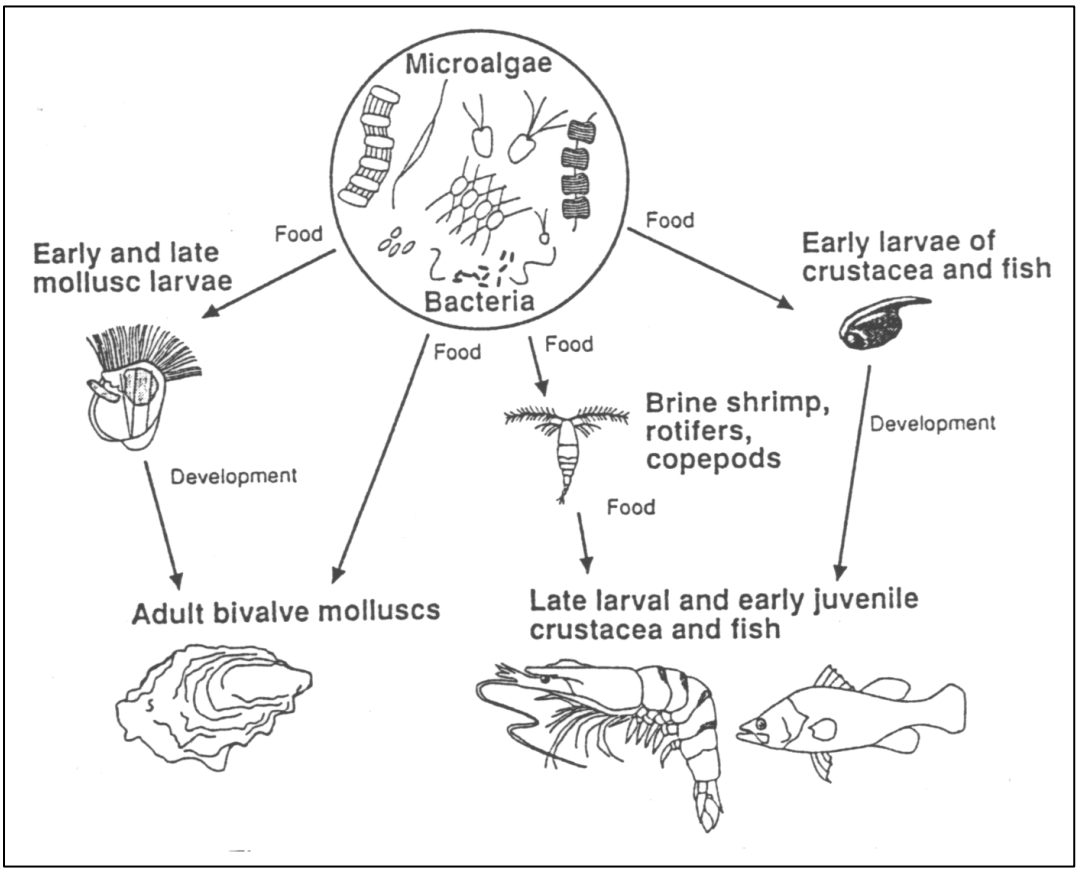

Fig 1: A simple food web representing larval feeding (Source: FAO Technical paper: 361)

Apart from providing essential nutrients to the larvae, many of the live feed organisms has the potential to prevent many of the diseases in the marine and brackishwater larviculture. Currently many studies are going on all over the world on the ability of live feeds (mostly microalgae) in health management in larviculture and on producing more number of healthy larvae in each cycle.

18.2. Satisfying the nutritional requirement

The live feeds provide most of the essential nutrients to the finfish and shellfish larvae, which are required for growth and development. Among the nutrients, lipids play a crucial role in the larval development, especially the essential fatty acids. There are some microalgae consist of high amount of essential fatty acids, lipids, proteins, vitamins and minerals.

The nutritional value of algal species mainly depends on its cell size, digestibility, production of toxic compounds and biochemical composition. The presence of highly unsaturated fatty acids (HUFA), in particular eicosapentaenoic

acid (20:5n-3, EPA), arachidonic acid (20:4n-6, ARA), and docosahexaenoic acid (22:6n-3, DHA), is of major importance in the evaluation of the nutritional composition of an algal species to be used as food for marine and brackishwater larval nutrition. Microalgae are primary producers of n-3 PUFA, and some Chlorophyta species such as *Nannochloropsis* and *Tetraselmis* are excellent sources of EPA, while some Haptophyta species *Isochrysis galbana* and *Pavlova lutheri* are outstanding sources of DHA. Besides being excellent n-3 PUFA producers, microalgae can be mass cultured and their EPA and DHA content can be modulated. Nutritionally rich microalgae can be used to enrich other live feeds (Rotifers, Artemia and Copepods) for better growth and survival of larvae.

