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FIELD GUIDE FOR
DIAGNOSIS PREVENTION AND CONTROL OF DISEASES OF
SHRIMP AND FINFISH
IN BRACKISHWATER AQUACULTURE

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Brackishwater Aquaculture for food, employment and prosperity

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PREFACE

It is 20 years since CIBA published a bulletin on diagnosis and prevention and control of disease shrimp in brackishwater aquaculture in India. Since then the aquaculture scenario in the country has undergone paradigm shift with regard to culture practices and issues faced in the farming sector. The finfish culture sector still is under nascent phase despite breeding technologies, and indigenous feed available. India's shrimp production recorded a phenomenal rise from less than 1.0 lakh metric tonnes in 1995-96 to over 3.80 lakh tonnes during 2014-15 with the total export value from marine and brackishwater sector reaching an all-time high of over US $ 5000 million. Availability of imported specific pathogen free (SPF) broodstock provided the much needed growth rate to India's brackishwater aquaculture sector. However, the intensification has exacerbated the epizootics and disease issues are becoming a major constraint affecting productions. Starting from 1994, the annual loss to shrimp culture industry of India has been estimated to be 300 crores and this is due to a single causative agent, white spot syndrome virus (WSSV). An assessment in 2009 by CIBA indicated a disease related loss to the tune of over 48717 metric tons of production valued at 1022 crores.

During the initial phase of brackishwater aquaculture development, which was traditional low stocking density culture, it was only the non-infectious (nutritional/toxin) or less dangerously endemic pathogen (bacteria, parasites) related diseases were prevalent. During during late 80's, diseases such as soft shell syndrome of shrimp in traditional aquaculture ponds of Kerala and West Bengal and epizootic ulcerative syndrome (EUS) of finish were plaguing our brackish water sector. In the initial years of semi-intensive brackishwater aquaculture development, bacterial diseases such as vibriosis caused limited problems in grow-out aquaculture. Since 1994 white spot disease (WSD) has been devastating our shrimp farming sector even today. Many brackishwater shrimp farmers shifted to scampi farming in brackishwater ponds, which also suffered setbacks due to white tail disease (WTD). Later on since 1998, the black tiger shrimp farming continued to face challenges such as loose shell syndrome (LSS), followed by the monodon slow growth syndrome (MSGS).

After introduction of the exotic Pacific white shrimp, *Penaeus vannamei,* following import risk analysis (IRA), the aquaculture sector in India has been booming. However, in addition to the already widespread WSD, the sector is being challenged with several uncharacterized disease syndromes. During the last five years, issues of concern in shrimp farming include stunted growth or growth variation, white faeces syndrome (WFS) low level daily mortalities (popularly called by shrimp farmers as running mortality syndrome or RMS) and white muscle syndrome (WMS). Stunted growth and WFS are often associated with the hepatopancratic microsporidiosis caused by *Enterocytozooan hepatopenaei* (EHP).In this context the present publication has been brought out now and is largely intended as a field guide for presumptive identification of diseases.

K.K. Vijayan

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CONTENT

1. INTRODUCTION

Brackishwater aquaculture has been playing a very significant role in terms of its contribution to the global seafood supply. From the ancient traditional status, the industry has evolved to the present developed form through several scientific interventions and thereby has been able to constantly increase its share in terms of both production and value. During 2014-15, India's seafood export reached all-time high of about US $5000 million. More than 70% of this share was from farmed shrimp alone and thereby signifying the importance of shrimp aquaculture.

Brackishwater aquaculture is predominated by shrimp. Several coastal states of India are involved in the culture practice of penaeid shrimps. Until 2009, it was totally dominated by the indigenous species, tiger shrimp, *Penaeus monodon*. Pacific white shrimp, *Penaeus vannamei* was introduced into India for culture in 2009, and presently, more than 80% of farmed shrimp constitutes this exotic shrimp.

India also has a great potential for the culture of brackishwater fin fishes. Biology and breeding of some of the species such as sea bass, Cobia and Milk fish etc has been taken up with success and culture of these finfish species has also been going on in certain areas. The potential areas for brackishwater aquaculture in India has not yet been exploited to full extent and thereby further scope for expansion of this industry is expected.

A number of biotic and abiotic factors influence aquaculture productivity. White spot disease (WSD), caused by white spot syndrome virus (WSSV) has often been the single most dreaded threat to the aquaculture industry, with global losses exceeding US $6 billion and an estimated annual loss over ₹300 crores to the Indian aquaculture sector. Besides, other important infectious agents including viruses, bacteria and parasites have been responsible for issues related to slow growth, mortalities and crop losses. In the recent past, emerging diseases such as early mortality syndrome, running mortality syndrome, white faces syndrome and zoea syndrome etc. are added problems to the already existing misery of farmers.

Many of these problems can be prevented using better management practices. Good pond preparation, maintenance of biosecurity, stocking ponds with healthy disease-free seed, regular health and water quality monitoring and judicious input application including feed are some of the key factors that decide successful harvest.

In this booklet, a brief outline on important diseases in brackishwater aquaculture, with special reference to shrimp and finfish, the causative agents, symptoms, mode of transmission, possible methods of prevention and control are provided for the information of the shrimp farmers. This publication is also intended to be useful to beginners who wish to understand disease issues in brackishwater aquaculture.

Considering the expertise, resource and infrastructure required for disease diagnostics, FAO/NACA (2000) has recommended the promotion of three levels of diagnostics according to existing resources (see Table 1). The different levels provide a broad-scale application to disease detection and diagnostics where countries can move from one level to the next as capacities are improved and as resources become available.

Table 1. Three levels of disease detection and diagnostics

Level	Site	Activity	Requirements
I	Field	Observation of animal and the environment Clinical examination	Little basic equipment or no equipment required. Training of field personnel on the basic biology and clinical examination and co-operation of culture-site managers/employees to access to information would help in achieving preliminary understanding and to some extent, diagnose the problem.
II	Lab	Parasitology, Bacteriology, Mycology, Histopathology	Significant investment in skill development / training of technical personnel, equipment and recurring costs. Access to current scientific information would enable technicians to achieve diagnosis of the problem.
III	Lab	Virology, Electron microscopy, Molecular biology, Immunology etc	Considerable investment in equipment and in skill development / training of manpower required to effectively utilise equipment with considerable recurring costs. Access to current information in all the activities required to achieve accurate diagnosis.

2. OIE LISTED DISEASES OF SHRIMP

More than 20 viruses have been identified that are known to infect penaeid shrimp. The OIE now lists two bacterial diseases, the acute hepatopancreatic necrosis disease (AHPND), necrotizing hepatopancreatitis (NHP) and five viral diseases, white spot disease (WSD), yellow head disease (YHD), Taura syndrome (TS), infectious myonecrosis (IMN) and infectious hypodermal and haematopoietic necrosis (IHHN) in the Aquatic Animal Health Code (OIE, 2015), which are considered to be transmissible and of significant socio-economic importance. All OIE member countries are obliged to report these diseases so that disease spread can be monitored and legislation instituted to prevent disease spread. IMN and TS have not affected shrimp aquaculture in India. Although YHV has been reported from India in one instance in farmed black tiger shrimp (using histopathological techniques), its economic impact was negligible. All these diseases are known to cause considerable loss to farmed *P. vannamei*.

White Spot Syndrome (WSD)

White spot disease (WSD) is the most serious threat faced by the shrimp farming industry worldwide. WSD was first reported in farmed *P. japonicus* from Japan in 1992-93, but was thought to have been imported with live infected post-larvae from mainland China. WSD has been identified from crustaceans in China, Japan, Korea, South-East Asia, South Asia, the Indian sub -continent, the Mediterranean, the Middle East, and the Americas. WSSV can infect a wide range of aquatic crustaceans including marine, brackish and freshwater penaeids, crabs and crayfish. All decapod crustaceans including crabs, crayfish, freshwater prawns, spiny lobsters and clawed lobsters in marine, brackish and freshwater sources are susceptible, but morbidity and mortality as a consequence of infection is highly variable. Penaeid shrimp species are highly susceptible to infection, often resulting in high mortality. Prevalence of WSSV is reported as highly variable, from <1% in infected wild populations to up to 100% in captive populations.

Fig 1a. Shrimp affected with white spot disease showing typical white spot on the carapace of black tiger shrimp and vannamei shrimp

What is the causative agent of WSD?

WSD is caused by a double-stranded DNA virus of 120-150 x 270-290 nm size, assigned to a new virus family, whispoviridae.

What are the symptoms of WSD?

The virus severely damages the stomach, gills, antennal gland, heart and eyes. WSSV affects organs of ectodermal and mesodermal origin and in later stages of infection, many cells get lysed and the organs are destroyed. Affected shrimp are lethargic show reddish body discolouration and during the advanced stage of disease, characteristic 1-2 mm diameter white spots could be seen on carapace, appendages and inside surfaces. Cumulative mortality typically reaches 100 percent within three to seven days of infection.

How WSD is Diagnosed?

WSD may be diagnosed based on gross signs such as the presence of the characteristic white spots, and rapid mortalities. White spots may not be always seen in the early stages of infection in shrimp. WSSV can be detected using polymerase chain reaction (PCR), or with molecular tools such as dot-blot and *in situ* hybridisation (ISH) tests. WSD can be also confirmed histologically (particularly in asymptomatic carriers) by the presence of large numbers of Cowdry type-A nuclear inclusions and hypertrophied nuclei in haematoxylin and eosine (H&E)-stained tissue sections, or simply by rapid fixation and H&E staining of gill tissue.

How is WSD transmitted?

