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RGCA Newsletter

QUARTERLY NEWSLETTER FROM RGCA

Management Strategies For Controlling "Emerging & Common Shrimp Diseases in Aquaculture Farms"

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About RGCA Newsletter

This issue of the RGCA Newsletter highlights the scientific innovations of the aquaculture programmes/events of RGCA. Online version of this issue is available at ***www.rgca.org.in***

Front cover photo: Fixation of shrimp samples for histology

Management Strategies For Controlling

"Emerging & Common Shrimp Diseases in Aquaculture Farms"

Shrimp aquaculture has witnessed rapid growth in the last couple of years. Incidence of diseases is also on the surge with the expansion of shrimp culture sector. The impact of diseases and its grave outcome impinges more on the shrimp farming community. As a part of RGCA's commitment in upholding the sustainability of the shrimp industry a brief account of the management strategies to control common and emerging shrimp diseases that can be routinely followed by the farmers is provided below:-

Hepatopancreatic Microsporidiosis (HPM)/EHP Infection

The disease caused by *Enterocytozoon hepatopenaei* (EHP) is known as Hepatopancreatic Microsporidiosis (HPM)

Signs of Disease

- Severe growth retardation/ growth stunting
- Disparate growth (high size variation)
- Floating white faeces (White faeces observed with other diseases also) in ponds
- Low level mortality associated with secondary bacterial infection

Disease Agent

The disease is caused by a microsporidian parasite - *Enterocytozoon hepatopenaei* (EHP).

Susceptible stages of the host

- All stages

Diagnostics

- PCR method
- Sample for PCR test – Hepatopancreas(HP) and faecal samples
- Histopathology

Host Range

- EHP infects *Penaeus monodon* and *L. vannamei*

Vector

- None identified

EHP infected L. vannamei with differential growth

Transmission mode

- Horizontal and vertical
- Primarily through oral route (through faeces and cannibalism of infected shrimp)
- EHP spore present in the pond sediment will cause fresh infection

Management

If a pond is affected by EHP then, the following pond soil treatment is suggested for subsequent crop

- The spores of EHP have thick walls and are not easy to inactivate.

- Even high levels of chlorine alone are not effective
- For pond sediment treatment apply CaO (quick lime, burnt lime, unslaked lime or hot lime) @ 6 Ton/ha
- Plough the CaO into the dry pond sediment (10-12 cm) and then moisten the sediment to activate the lime
- Leave for one week before drying or filling
- After application of CaO, the soil pH should rise to 12 or more for a couple of days and then to the normal range as it absorbs carbon dioxide and forms $CaCO_3$
- Hatcheries should test fresh feeds & artemia by PCR method
- Stock PCR negative seed (tested in reputed labs)
- Select healthy and strong seed
- Stock seed in ponds with good plankton/bloom

Acute Hepatopancreatic Necrosis Disease (AHPND)/ Early Mortality Syndrome (EMS)

Signs of Disease

- Often pale to white hepatopancreas (HP)
- Significant atrophy (shrinkage) of HP
- Often soft shells and guts with discontinuous contents or no content
- Blackspots or streaks sometimes visible within the HP
- HP does not squash easily between thumb & finger
- Onset of clinical signs and mortality from the tenth day of stocking
- Moribund shrimps sink to bottom or floats sometimes

Disease Agent

The disease is caused by a pathogenic strain of *Vibrio para haemolyticus containing* a virulence plasmid/toxin gene

Susceptible stages of the host

- Juveniles

Diagnostics

- PCR method targeting the toxin gene
- Histopathology

Host Range

- AHPND affects *Penaeus monodon* and *L. vannamei*

Vector

- None identified

Transmission mode

- Horizontal and vertical

Management

- When signs of AHPND / EMS disease is observed immediately submit samples for analysis immediately to reputed labs and inform appropriate authorities
- Stock PCR negative seeds (Tested in reputed Lab)
- Select healthy and strong seeds
- Nursery rearing of PL
- Stock seed in ponds with good plankton/bloom
- Monitor and maintain Vibrio load under control

The following products/technology reported to help managing AHPND: Probiotics, Tilapia culture water, use of bacterial biofloc, bioremediators, toxin binders, immunostimulants, vitamins, phage therapy etc.