18.3. Creating a healthy environment for the larvae

The presence of microalgae in the larval rearing tank will improve and stabilize the water quality in static systems (eg: Remove metabolic by-products and produce oxygen). They can be a direct food source through active uptake by the larvae and the polysaccharides present in the algal cell walls may possibly stimulate the non-specific immune system in the larvae. Apart from that, they can be an indirect source of nutrients for fish larvae (i.e. by enhancing the nutritional value of the live prey organisms in the larval rearing tank). Interestingly the green water system (larval rearing tanks with microalgae- unialgal or mixed algal) may control microbial load in tank water and/or larval gut (FAO fisheries technical paper: 361).

There are certain factors, which determine the larval health and survival such as proper environment, suitable feed at right time, absence of pathogens and overall biosecurity (Fig. 2). All these factors are connected each other. A green water system or the presence of certain microalgae can make all the essential requirements for better larval health.

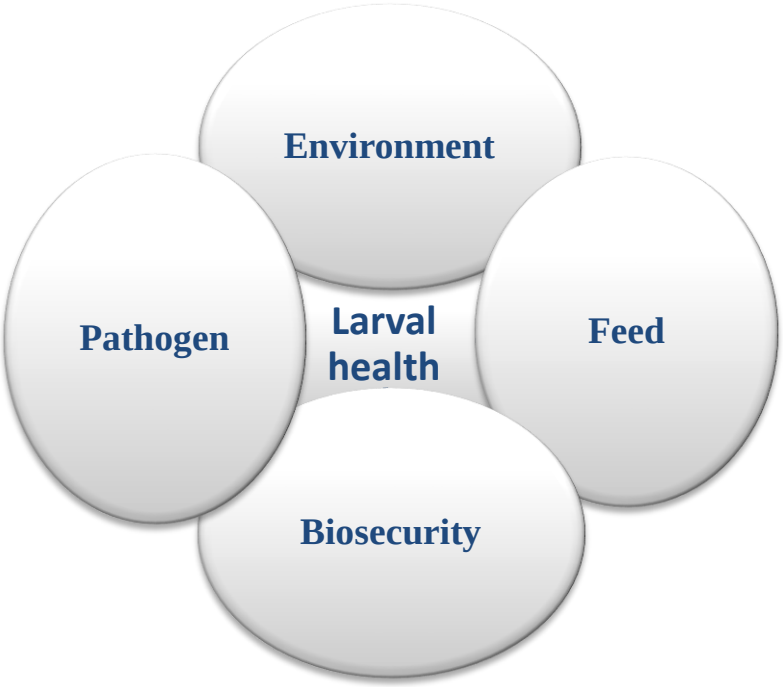

Fig. 2: Factors controlling finfish and shellfish larval health

Maintaining water quality parameters in the optimal range is an important task in all hatchery operations. Among various water quality measures, ammonia and CO_2 will make more problems to the larvae when their concentrations are above certain limits. Microalgae culture is an effective method for mitigation of ammonia and CO_2 from the larval rearing systems. The experiments showed that a microalgae *Scenedesmus dimorphus* can assimilate up to 98.6% of ammonia from the system, with an ammonia removal rate of 51mg/L/day (Kang and Wen, 2015). The photosynthetic microalgae will use the CO_2 and produce oxygen.

Different algal species used in green water systems in larval rearing has proved to be beneficial for larval performance among marine finfish species like turbot, Atlantic halibut, sole, sea breams, sea bass, striped mullet, damsels, flounders and crabs (*e.g.* mud crab- *Scylla serrata*). The effect of microalgae in fish larval rearing system is not completely understood. The hypotheses listed in scientific studies include improvement of water quality, direct nutrition through active ingestion, indirect nutrition by enriching the live prey in the rearing tank, micronutrient stimulus for feeding behavior or physiological processes, and regulation of opportunistic bacteria by antibacterial or probiotics action (Muller-Feuga et al., 2003). The selection of suitable algal species for larval rearing is also important.