WSD can be transmitted vertically and horizontally by cannibalism, predation, etc. and by water-borne routes. Dead and moribund animals can be a source of disease transmission. Outbreaks are usually triggered by latent carriers due to environmental changes, such as osmotic stress induced through changes in salinity or hardness or rapid fluctuations in temperature.

How WSD can be prevented / controlled?

So far, no measures are known for controlling WSD in aquaculture. Pathogen exclusion or biosecurity and adoption of best management practices (BMPs) are the only means of prevention of WSD. Specific pathogen free (SPF) broodstock only need be used in the hatcheries. Hatcheries should quarantine broodstock and screen for WSSV by PCR before breeding. PL should also be screened for WSD by PCR before stocking ponds. Washing and disinfection of eggs and nauplii may prevent vertical transmission of WSSV from infected broodstock to larval stages. Live feed including polychates, should be ensured to be free from WSD before using in the hatchery. Aquafarms should provide reservoirs and disinfect water by chlorination prior to use in the farms. All effluent from farms or processing plants should be disinfected with formalin or chlorine prior to discharge to avoid transmission of disease to other farms.

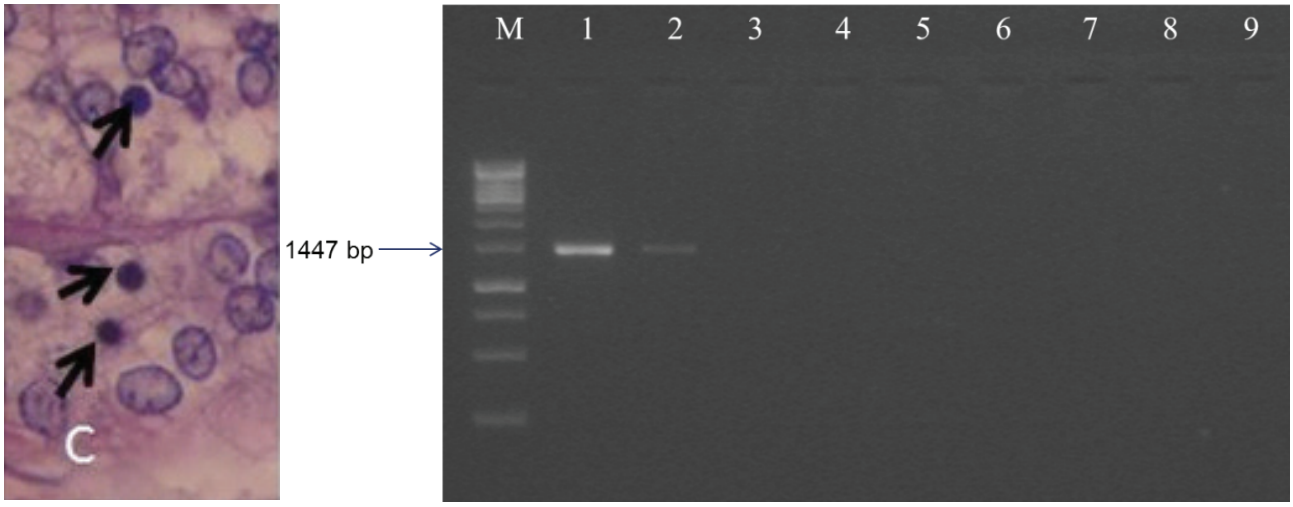


Fig.1 b Histopathological demonstration of basophilic hypertrophied nuclei in gill and diagnosis of WSD by PCR

Infectious Hypodermal and Haematopoietic Necrosis

Infectious hypodermal and haematopoietic necrosis (IHHN) was first discovered in *P. vannamei* and *P. stylirostris* in the Americas in the year 1981, possibly introduced along with live *P. monodon* from Asia. IHHN probably existed for some time in Asia without detection due to its insignificant effects on *P. monodon*, the major cultured species in Asia. Recent studies have revealed geographic variations in infectious hypodermal and haematopoietic necrosis virus (IHHNV) isolates, and suggested that the Philippines was the source of the original infection in Hawaii, and subsequently in most shrimp farming areas of Latin America. Large-scale epizootics were responsible for multi-million dollar losses in *P. vannamei* culture in the Americas during the 1990s.

What is the Causative agent of IHHN?

IHHN is caused by a small (20-22 nm) single-stranded DNA-containing parvovirus.

What are the Symptoms of IHHN?

Gross signs of disease are not specific to IHHN, but may include reduced feeding, elevated morbidity and mortality rates, fouling by epicommensals and bluish body coloration. Larvae, post-larvae (PL) and broodstock rarely show symptoms. In *P. vannamei*, IHHNV can cause runt deformity syndrome (RDS),

Fig 2. *IHHN infection in shrimp showing rostrum (source: http://bioaqua.vn/en/the-infectious-hypodermal-and-haematopoietic-necrosis-virus-a-brief-review-of-what-we-do-and-do-not-know/) and abdominal deformity characteristic of runt deformity syndrome (RDS)*

which typically results in cuticular deformities (particularly bent rostrums), slow growth, poor food conversion ratio (FCR) and a greater size variation at harvest, contributing substantially to reduction in profits. These effects are typically more pronounced when the shrimp are infected at larval stages. IHHN typically causes no problems for *P. monodon* since they have developed a tolerance to it over a long period of time, but they may occasionally suffer with RDS. *P. merguiensis* and *P. indicus* appear refractory to the IHHNV.

How is IHHN Diagnosed?

IHHN can be diagnosed by histological demonstration of intracellular Cowdry type A inclusion bodies in ectodermal and mesodermal tissues such as cuticular epithelium, gills, foregut, hind gut, lymphoid organ and connective tissues of H&E-stained sections. Sensitive specific and rapid methods such as PCR, dot blot, ISH are also available for IHHN diagnosis.

How IHHN is transmitted?

Transmission of IHHN is known to occur rapidly by cannibalism of weak or moribund shrimp, cohabitation and waterborne route. Vertical transmission from broodstock to larvae is common and has been shown to originate from the ovaries of infected females. Insects and birds have been shown to act as mechanical carriers of IHHN and may also transmit the disease.

How IHHN can be prevented / controlled?

Strict hatchery biosecurity including screening out broodstock by PCR, or the use of SPF broodstock, washing and disinfecting of eggs and nauplii are useful in combating this disease. IHHNV is reported to be highly resistant to all the common methods of disinfection including chlorine, lime and formalin. One of the big problems with IHHNV is its eradication in infected facilities. Complete eradication of all stocks, complete disinfection of the culture facility and avoidance of restocking with IHHNV-positive animals may bring down incidences of IHHNV infections.

Taura Syndrome

Taura Syndrome (TS) was first identified in shrimp farms around the Taura River in Ecuador in 1992 and the disease spread rapidly to the whole of Latin and North America within three years. Subsequently, TS was also reported from Asia including Mainland China and Taiwan (from 1999), and in late 2003 in Thailand, probably through the regional and international transfer of live PL and broodstock of P. vannamei. TS is so far not reported from India.

What is the causative agent of TS?

Initial work suggested that TS was caused by a toxic pesticide. However, it is now known that a single or perhaps several very closely related mutant strains of the Taura syndrome virus (TSV) are responsible for the TS. TSV is a single stranded RNA virus of 32 nm size, non-enveloped icosahedrons and more prone to mutations causing more concern.

What are the symptoms of TS?

TSV infections occur in juvenile shrimp (0.1-1.5 g body weight) within two to four weeks of stocking ponds and occur largely within the period of a single moult cycle. In the acute phase of the disease, during pre-moult stage, the shrimp are weak, soft-shelled, have empty gut and diffuse expanded chromatophores that appear red, particularly in the tail (hence the common name - red tail disease). Such animals will usually die during moulting (5-95 percent). Adult shrimp are known to be more resistant than juveniles. Shrimp that survive infection show signs of recovery and enter the chronic phase of the disease. Such shrimp show multiple, randomly distributed, irregular, pitted, melanised lesions of

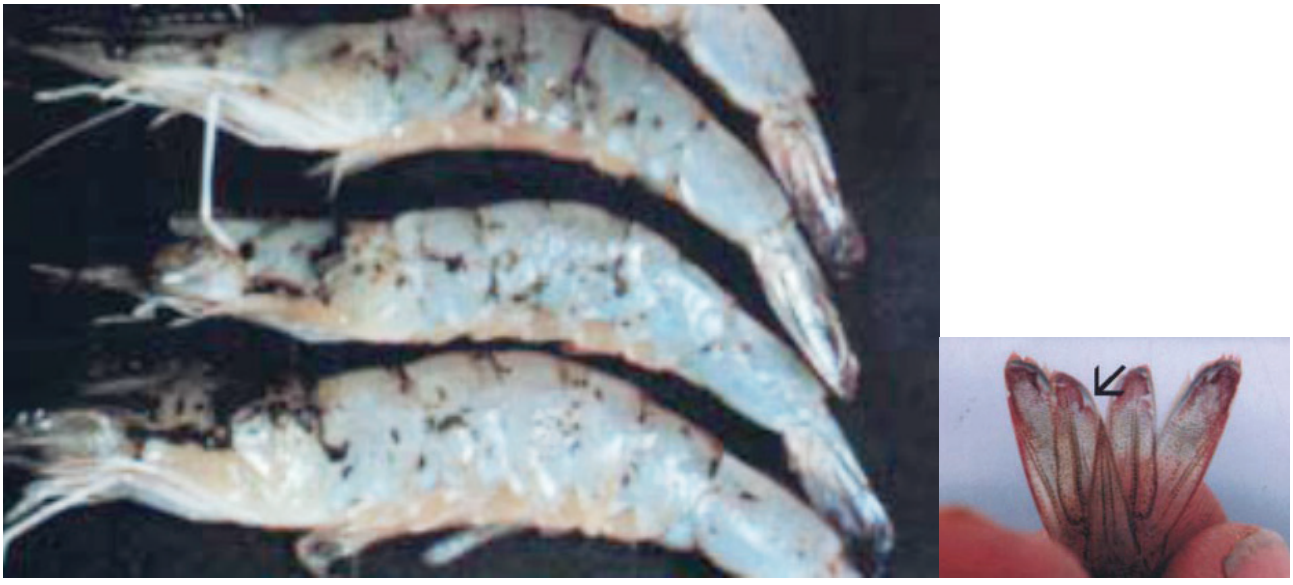


Fig 3. Juvenile, pond-reared P.vannamei showing melanized foci mark sites of resolving cuticular epithelium necrosis (left) and tail fan showing reddish discoloration and rough edges of the cuticular epithelium in the uropods suggestive of focal necrosis (right) due to TSV infection (Pictures from Bondad-Reantaso et al, 2001)

the cuticle. These gross lesions will persist, but may be lost during moulting, and the shrimp thereafter appear normal.