Infrastructure improvements

- Provide central drain in ponds to flush out organic wastes
- Position aerators in the pond so as to concentrate and help to remove organic wastes towards central drain
- Provide nursery in the farm to rear PL for 25 to 35 days
- Remove sludge after every crop
- Stock salinity resistant strain of Tilapia in cages @ 500 to 800 nos per ha in nursery
- Use tilapia culture water to manage AHPND

Shrimp with AHPND (left) and normal shrimp (right)

Covert Mortality Disease (CMD)

Clinical Signs of disease

- Atrophic or pale HP
- Whitish abdominal muscle
- Partial/Empty stomach and guts
- Soft Shell , slow growth

Mortality Pattern

- Shrimp dies at the pond bottom
- Mortality during 30 to 80 Days of Culture (DOC)
- Moribund and dead shrimp can be found daily
- Disease worsens after 60 - 80 DOC accompanied by high NO_2-N and temperature above 28° C
- Cumulative mortality up to 80 %

Disease agent

- Covert Mortality Noda Virus

Diagnostics

- Reverse transcription (RT)-PCR method
- Sample for PCR test - Haemolymph, Hepatopancreas(HP), muscle
- Histopathology

Host Range

- CMNV infects *L. vannamei*

Vector

- None identified

Transmission mode

- Horizontal

Management

- Stock PCR negative seed (tested in reputed labs)
- Stock healthy and strong seeds

Infectious Hypodermal and Haematopoietic Necrosis (IHHN)

Signs of Disease

- Juveniles with acute IHHN show a marked reduction in food consumption, followed by changes in behaviour and appearance
- Runt Deformity Syndrome (RDS), a chronic form of IHHN disease, occurs in wild or inbred L. *vannamei* as a result of IHHNV infection
- Juvenile shrimp with RDS may display a bent or otherwise deformed rostrum, a deformed sixth abdominal segment, wrinkled antennal flagella, cuticular roughness, bubble-heads and other cuticular deformities
- Populations of juvenile shrimp with RDS display disparate growth with a wide distribution of sizes and much smaller than the normal size at harvest.

Disease agent

- *Penaeus stylirostris* densovirus / Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)

Vectors

- No vectors are known in natural infections

Known or suspected wild aquatic animal carriers

- IHHNV is common in wild *P. monodon*, *L. vannamei*, and other wild penaeid species

Transmission mode

- Transmission of IHHNV is by horizontal or vertical modes. Horizontal transmission of the pathogen occurs through cannibalism or by contaminated water and vertical transmission via infected eggs

Diagnostics

- PCR method
- Histopathology

Management

- Use of specific pathogen free (SPF) or polymerase chain reaction (PCR)-negative seed stocks
- Other management measures similar as for White Spot Disease

Note

- Seed derived from selectively bred *L. vannamei* are resistant to IHHNV

L. vannamei with Bent Rostrum, one of the clinical signs of IHHNV

White Spot Disease (WSD)

Signs of Disease

Important: Animals with disease may show one or more of the signs listed below, but disease may still be present in the absence of any signs.

Disease signs at the farm level

- High and rapid mass mortality
- Can occur at any stage of the grow-out period

Disease signs at the tank and pond level

- Shrimp with white spot disease sometimes do not show distinctive clinical signs
- Lethargy/ weakness
- Shrimp with WSD stop feeding
- A few days later, moribund/about to die prawns observed near surface /at the edge of rearing pond

Clinical signs of disease in an infected animal

- White calcium deposits embedded in shell, causing white spots of 0.5 - 2.0 mm in diameter (but white spot disease can occur without these signs)
- Darkened (red or pink) body surface and appendages
- Heavy surface and gill fouling by external parasites
- White midgut line could be observed through abdomen of severely affected shrimp

Disease agent

- White Spot Syndrome Virus (WSSV)

Susceptible stages

- Any stage of grow out period
- Even SPF *P. monodon* and *L. vannamei* are susceptible at farm if proper biosecurity is not maintained

Vectors

The virus can transmit from host to host and does not need a biological vector

Known or suspected wild aquatic animal carriers

- Wild decapods can be easily infected by WSSV and may express the disease under suitable environmental conditions

Shrimp carapace with white spots

- However, non-decapodal crustaceans such as copepods, rotifers, Artemia may become wild aquatic animal carriers by latent infection without disease
- Other marine molluscs, polychaete worms, as well as non-crustacean aquatic arthropods such as sea slaters (Isopoda) and Euphydradae insect larvae can mechanically carry the virus without evidence of infection

Transmission mode

- Horizontal - infected shrimp to healthy shrimp through cannibalism or water and vertical from infected broodstock to larvae/seed

Management

- Use of farm specific implements like cast net, crates and check trays
- Biosecurity measures: Bird netting, crab fencing, hand dips, foot bath / tyre bath
- Tested seed from reputed SPF shrimp seed supplier / use of specific pathogen free (SPF) or polymerase chain reaction (PCR)-negative seed stocks
- Follow Good Aquaculture Practices
- Avoid stocking during cold season
- Use of biosecure water and culture systems