Fig. 3 & 4: Green water sstems in larval rearing tanks

18.4. Antimicrobial property of microalgae

Widespread antibiotic resistance among pathogenic bacteria and the low specificity of these drugs made an urgent need for the development of novel antibacterial agents. Addition of microalgae (green water system) often has a positive effect on the survival rates of finfish and shellfish larvae. Fishes are susceptible to a wide variety of bacterial pathogens which can cause variety of diseases. These bacteria only become pathogenic when fishes are physiologically unbalanced, nutritionally deficient, or under various stress such as poor water quality, overstocking etc. (Anderson, 1995). Antimicrobial activity of microalgae has been studied by many researchers (Austin et al., 1992). Microalgae produce compounds at intracellular and extracellular levels, however, as a large proportion of these compounds is not excreted but remains within the cells (Guedes et al., 2011). Microalgae have been explored for their bioactive compounds with promising applications consisting antibacterial, antiviral and antifungal activities.

18.5. Health management by other live feeds

Other live feeds like rotifers, artemia and copepods are also widely used in larviculture in different larval stages of different species. The rotifers and artemia can be enriched with suitable microalgae or commercial enrichment product to get better larval survival and health. The copepods are nutritionally superior to artemia and rotifers. The broad range of size (30 µm to 1 mm) of copepods is also suitable for marine larviculture. Unfortunately the usage of copepods in hatchery operations are limited as the single species mass culture of copepods are not yet standardized in many countries and it is very difficult.

18.6. Live feeds as immune-stimulants

The production of fish larvae is often hampered by high mortality rates, and it is believed that most of this economic loss due to infectious diseases. The immunomodulation of fish larvae has been proposed as a potential method for improving larval survival by increasing the innate responses of the developing

animals. Conversely, there is a school of thought that raises the concern of immunomodulating a larvae before its immune system is fully formed as this may adversely affect the development of a normal immune response.

Microalgae play a crucial role in present shrimp culture management strategies. Algal compounds and their metabolites have been shown to improve the immune system of the shrimp and increase its resistance against pathogens. Some of these compounds have been known to exhibit either antiviral or antibacterial activities.

18.7. Application of transgenic microalgae

There are researches going on in the production of transgenic microalgae and its application in aquatic health management. Researchers developed a safer, more effective and less expensive biological bactericide for aquaculture use (Li and Tsai., 2009). A stable transgenic line of *Nannochloropsis oculata,* which has bactericidal activity, was developed. By feeding this transgenic *N. oculata* to a small model fish medaka, significant increase in the survival rate was observed 24 hrs after bacterial pathogenic infection with *Vibrio parahaemolyticus* in the fish's digestive tract.

Table 1: Commercial algal culture and its applications:

Source: Hemaiswarya et al., 2011

Species	Use in aquaculture
Nannochloropsis	Growing rotifers and in fin fish hatcheries;very high EPA level
Pavlova	Used to increase the DHA/EPA levels in oysters, clams,mussels and scallops
Isochrysis	Enrichment of zooplankton such as artemia, used in shellfish hatcheries and used in some shrimp hatcheries, good size for feeding brine shrimp and copepods, oysters, clams, mussels, and scallops
Tetraselmis	Excellent feed for shrimp larvae and contains natural amino acids that stimulate feeding in marine animals, used in conjunction with *Nannochloropsis* for producing rotifers, excellent feed for increasing growth rates and fighting zoea syndrome
Thalassiosira weissflogii	Used in the shrimp and shellfish larviculture, considered by several hatcheries to be the single best alga for larval shrimps, also good for feeding copepods and brine shrimps
Dunaliella	Used as source of vitamins and pigments for shrimp larval forms.
Chaetoceros	Used as source of vitamins for shrimp larval forms

18.8. Conclusion

According to the scientific literature, live microalgae with high nutritive value and appropriate physical properties can provide a healthy rearing environment to the aquaculture system. Finally, a better understanding of the mechanism of *green water systems* both in intensive and extensive culture will aid in optimizing the usage of microalgae in larval culture. A broader range of microalgae species, especially mixtures and including species rich in DHA, should be assessed in green water systems. Further research has to be done on the exact mechanisms on the improvement of growth, survival and health of finfish and shellfish larvae by different microalgae.

18.9. Further reading

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