How is TS Diagnosed?

TS can be diagnosed using standard histological and molecular methods of detection. Reverse-transcriptase PCR (RT-PCR) assay is commonly used. Specific DNA probes applied to ISH assays with paraffin sections and histological demonstration of enlarged lymphoid organs (LO) with multiple LO spheroids and multifocal areas of necrosis in the cuticular epithelium of the general body surface, appendages, gills, hindgut, and foregut (the oesophagus, anterior and posterior chambers of the stomach) provide the confirmatory diagnosis

How is TS transmitted?

TSV is horizontally transmitted through infected animals, cohabitation and water borne routes. Recently it has been shown that mechanical transfer through insect and avian vectors is likely route of infection. Shrimp-eating seagulls can transmit TSV through their faeces. Hence birds are likely to transmit TSV.

How to prevent / control TS?

The disease can be prevented by use of SPF broodstock in hatcheries and stocking ponds with seed tested to be free of TS. Precaution should be taken to prevent reintroduction of the virus from nearby facilities, wild shrimp and carriers. Other methods suggested for controlling the virus include BMPs and maintenance of optimal environmental conditions, weekly applications of hydrated lime (CaOH) at 50 kg/ha, polyculture with fish (to consume dying and dead carriers). Infected stocks must be totally destroyed and the culture facility must be disinfected.

Yellow head disease

Fig 4. Gross sign showing yellow cephalothorax of YHD in Penaeus monodon (Picture from Bondad-Reantaso et al, 2001)

Yellow head disease (YHD) was the first major viral disease that caused extensive losses to black tiger shrimp farms in Thailand during 1990-91. YHD has been reported in China, Taipei, Indonesia, Malaysia, Philippines, Sri Lanka, Thailand and Vietnam.

What is the causative agent of YHD?

YHD is caused by a rod-shaped enveloped virus of 40-60 nm by 150-200 nm size, containing single stranded RNA, the yellow head virus (YHV). Less virulent related genotypes known as gill-associated virus (GAV) are reported to be highly prevalent in farmed and wild populations in Australia, Asia, East Africa and Mexico.

What are the symptoms of YHV?

YHD principally affects pond reared juvenile stages of 5 -15 g. Affected shrimp typically feed voraciously for two to three days and then stop feeding abruptly and are seen swimming near the periphery of the pond. YHD can cause up to 100% mortality in infected *P. monodon* ponds within 3-5 days of the first appearance of clinical signs. GAV has been reported to be associated with mortalities of up to 80% in *P. monodon* ponds in Australia. YHV infections can cause swollen and light yellow colored hepatopancreas in infected shrimp, and a general pale appearance, before dying within a few hours. YHV affects tissues of ectodermal and mesodermal origin such as lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia.

How is YHV Diagnosed?

YHD can be diagnosed using RT-PCR or with a probe designed for dot-blot and ISH tests. It can also be diagnosed histologically in moribund shrimp by microscopic demonstration of intensely basophilic inclusions in H&E stained sections of stomach or gill.

How is YHD transmitted?

The primary mechanism of spread of YHD is through horizontal route, either water-borne or carrier

organisms, cohabitation and mechanical means. YHV is reported to remains viable in aerated seawater for up to three days. Other shrimp such as *P. merguiensis*, *P. indicus*, *Metapenaeus ensis*, *Palaemon styliferus* and *Acetes spp*. may become infected and act as carriers. Other crustaceans, such as *Macrobrachium rosenbergii* and many crab species and *Artemia* appear to be refractive to YHD. Infected broodstock can pass on the virus to larvae in the maturation/hatchery facilities if thorough disinfection protocols are not strictly adhered to.

How to prevent / control YHD?

YHD eradication in ponds is similar to that for other viruses and involves practicing BMPs that include pond preparation by disinfection and elimination of carriers, chlorination of reservoir water, filtering inlet water with fine screens, avoidance of live feeds, maintenance of stable environmental conditions, disinfection of infected ponds before discharge, and routine monitoring.

Infectious Myonecrosis (IMN)

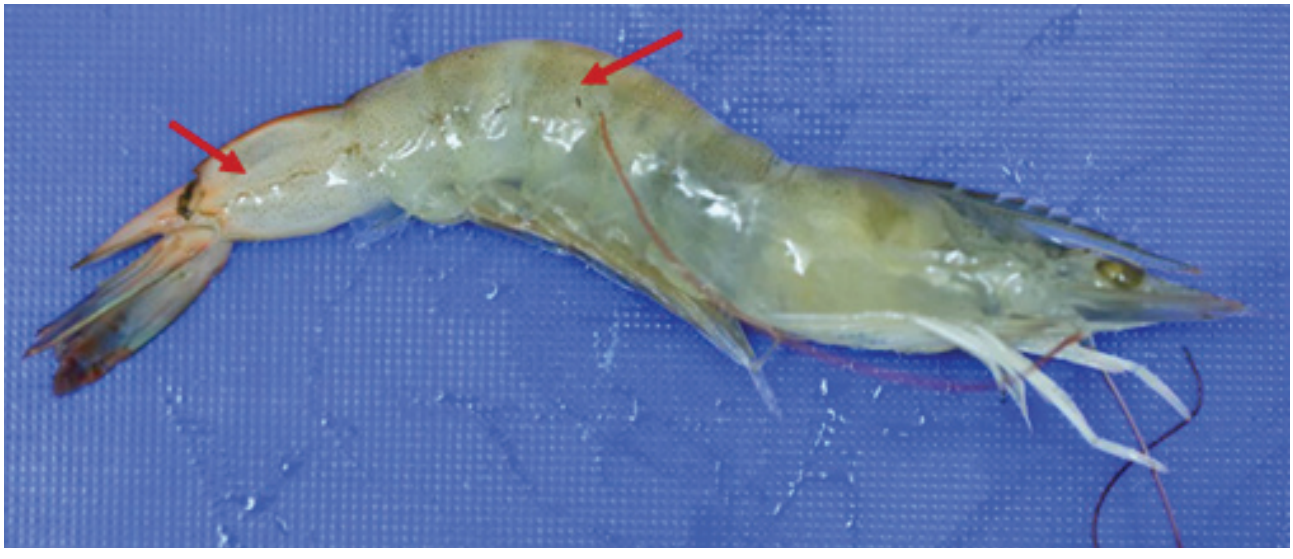
Infectious myonecrosis was first detected in Brazil during 2004 in *P. vannamei* and then in Indonesia in 2006. To date, IMN has been detected in East Java, Bali, and West Nusa Tenggara provinces.

What is the causative agent of IMN?

IMN is caused by a putative totivirus. IMNV particles are icosahedral in shape and 40 nm in diameter.

What are the symptoms of IMN?

IMN disease causes significant mortality in grow out ponds and is characterised by acute onset of gross signs including focal to extensive whitish necrotic areas in the striated muscle, especially of the distal abdominal segments and the tail fan, which may become necrotic and reddened similar to the colour of cooked shrimp. Juveniles and sub-adults of *P. vannamei*, farmed in marine or low salinity brackish water, appear to be the most severely affected by IMN disease. The principal target tissues for IMNV include the striated muscles, connective tissues, haemocytes and the lymphoid organ parenchymal cells. Severely affected shrimp become moribund and mortalities can be instantaneously high and continue for several days. Mortalities from IMN range from 40 to 70% in cultivated *P. vannamei*, and FCR of infected populations increase from normal values of ~ 1.5 to 4.0 or higher.