For Further Reading

- Briggs, Mathew et al., 2005.Introductions and movement of two penaeid shrimp species in Asia and the Pacific, *FAO Fisheries Technical paper*, 476, 78p.
- FAO., 2013. Report of the FAO/MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp (under TCP/VIE/3304), *FAO Fisheries and Aquaculture* Report No. 1053, 54p.
- Lightner, D.V. 2011. Status of shrimp diseases and advances in shrimp health management, pp. 121-134. In: Bondad-Reantaso, M.G., Jones, J.B., Corsin, F. and Aoki, T. (eds.). *Diseases in Asian Aquaculture VII*. Fish

Highlights of Good Aquaculture Practices (GAP) and biosecurity maintenance in shrimp farms

Implementation of Good Aquaculture Practices (GAP) and biosecurity maintenance in farming systems depend greatly on the system, the species, recommended stocking season and the region where farming is conducted. However, there are some common elements when dealing with GAP in shrimp pond farming. There are also many examples and references on good management practices. Some of them are provided below:

Pond and pond bottom preparation and water management prior to stocking

- Dry the pond for a period of two or more weeks
- Remove the sludge and dispose it away from the pond site
- Plough wet soil if the sludge has not been removed completely
- Lime the pond
- Filter the intake water using twin bag filters of 300 mm mesh size
- Maintain the water depth to at least 80 cm at shallowest part of pond
- Fertilize and condition the water for 10 to15 days before stocking
- Control undesirable species (e.g., finfish, crustaceans, mollusks, amphibians, reptiles, birds, mammals)through physical, chemical and other means
- Provide additional reservoir in the farm, in which salinity resistant strain of Tilapia or *Chanos chanos*(Milk fish) are reared. Use water from this reservoir to fill the culture ponds

Seed selection and stocking

- General health checks of PLs to be done before stocking using traditional and molecular-based methods
- Use PLs of uniform size and colour which actively swims against the water current
- Eliminate weak PL before stocking, using formalin (100 ppm) stress for 15 - 20 minutes in continuously aerated water
- Rear PLs for 20 to 30 days in on farm nursery systems.
- Stock seeds in green water and avoid transparent water during stocking
- Strictly adhere to the stocking density recommended by the competent authority
- Stock seeds in a single area at a time to avoid disease transmission from infected adult shrimps/juveniles to freshly stocked seeds

Post-stocking management

- Use water reservoirs, and 10 to 15 days aged water in grow-out ponds
- Regularly use agricultural lime, especially after water exchange and rain
- Do not use any harmful/banned chemicals
- Use feed check trays to ensure sufficient feeding of shrimps based on demand
- Feed across the pond using boat/floating device to avoid local waste accumulation
- Install auto feeder to help better feed management which would minimize pond deterioration thus reducing incidence of diseases
- Regularly remove benthic algae
- Monitor water quality to ensure appropriate pH, alkalinity and DO levels
- Exchange water only during critical periods
- Check the pond bottom mud for blackish organic waste accumulation and bad smell
- Monitor growth of shrimps at regular intervals and adjust the feed according to the estimated biomass