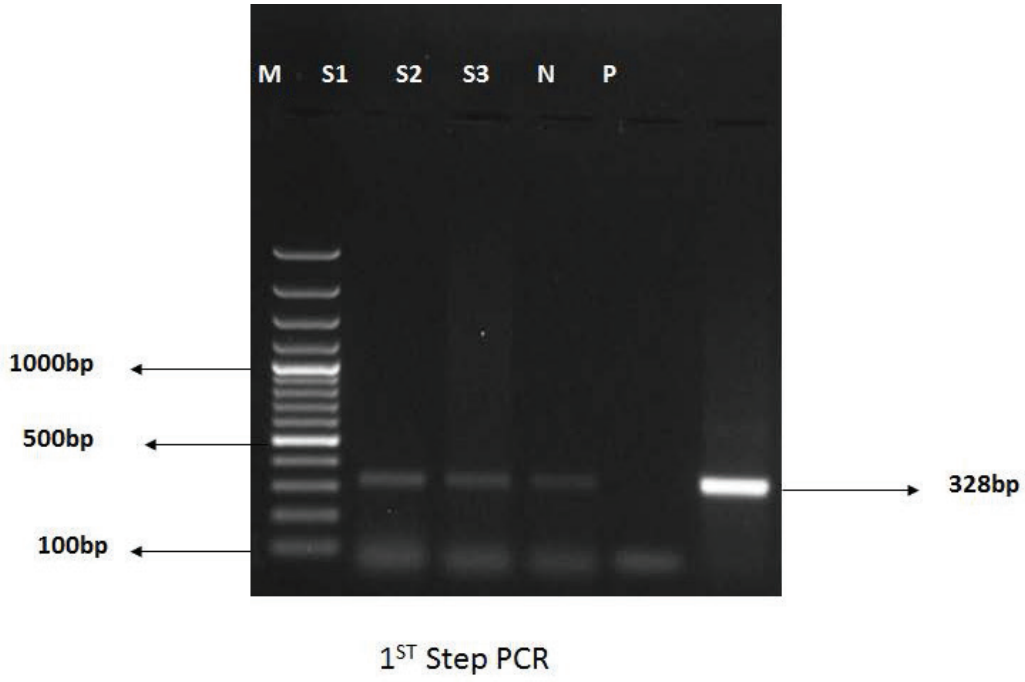


Fig 5. P. vannamei affected with IMNV showing whitish necrotic areas of the distal abdominal segments, diagnosis of IMNV by RT-PCR

How is IMN Diagnosed?

IMN can be confirmed by histopathology using routine H&E stained paraffin sections and demonstrating characteristic coagulative necrosis of striated skeletal muscle fibers, often with marked oedema among affected muscle fibers. IMN may be also rapidly diagnosed using a nested RT-PCR. Published methods available are ISH, nested RT-PCR and real-time RT-PCR for the molecular detection of IMNV.

How is IMN transmitted?

IMNV has been demonstrated to be transmitted through cannibalism. Transmission via water and vertical transmission from broodstock (trans-ovarian or by contamination of the spawn eggs) to progeny is also likely to occur. IMNV may also be transmitted among farms by feces of seabirds or shrimp carcasses. Outbreaks of IMN with sudden high mortalities may follow stressful events such as capture by cast-net, feeding, sudden changes in salinity or temperature, etc., in early juvenile, juvenile, or adult *P. vannamei* in regions where IMNV is enzootic.

How IMN can be prevented /controlled?

IMN can be prevented using SPF broodstock and practicing BMPs. No effective therapeutants have been reported for IMN.

Acute hepatopancreatic necrosis disease

Acute hepatopancreatic necrosis disease (AHPND) earlier known as early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) has been causing significant losses in shrimp farms in China, Vietnam, Malaysia and Thailand since 2009. The disease affects both black tiger shrimp and Pacific white shrimp and is characterised by mass mortalities during the first 20-30 days of stocking. In Vietnam, the disease was observed since 2010, but the most widespread devastation due to EMS was reported since March 2011 in the Mekong Delta (South Vietnam). In China, the occurrence of EMS in 2009 was initially ignored by most farmers. But in 2011, outbreaks became more serious especially in farms with culture history of more than five years and those closer to the sea using high saline water. Shrimp farming in Hainan, Guangdong, Fujian and Guangxi suffered during the first half of 2011 with almost 80% losses. In Malaysia, EMS was first reported in mid-2010 in the east coast of peninsular states of Pahang and Johor. Thailand also suffered serious setback due to AHPND.

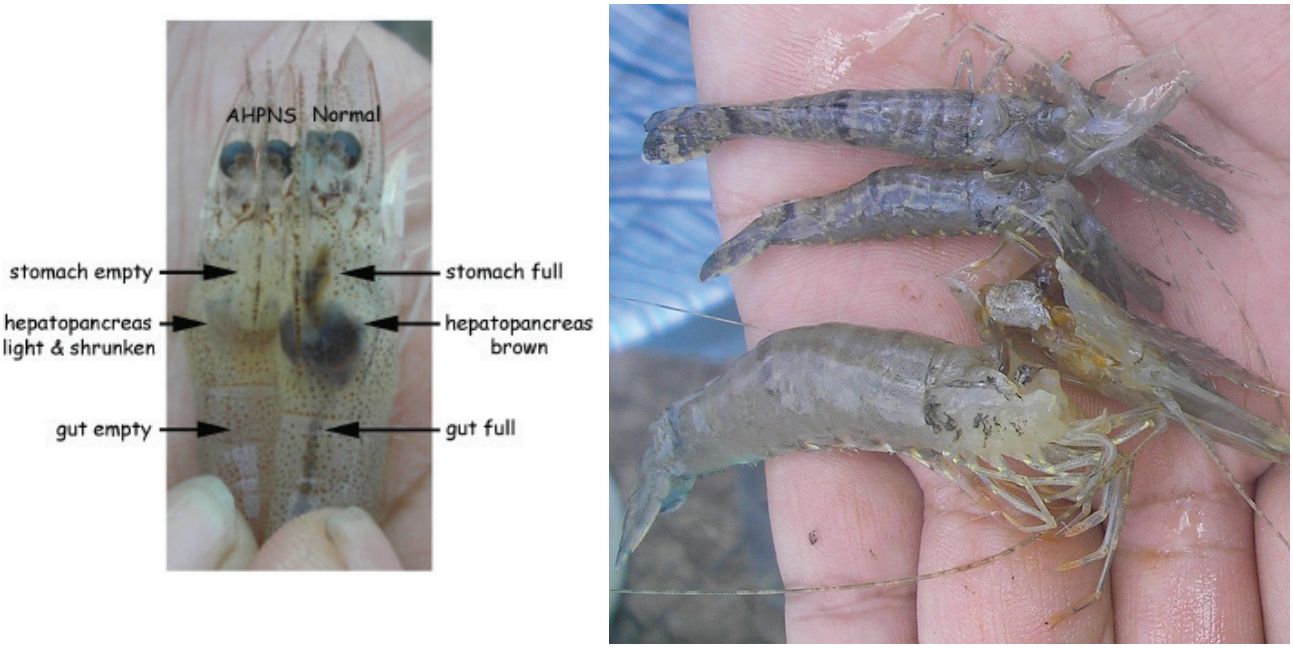


Fig 6. Gross clinical signs of Penaeus vannamei affected by acute hepatopancreatic necrosis disease (left) compared to normal shrimp (right). Source: AHPND disease card (http://www.enaca.org/publications/health/disease-cards/ahpnd-disease-card-2014.pdf) and Loc Tran (2014)

What is the causative agent of AHPND?

The disease is caused by a unique strain of *Vibrio parahaemolyticus*, that can produce toxins responsible for the primary pathology in affected shrimp.

What are the symptoms of AHPND?

The clinical signs such as significant atrophy of the hepatopancres (HP), which may be often pale to white due to pigment loss, sometimes with visible black spots or streaks, which does not squash easily between the thumb and forefinger could be used for presumptive diagnosis in cases of shrimp mortality starting as early as 10 days post-stocking. Progressive degeneration and dysfunction of the

HP tubule epithelial cells progressing from proximal to distal ends of HP tubules and its degenerative pathology suggested of a toxic etiology.

How to Diagnose AHPND?

AHPND can be diagnosed based on gross signs along with histopathological examination and culture and isolation and *of V. parahaemolyticus*, supplemented by bioassay studies following the challenge tests. AHPND can be confirmed using PCR method (AP3 or AP 4 primers). Commercial diagnostic kits are also available (e.g., IQ Plus POCKIT by M/s GeneReach Technologies, Taiwan)

How is* AHPND *transmitted?

Vibrios are ubiquitous organisms in marine ecosystem and transmission of AHPND through oral route is most likely. AHPND has been transmitted experimentally by immersion. Transmission by cohabitation is expected. *P. monodon*, *P. chinensis* and *Penaeus vannamei* are known to be susceptible. Infected live shrimp and fresh (never frozen) shrimp tissues can effectively transmit the disease to "clean" shrimp

How to prevent / control AHPND?

AHPND can be prevented by use of SPF broodstock and strict adoption of BMPs in hatcheries and grow-out farms. The practice of nursing post larvae to a larger size before stocking into ponds has been strongly encouraged. Nurseries have to be physically separated from the grow-out area and very strict biosecurity measures have to be in place. Applying disinfectant during pond preparation reduces the risk of horizontal transfer. Pond sludge management is another important strategy, as *V. parahaemolyticus* can persist in the organic matter that accumulates in pond bottom. Affected ponds must be disinfected before release of pond water into wild.

Necrotizing Hepatopancreatitis (NHP)

This disease is also known as Texas necrotizing hepatopancreatitis (TNHP), Texas pond mortality syndrome (TPMS), Peru necrotizing hepatopancreatitis (PNHP). NHP has been reported as an important disease since its first diagnosis in 1985. It has been reported to cause mass mortalities to the tune of 20-90 percent of P. vannamei in highly saline commercial grow-out ponds nearly every year since then. By 1993, NHP spread to Ecuador, Guatemala, Honduras, Mexico, and Peru and by 1995, coincided with warm waters with high salinity associated with El Nino, and caused severe mortalities (60-80 percent mortality) of P. vannamei and P. stylirostris throughout Ecuador. NHP has not yet been reported in Asia, but could cause significant damage were it to be transferred here with untested shrimp introduction.

What is the causative agent of NHP?

Necrotizing hepatopancreatitis is caused by obligate intracellular Rickettsia-like bacterium, a member of the order □-Proteobacteria (Gram-negative, pleomorphic, rod-shaped or helical-shaped bacterium).

What are the symptoms of NHP?

Affected shrimp are lethargic, anorexic with empty gut and show epibiotc fouling. Exoskeleton becomes soft and show abdominal muscle atrophy. Affected ponds have increased FCR and growth of affected shrimp is retarded. The hepatopancreas becomes watery with white or black streaks. Mortality rates reach up to 90% within 30 days of the appearance of clinical signs.

How NHP is diagnosed?

NHP can be diagnosed by microscopic demonstration of lipid droplets and melanisation of hepatopancreas in wet mount of preparations. It may be confirmed by histopathological examination showing atrophy and

the presence of granulomata in the hepatopancreas, and haemocyte aggregations around the hepatopancreatic tubules. Intracytoplasmic Rickettsia-like bacteria may be prominently seen in the cytoplasm. Molecular diagnostic tools such as in situ hybridization, dot blot hybridisation, and PCR for specific □-Proteobacterial DNA are also available.

How is NHP transmitted?

NHP could be transmitted horizontally with infected PL.

How NHP can be prevented /controlled?

Strict adoption of BMPs, use of SPF broodstock and stocking NHP free seed in the farms is the best way to prevent NHP. Adhering to strict biosecurity protocols and practicing BMPs will be useful in preventing NHP.

Fig 7. Juvenile P. vannamei affected with NHP showing markedly atrophied hepatopancreas (Picture from Bondad-Reantaso et al, 2001)

3. Diseases prevalent in brackishwater aquaculture in India

Among the diseases explained in the earlier section, only two diseases, viz., WSD and IHHNV have been reported from Indian aquaculture sector. Other OIE listed diseases have so far not been reported in Indian aquaculture so far. However, during the recent times, several shrimp diseases have emerged in Indian brackishwater aquaculture the cause of which are yet to be determined. Many of the farms suffer serious morbidity and mortality of stock, resulting in economic losses due to these diseases.

3.1. **Shrimp diseases**

Hepatopancreatic microsporidiosis or Enterocytozoon hepatopenaei

Hepatopancreatic microsporidiosis (HPM) is caused by *Enterocytozoon hepatopenaei* (EHP). It was first reported as an unnamed microsporidian from growth retarded black tiger shrimp *Penaeus monodon* from Thailand in 2004. It also has smaller spores (approximately 1 µm in length) and is currently known to infect both *P. monodon* and *P. vannamei*. It has been found that EHP can be transmitted directly from shrimp to shrimp by cannibalism and cohabitation. EHP is confined to tubule epithelial cells of the shrimp HP and shows no gross signs of disease except retarded growth. It is urgent that these possibilities be explored in order to improve control measures. Although EHP does not appear to cause mortality in *P. monodon* and *P. vannamei*, information from shrimp farmers indicates that it is associated with severe growth retardation in *P. vannamei*. The best approach for maturation and hatchery facilities to avoid EHP is not to use wild, captured, live animals (e.g., live polychaetes, clams, oysters, etc.) as feeds for broodstock. Better would be pasteurization (heating at 70°C for 10 minutes). Another alternative would be to use gamma irradiated frozen feeds. Alternatively, polychaetes could be selected and tested for freedom from shrimp pathogens and then reared as broodstock feed in biosecure settings designed to maintain their freedom from shrimp pathogens.

Fig 8. Shrimp, P. monodon and P. vannamei affected with growth retardation and thereby showing wide range of size variation due to hepatopancreatic microsporidiosis,

White faeces syndrome

White faeces syndrome (WFS) has been reported in shrimp farms since last decade, however during recent times, it has become widely prevalent in *P. vannamei* farms throughout the shrimp farming countries. 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. WFS has been found to be associated with presence of vermiform like gregarine bodies, vibriosis, *Enterocytozoan hepatopenaei*, blue green algae and loose shell syndrome. Microscopy of squash preparations of gut or faecal strings reveal masses of vermiform bodies that superficially resemble gregarines. Bacteriological studies indicate that total bacteria and *Vibrio* spp. are significantly higher in haemolymph and intestine of WFS affected shrimp. Six species of fungi (*Aspergillus flavus, A. ochraceus, A. japonicus, Penicillium* spp., *Fusarium* spp., and *Cladosporium cladosporioides*) have been isolated from shrimp naturally infected with white faeces syndrome. Histopathological examination reveals diffused haemocyte encapsulation and dilated hepatopancreatic tubules accompanied by necrosis. Furthermore it has been estimated that the Thai production losses due to WFS in 2010 was estimated to be of the order of 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 WFS.

Fig 9. Shrimp showing white gut, white faecal strings floating on pond surface, and aggregated, trans-formed microvilli (ATM) in microscopic wet mount of shrimp white faeces.

Running Mortality Syndrome (RMS)

Running mortality syndrome (RMS) is named by shrimp farmers for continuous low-level mortalities during the culture period, resulting in low survival and productions. The syndrome is widely prevalent in the vannamei farms since 2011 in Andhra Pradesh (AP) and Tamil Nadu (TN). Generally mortalities start after a month or 40 days of culture (DOC); a portion of shrimp continue to survive and can grow to fully harvestable size. Affected shrimp show patches of whitish musculature in the abdominal segments as a clinical sign. Investigations carried out at CIBA have revealed no association with known shrimp viral infection. Shrimp from RMS affected ponds tested negative for WSSV, IHHNV, IMNV, TSV, YHV, MBV, HPV and PvNV. Bacteriological examination of haemolymph samples of RMS affected shrimp indicated predominance of Vibrio spp., such as Vibrio parahaemolyticus and Vibrio azureus. The hepatopancreas was largely normal as revealed by histological techniques. However, some samples showed enlargement of nucleus and increased inter hepatopancreatic tubular space with haemocytic infiltration. Muscle necrosis was indicated by haemocytic infiltration. Lymphoid organ (LO) tubules had constricted lumen. Bioassay experiments carried out by feeding RMS affected shrimp tissue to healthy shrimp (13-14 g) did not elicit any disease in the experimental shrimp. Due to low survivability, the FCR becomes very high and thus the farmers face considerable loss. RMS affected shrimp showed recovery and appeared healthy and active after 6 days 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. The study could not attribute any infectious aetiology to RMS.

Fig 10. a Clinical signs of running mortality syndrome: moribund shrimp swimming sluggishly in the edge of the pond, dead floating shrimp collected from pond with net

Fig 10. b Shrimp showing muscle necrosis (white patches) and histological section showing necrosis of muscle tissue.

Vibriosis

Epizootics due to vibrio infections in occur in all life stages of shrimp, but are more common in hatcheries. It is a severe systemic bacterial disease. Major epizootics of vibriosis have been reported in *P. japonicus* from Japan, *P. monodon* from the Indo-Pacific region and *P. vannamei* from Ecuador, Peru, Colombia and Central America. In Latin America vibriosis is also called Sea Gull Syndrome or Sindroma de Gaviota.

What is the causative agent of Vibriosis?

Vibriosis is caused by gram-negative bacteria in the family Vibrionaceae such as *V. harveyi, V mimicus, V. splendidus, V. vulnificus, V. parahaemolyticus, V. alginolyticus*.

What are the symptoms of Vibriosis?

General signs of vibriosis in shrimp include lethargic, abnormal swimming behaviour, loss of appetite, red discoloration, brown gills, soft shell, atrophied hepatopancreas and necrosis of the sub cuticular tissue in the tail and appendages region. In severely affected shrimp, the gill covers appear flared up and eroded and extensively melanised black blisters can be seen on the carapace/abdomen. Moribund shrimp appear hypoxic and often come to the pond surface or edge.

Symptoms of luminescent bacterial disease (LBD) include lethargy, slow larval metamorphosis and body malformations, bioluminescence, muscle opacity, melanisation, empty midgut and anorexia.

Fig 11. Shrimp exhibiting signs of vibriosis: flared up branchiostegites (gill covers), blister on the branchiostegites, necrosis of appendages, melanisation of carapace (black spots).

In Latin America *V. harveyi* is reported to cause Bolitas negricans in penaeid shrimps which is the spanish term for small ball and the diseased shrimp show balled epidermal tissue blocking the digestive tract. There will be high mortalities in PL's and young juveniles.

How is vibriosis diagnosed?

Vibriosis can be diagnosed based on gross signs and confirmed by isolation of bacterial pathogen from haemolymph by standard microbiological methods.

How vibriosis is transmitted?

Vibrio species normal microflora of marine and brackishwater ecosystems and are widely distributed in aquaculture facilities. Being opportunistic pathogens, in heavily stocked culture systems, *Vibrio*-related diseases spread rapidly. Stressful environment conditions like poor water quality, nutrition, improper handling, overcrowding and parasitic infestations act as predisposing factors.

How to prevent / control vibriosis?

Strict adoption of BMPs, maintaining optimal stocking densities would help in preventing occurrence of vibriosis. When cases of vibriosis are detected, use of medicated feeds such as oxytetracycline @ 1.5g/ Kg, fed at 2-10% of body weight for 10-14 days along with proper water and pond management may be helpful. Antibiotics should be used with utmost care. Sufficient withdrawal period (about 25-30 days) should be allowed for the antibiotic to become inactive or harmless.