Health management

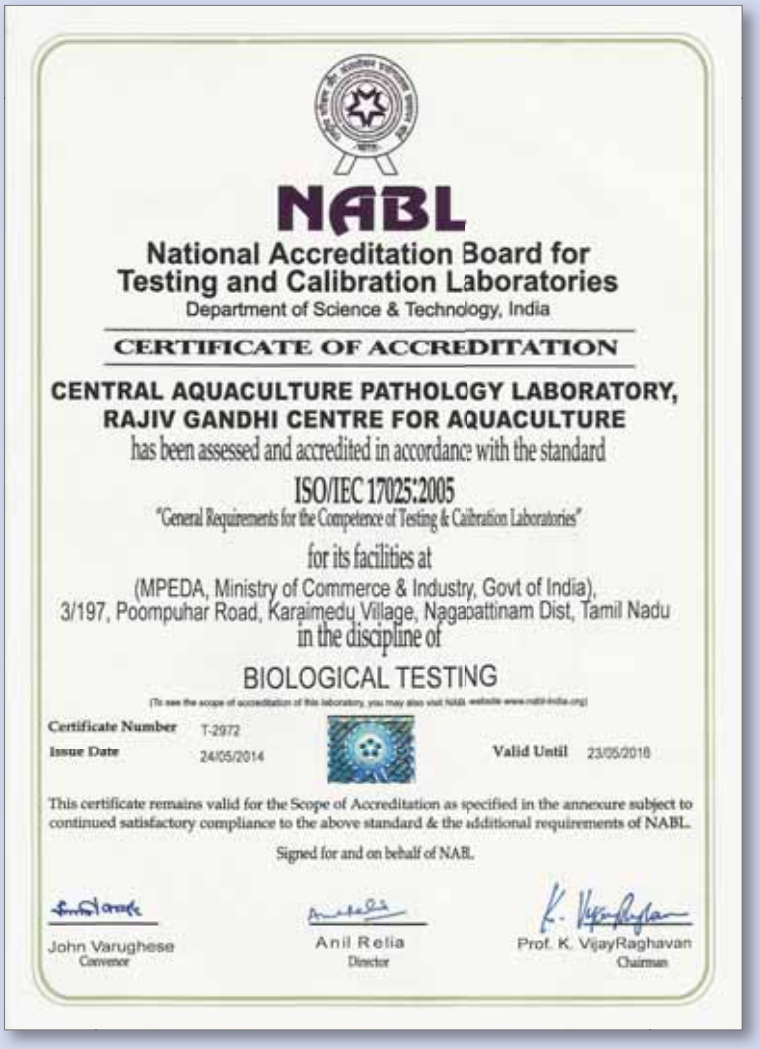
- Monitor shrimp behaviour and assess general health condition of the shrimps
- Apply right diagnostic procedures when a health problem occurs
- If the stock is affected by general vibriosis, feed should be reduced, water and pond bottom quality should be improved by adopting necessary steps.
- If a disease outbreak occurs due to an infectious disease, the risk of spread of the disease to other farms should be prevented (Eg., no water discharge, no movement of infected stocks) and should be informed to neighbouring farmers and authorities. Dead shrimp if any should be burnt or buried with bleach
- Emergency harvesting to be done after proper planning
- Do not drain or abandon diseased/affected stocks
- Destruct infected/dead stocks as per standard protocols

(Compiled by : Biju, V.N, Karthick Kannan K, Mithun Raj and Babu, B)

Health Section, Asian Fisheries Society, Selangor, Malaysia, 385p.

- Tourtip S, Wongtripop S, Stentiford GD, Bateman KS, Sriurairatana S, Chavadej J, Sritunyalucksana K, Withyachumnarnkul B.2009 *Enterocytozoonhepatopenaei* sp. nov. (Microsporida: Enterocytozoon idae), a parasite of the black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae): Fine structure and phylogenetic relationships. *Journal of Invertebrate Pathology*, 102(1): 21- 29.
- *OIE., 2015. Manual of Diagnostic Tests for Aquatic Animals.*
- Urgent appeal to control spread of the shrimp microsporidian parasite *Enterocytozoon hepatopenaei* (EHP), NACA News Article, Posted on 24/11/2014 at http:/www.enaca.org/modules/news/article.php?article_id=2039.

CAPL of RGCA becomes the first and only NABL accredited Aquaculture Patho lab in the country



NABL

National Accreditation Board for Testing and Calibration Laboratories

Department of Science & Technology, India

CERTIFICATE OF ACCREDITATION

CENTRAL AQUACULTURE PATHOLOGY LABORATORY, RAJIV GANDHI CENTRE FOR AQUACULTURE

has been assessed and accredited in accordance with the standard

ISO/IEC 17025:2005

"General Requirements for the Competence of Testing & Calibration Laboratories"

for its facilities at

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in the discipline of

BIOLOGICAL TESTING

Certificate Number T-2972

Issue Date 24/05/2014

Valid Until 23/05/2016

This certificate remains valid for the Scope of Accreditation as specified in the annexure subject to continued satisfactory compliance to the above standard & the additional requirements of NABL.

Signed for and on behalf of NABL

John Varughese, Convenor

Anil Relia, Director

Prof. K. VijayRaghavan, Chairman

The Central Aquaculture Pathology Laboratory (CAPL) of RGCA at Sirkali has achieved the distinction of becoming the first and only aquaculture pathology laboratory in the country accredited by NABL (National Accreditation Board For Testing & Calibration Laboratories).

The state-of-the-art laboratory was established during September 2011. Since then, the laboratory has been serving the aquaculture industry of the country by providing timely and reliable diagnosis on various diseases encountered during the culture of finfish, shrimp and freshwater prawns.

Strengths of RGCA Central Aquaculture Pathology Laboratory

- Expertise to diagnose new and emerging shrimp diseases
- Capability to diagnose about 18 shrimp pathogens by molecular method
- Expertise in diagnosis of diseases encountered in export oriented aquaculture species
- Expertise in research on new diseases encountered in aquaculture

Amplification room

Automated Nucleic Acid Extraction system

Real time PCR system

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