Black gill disease

Affected shrimps have gills with black to brown discoloration, in acute cases necrosis and atrophy of the gill lamellae may be apparent. The blackening is due to the deposition of melanin at sites of massive haemocyte accumulation, followed by dysfunction and destruction of gill processes. A small percentage of shrimp population in ponds occasionally suffer with black gill disease. However, during recent vannamei crops, incidences of black gill disease are on the rise. A number of abiotic and biotic reasons have been attributed to the black gill in shrimps. Presence of excessive levels of toxic substances such as nitrite, ammonia, heavy metals, crude oils etc. in the culture water may lead to black gill disease. High organic load, heavy siltation and reducing conditions in rearing pond can also cause this disease in shrimps. Infection with certain bacterial, fungal and protozoan pathogens can also cause black gill condition in shrimp. Treatment of the black gill disease depends upon the cause of the disease. Preventive or corrective measure may be adopted to avoid or reduce the biotic / abiotic factors in the rearing pond to control the disease condition.

Fig 11. P. vannamei shrimp affected with black gill disease.

Protozoan fouling

Affected shrimps are restless and their locomotion and respiratory functions are hampered. In heavily infected juvenile and adult shrimps, one can observe fuzzy mat-like appearance due to ciliate fouling. Protozoans such as Vorticella, Zoothamnium, Epistylis, Acineta and Ephelota are involved. Maintaining good water quality and reducing organic load and silt in water exchange with good quality water would help in prevention of protozoan fouling. Formalin of affected individuals can be used for controlling protozoan infestation.

Fig 12. Tiger shrimp affected with protozoan fouling

3.2. **Finfish Diseases in India**

Considering the significance and recorded to be present in India, description of only two viral diseases affecting brackishwater finfish is described here.

Iridovirus (Rana virus) infection

Iridovirus infection is a significant cause of mortality in farmed red sea bream *(Pagrus major)* and more than 30 other species of cultured marine fish belonging mainly to the orders Perciformes and Pleuronectiformes. The infection has also been detected in Asian sea bass. It affects all the stages of fish but the susceptibility of juveniles is generally higher than that in adults.

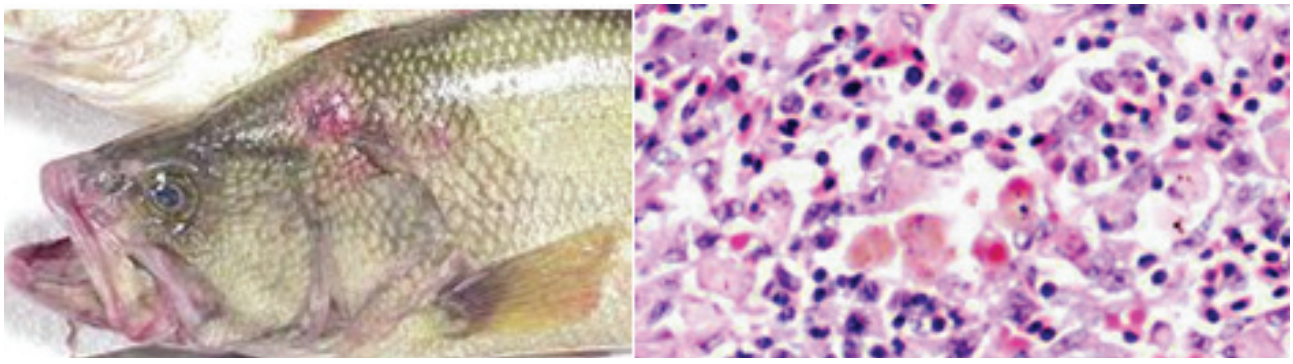


Fig 13. Iridovirus infection with typical skin lesions (Source: https://nas.er.usgs.gov/queries/greatlakes/FactSheet.aspx?SpeciesID=2657&Potential=N&Type=0&HUCNumber=DHuron) and histopathological changes

What is the causative agent of iridoviral disease?

The disease is caused by double stranded DNA virus of genera Lymphocystivirus and Ranavirus. Ranaviruses causes systemic disease in infected fish and are associated with high morbidity and mortality.

What are the symptoms of iridoviral disease?

Outbreaks of the disease have been mostly reported in the summer season at water temperatures of 25°C and above. Depending on host fish species, fish age, water temperature, and other culture conditions, mortality rates ranges between 0 and 100%. Affected fish become lethargic, exhibit severe anaemia, petechiae of the gills, and enlargement of the spleen.

How is iridoviral disease diagnosed?

The sample should be collected from moribund fish. Gill and visceral organs such as spleen, heart, kidney, liver and intestine should be collected. However, the spleen and/or kidney tissues are the most appropriate organ for pathogen detection by immunofluorescent antibody test (IFAT). The fish sample should be stored at 4°C for use within 24 hours or -80°C for longer periods. The disease is characterised by the appearance of abnormally enlarged cells stained deeply with Giemsa solution in the histopathological observations of the spleen, heart, kidney, intestine and gill of infected fish. Electron microscopy confirms the presence of virions (200-240 nm in diameter) in these cells. PCR is able to detect Iridovirus infection with high degree of sensitivity in short time. Recently real time PCR has been developed which shown improved rapidity, sensitivity, reproducibility, and the reduced risk of carry over contamination over normal PCR. An antibody based enzyme-linked immunosorbent assay (ELISA) has also been developed to detect iridovirus infection.

How iridoviral disease is transmitted?

The principal mode of transmission of Iridovirus infection is horizontal via water. The vertical mode of transmission has not yet been established.

How to prevent / control iridoviral disease?

Strict adoption of biosecurity protocols, screening fish prior to inducting them as broodstock in finfish hatcheries and stocking screened seed in grow-out culture, strict adoption of BMPs help in preventing occurrence of iridoviral disease.

Viral nervous necrosis

Viral nervous necrosis (VNN) also known as viral encephalopathy and retinopathy (VER) is a devastating disease of marine fish species cultured worldwide. It affects more than 50 wild and cultured marine fish species, especially the larval and juvenile stages which records high mortality. In India, betanodavirus infection has been observed in cultured and wild population of brackishwater/ marine fish species such as *Lates calcarifer, Mugil cephalus, Chanos chanos* and *Epinephelus tauvina* etc. VNN has also been reported from low saline and freshwater environments.

Fig 14. Sea bass affected with VNN

What is the causative agent of viral nervous necrosis disease?

The disease is caused by piscine nodavirus of the genus betanodavirus of the family Nodaviridae. The virus contains single stranded, bipartite positive sense RNA genome.

What are the symptoms of viral nervous necrosis disease?

The major clinical signs of VNN are common behavioural changes such as lack of appetite, erratic, spiral or belly-up swimming and dark coloration of body. Clinically, the affected animals show spiral or looping swim pattern, swim bladder hyperinflation and later wasting. The severity of disease is more in juveniles with equal higher rate of mortality. In sea bass, the earliest onset of clinical signs of the disease is during 16-21 day post hatch. Vacuolation is seen in the grey matter of brain and retina of eye. Necrosis is observed in the spinal cord, brain and retina while intra-cytoplasmic inclusion in nervous cells.

How is viral nervous necrosis disease diagnosed?

Viral nervous necrosis can be diagnosed by demonstrating characteristic lesions in the brain and/or retina by light microscopy. Detection of virions by electron microscopy, viral antigens or antibodies by serological methods such as indirect fluorescent antibody test, and enzyme linked immune-sorbent assay or detection of viral nucleotides by molecular techniques such as RT-PCR and by tissue culture are different ways for pathogen detection. The RT-PCR targeting capsid protein gene sequence is the most sensitive test and has become the main diagnostic method for fish nodaviruses using blood, sperm, as well as nervous and ovarian tissues.

How is viral nervous necrosis disease transmitted?

Betanodaviruses are quite resistant to environmental conditions, which make it possible to get translocated by commercial activities via influent water, juvenile fish, utensils, vehicles, etc. Translocation of species for stocking purpose from one location to another may be another way but it is yet to be validated. Latent infection among wild fishes also serves as source of infection. Apart from all these means of horizontal transmission, vertical transmission is highly suspected to take place from infected spawners to fry. Instances of asymptomatic/ sub-clinical infection among wild fishes may possibly act as potential carriers.

4. Level 1 Diagnosis of table on diseases of shrimp and finfish

Table 1. Filed level Diagnostic table on diseases of shrimp

	Shrimp Diseases Probable causative agent	Exoskeletal deformities	Irregular, pitted, melanised lesions on cuticle	Bent rostrums	Shrimp Lethargic	Black gills	Fouling by epicommensals	Black spots on exoskeleton	Flaring up of branchiostegites	Gathering at surface/edges of pond /tank	Bluish body coloration	Reddish discoloration	Soft-shell (exoskeleton)	Loose shell (exoskeleton)	White spots on exoskeleton	Hepatopancreas swollen and light yellow	Granular hepatopancreas	Empty gut	Whitish necrotic areas in distal abdominal segments	Whitish midgut line	White faecal strands	Reduced feeding	Shrimp feed voraciously for two to three days and then stop feeding abruptly	Reduced growth/ stunted growth	Mortality within 30-40 days of stocking	Mass mortality - 70-100% within 3-10 days	Daily mortality	Low-level mortalities
1	White spot disease (WSD)				+					+		+			+							+			+	+	+	
2	Infectious hypodermal and haematopoietic necrosis (IHHN)	+		+			+	+														+		+				+
3	Yellow head disease (YHD)				+											+							+				+		
4	Taura syndrome (TS)		+		+		+	+				+	+									+					+	+
5	Infectious myonecrosis (IMN)				+		+						+	+			+		+	+							+	+
6	Necrotising hepatopancreatitis (NHP)				+																	+							
7	Vibriosis	+			+	+	+	+	+			+																+	+
8	Acute hepatopancreatic necrosis disease (AHPND)				+		+			+			+	+			+	+					+			+	+	+	
9	Hepatopancreatic microsporidiosis or *Enterocytozoon hepatopenaei* (EHP)				+															+	+			+					+
10	Chronic (running) mortality syndrome (CMS)						+			+												+							+
11	White faeces syndrome (WFS)																							+					+

Table 2. Filed level Diagnostic table on diseases of finfish

Diseases of Finfish in India Probable causative agent	Epizootic ulcerative syndrome (EUS)	Viral encephalopathy and retinopathy (VER) or Viral nervous necrosis (VNN)	Iridoviral disease	Vibriosis
lethargy	+	+	+	+
gill anaemia or haemorrhage			+	
pop eyeB/or ocular haemorrhage			+	+
external haemorrhages			+	+
internal haemorrhages			+	
whirliing/corkscrew behaviour		+		
erratic swimming/jumping from water/		+		
scraping or other motor skill disfunction				
skin/body lesions /ulcers	+			+
external growths or cysts				
gathering at surface/edges of waterbody				+
swollen or colour change of internal organs		+	+	
(kidney/spleen/other hapatic)				
secondary bacterial/fungal/parasitic infection	+			
ulceration /lesions in tissue				+
reduced feeding			+	
mass mortality	+		+	

5. Disease Prevention and Control in Brackishwater aquaculture

A number of "component causes" (risk factors) along with the "necessary cause" might become "sufficient cause" to produce disease outbreaks. It is extremely difficult to control disease once it strikes and so far not successful stories have been documented in treating diseases during aquaculture operation. Hence "prevention is always better than cure". Disease prevention in brackish water aquaculture and achieving sustainability of shrimp farming largely depends on the implementation of biosecurity principles and best management practices (BMPs) in the farms. BMP encompasses policy, regulatory and programme frameworks in response to managing risks associated with diseases. BMPs are simple and practical but science based International Principles for Responsible Shrimp Farming which covers farm management practices that include farm selection, farm design, water use, broodstock and post-larvae, feed management, health management, food safety and social responsibility. BMPs minimize the potential of farm-raised fishery products from being contaminated with pathogens, chemicals, or unapproved or misused animal drugs.

Risk factors occur throughout the shrimp cropping cycle and include pond preparation, stocking densities, seed quality, water management, pond bottom management, feed management and several others. Effective management of these factors can help in reducing the risks of disease occurrence. BMP is the ability to prevent losses to disease through effective elimination of pathogens and their carriers. The basic elements of a BMP programme in a shrimp hatchery include the physical, chemical and biological methods necessary to protect the hatchery from all diseases of high risk. The most important BMP for hatcheries would be use of specific pathogen free (SPF) or high health (HH) shrimp broodstocks. BMPs in aquaculture include a number of components starting with pond preparation to provide the aquatic animal with a clean pond base and appropriate stable water quality. Before initiating a second crop, the pond has to be prepared by drying, removing the organic matter accumulated from the earlier crop to ensure sustained production. After the harvest, the pond bottom is allowed to dry and crack, primarily to oxidize the accumulated organic components. The pond bottom should be thoroughly dried to allow the soil should crack to a depth of 25 - 50 mm. Tilling pond enhances drying and accelerates decomposition of organic matter and oxidation. Where complete drying is not possible, formalin, potassium permanganate, benzyl chromium chloride, provodone iodine etc can be used disinfection. Liming of aquaculture ponds is done to neutralize soil acidity and increase total alkalinity and total hardness concentrations in water.

Pathogen exclusion or biosecurity is an important means of prevention of dreaded diseases such as WSD. 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 the 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. Disinfection of aquaculture facilities is a common disease management practice to ensure biosecurity. Methods for disinfection of aquaculture establishments have been outlined in OIE Aquatic Manual. Following practice will help in ensuring biosecurity from WSSV.

1. Source water: Ideally farms must have reservoirs of adequate capacity of seawater for operation of hatchery or aquafarm. Source water should be filtered first through coarse screens to remove larger aquatic animals and debris and then pumped into the supply/settling canal. Then, the water is passed through a series of progressively finer screens, through a fine mesh (150 250 µm mesh size) bag screen before being introduced into the reservoir.

2. Water should be chlorinated with appropriate dose of chlorine (10 ppm) to kill any potential vectors or carriers in the source water collected in the reservoir. For one ha reservoir / pond of one meter depth, 150-160 kg of calcium hypochlorite providing 65% active chlorine would give a final concentration of 10ppm. Personnel handling such chemicals should take precautions to protect their skin and eyes. Since commercial bleach powders vary in active chlorine content, dosages need to be adjusted accordingly. Reservoir should be vigorously aerated at least for 48 h to remove residual chlorine.

3. Disinfection of WSD affected grow-out ponds: Do not discharge water from WSD affected ponds. Remove aeration devices and implements and disinfect separately. Disinfect by evenly distributing calcium hypochlorite to provide a minimum final free chlorine concentration of 10 ppm within the entire system's water. Allow the system to stand for a minimum of 24 48 hours at this minimal chlorine concentration by adding hypochlorite if required.

4. Disinfection of effluent water: Chlorinate (50 ppm chlorine) for 24-48h. Vigorously aerate reservoir at least 48 h for de-chlorination to remove residual chlorine. Alternatively, ozone treatment may be carried out if available at levels of 0.08 1.0 mg per litre to significantly reduce microbial load.

5. Following harvest after a crop, the deposits of organic debris in the pond bottom should be removed or treated, ploughed and tilled. Level the ponds and ensure that there are no wet patches in the ponds, especially at the centre or near the sluice gates. All parts of ponds should be thoroughly sun dried for at least three weeks.

6. Disinfection of dried earthen ponds can be further carried out with quicklime (calcium oxide) at a rate of 4000 5000 kg per ha. Quicklime causes desiccation / dehydration of organic matter.

6. Anatomy of shrimp and fish and sampling for disease investigation

Anatomy of shrimp

Shrimp can be divided into two parts, head and abdomen. The head region is called cephalothorax. The head is covered by a hard cuticle called carapace. The carapace contains spines and a large rostrum extending straight. Towards the upper end of head on both sides, two compound eyes are present. Other important organs in the frontal part include antenna, antennule and maxilliped. The down part of the head contains 5 pairs of walking legs called as periopods. The abdomin is divided into six segments. At the end of the abdominal segment, a apine like structure called telson is flanked by two fan like structures called uropods. On the lateral side, a pair of legs on each of the first five abdominal segments, pleopods or swimming leg helps the shrimp to swim.

Major internal organs of shrimp include hapatopancreas, heart, digestive system, lymphoid organ, nerves and arteries. The hepatopancreas is an important part, which secretes digestive enzymes and functions both as liver and pancreas. Heart of shrimp contains hemolymph which becomes green in colour because of a copper containing pigment called haemocyanin. Digestive system includes mouth, mandibles, cardiac stomach, pyloric stomach, midgut, hindgut and anus.

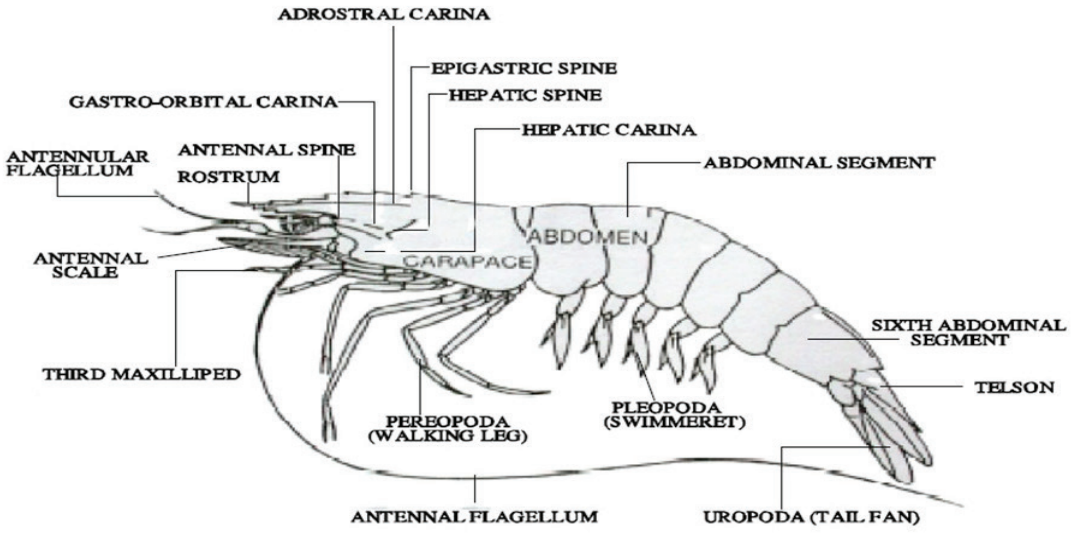


Fig.15 a. General Morphology of shrimp

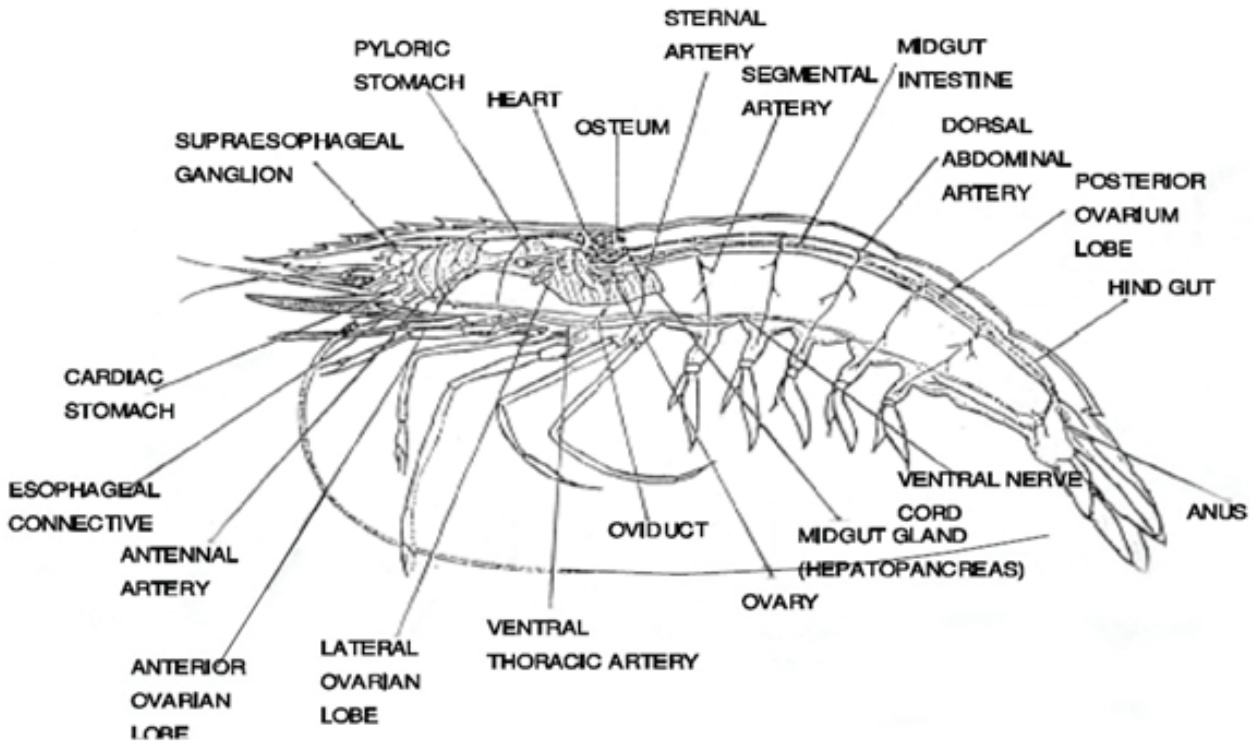


Fig.15 b. Anatomy of shrimp

Anatomy of Fish

The body of fish is divided into a head, trunk and tail, although the divisions between the three are not always externally visible. The main skeletal element is the vertebral column, composed of articulating vertebrae. The ribs attach to the spine and there are no limbs or limb girdles. The main external features of the fish, the fins, are composed of either bony or soft spines called rays which, with the exception of the caudal fins, have no direct connection with the spine. They are supported by the muscles which compose the main part of the trunk. The heart has two chambers and pumps the blood through the respiratory surfaces of the gills and on round the body in a single circulatory loop. The eyes are adapted for seeing underwater and have only local vision. There is an inner ear but no external or middle ear.

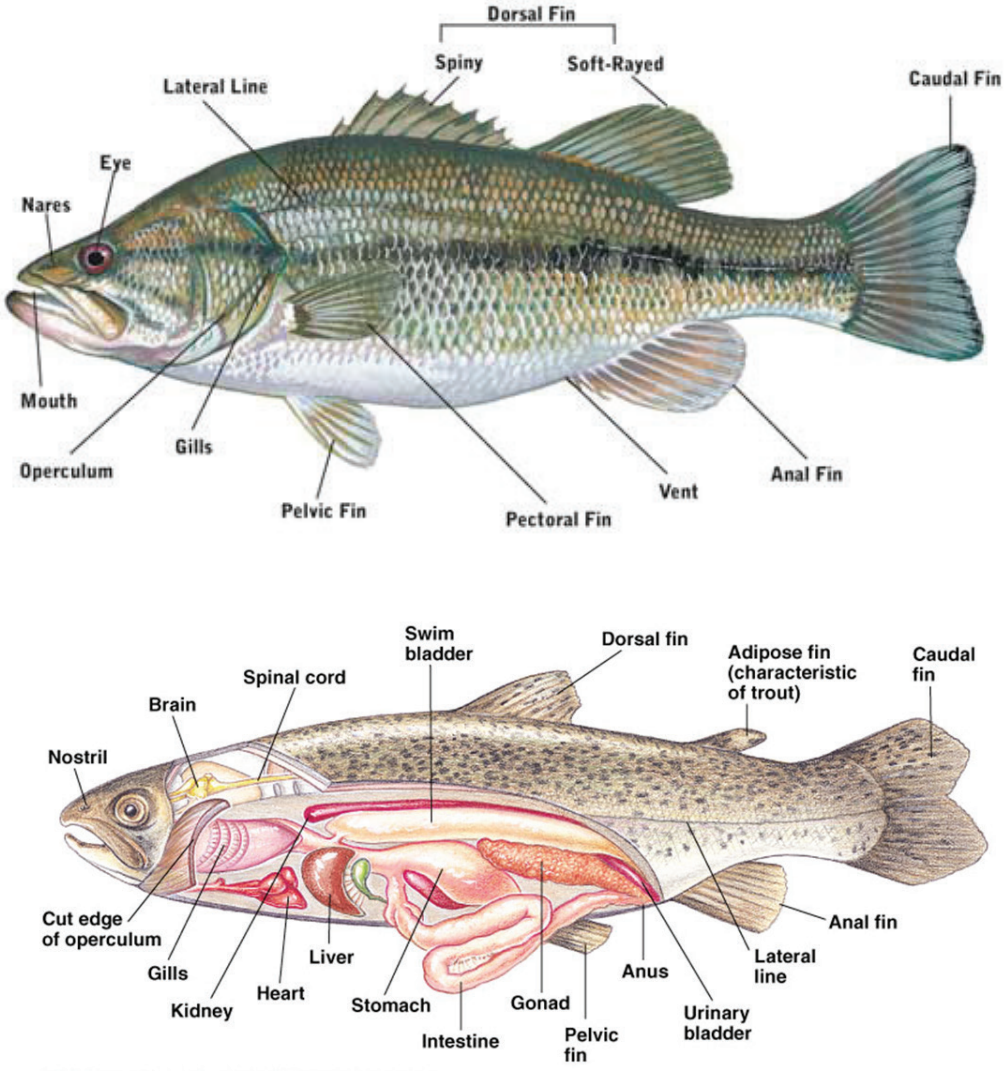


Fig.16. Morphology and anatomy of finfish

Sampling for disease investigation

Accurate disease diagnosis is an important aspect for health management. Since disease investigation facilities may not be available in shrimp / fish farming areas, it would be essential that the samples will have to be sent to diagnostic laboratories or the referral laboratories for diagnosis of the diseases. For investigation of disease in aquaculture, background information on the disease and appropriate samples are essential. For investigating disease, moribund sample (infected animals those are about to die) are best samples for diagnosis. However, individuals from the same pond may also be collected fixed appropriately and provided to the diagnostic laboratory.

For shrimp, samples of pleopods or haemolymph or gills are ideal testing for WSSV, IHHNV, TSV and YHV. For testing IMNV, telson is preferred. For

testing hepatopancreatic viruses, AHPND and EHP, hepatopancreas, stomach, midgut etc need to be collected. Lymphoid organ is a preferred sample for many of the shrimp viruses. Samples can be collected either on alcohol, RNAlater for molecular diagnostics and in Davidson's fixative for histopathological examination. Proper sampling procedure is very much important for appropriate diagnosis of fish and shrimp diseases. 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 (Table). Assumptions of 2% and 5% prevalences are most commonly used for surveillance of presumed exotic pathogens, with a 95% confidence limit).

Table 3. Sample sizes needed to detect at least one infected host in a population of a given size, at a given prevalence of infection.

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

7. CIBA's Referral laboratory services

As a National referral laboratory for brackishwater aquatic animal diseases (NRLD), CIBA with its capacity in diagnosing all OIE listed aquatic animal pathogens including emerging pathogens such as Acute Hepatopancreatic Necrosis Disease (AHPND) and *Enterocytozoon hepatopenaei* (EHP), has addressed several issues in aquatic animal health sector. The NRLD of CIBA has been providing valuable services to various agencies such as Animal Quarantine and Certification Services, Southern Region (AQCS-SR), Chennai, Aquatic Quarantine Facility (AQF), Rajiv Gandhi Centre for Aquaculture (RGCA), for screening live imported aquaculture inputs such as *Artemia* cyst, exotic and specific pathogen free (SPF) *P. vannamei* brooders and PLs etc.

Table 4. Protocols used for the screening of shrimp pathogens in the National Referral Laboratory for Brackishwater Aquatic Animal Diseases (NRLD) of CIBA

S.No.	Shrimp and Fish Pathogens	Reference
1.	White Spot Syndrome Virus (WSSV)	OIE*; Kimura et al, 1996
2.	Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)	OIE
3	Monodon baculovirus	OIE
4	Hepatopancreatic Parvo virus (HPV)	OIE
5.	Yellow Head Virus (YHV)	OIE
6.	Taura Syndrome Virus (TSV)	OIE
7.	Infectious Myonecrosis Virus (IMNV)	OIE
8.	Acute Hepatopancreatic Necrotic Disease (AHPND)	Sirikharin et. al, 2014; Itsathitphaisarn *et al*. 2016
9.	Necrotising Hepatopancreatitis Bacteria (NHPB)	IQ 2000 kit (Gene Reach)
10.	*Enterocytozoon hepatopenaei* (EHP)	Tangprasittipap et al. (2013)
11.	Viral Nervous Necrosis (VNN)	OIE
12.	Iridovirus	Jeong et al., 2006

*http://www.oie.int/international-standard-setting/aquatic-manual/access-online/