

White Spot Disease

White spot disease

White spot disease (WSD) is a serious viral disease of farmed shrimp globally. Most crustaceans including all penaeid shrimps (monodon, vannamei, indicus etc.) and crabs can be affected by WSD. All the life stages of shrimp may get infected by this virus.



Circular White spots on the carapace of P. monodon

What is the causative agent of WSD?

White spot disease is caused by a virus called as White Spot Syndrome Virus (WSSV). This is a double stranded DNA virus of the genus *Whispovirus* and family Nimaviridae.



P. vannamei infected with WSSV

White spots on the carapace

What are the symptoms of WSD disease?

Affected shrimp exhibit anorexia, lethargy, reddish discoloration and presence of circular white spots on the carapace and other exoskeletal parts. In Pacific white shrimp, or vannamei shrimp, white spots may not be clearly visible. Mortality of shrimp may start 2-3 days after infection and reach 80-90 per cent within 5-7 days of onset of first mortalities.





How WSD is transmitted?

WSD can be transmitted both horizontally as well as vertically. The vertical transmission of WSD occurs from infected brood stock to postlarvae. Hence, it is always advisable that PCR tested seeds only be stocked in the ponds. The major route of horizontal transmission is through carrier animals or through cannibalism of infected organisms. Many crustaceans such as crabs, squilla, copepods from marine and brackishwater are either hosts or carriers of WSSV. Crawfish and freshwater prawn, scampi can also serve as carrier for WSSV. Nonarthropod crustaceans such as Balanus sp and annelid such as polychaete worms can also act as carriers. These carrier animals are capable to transmit WSSV virus to cultured shrimp. Therefore, it is advisable to prevent the entry of carrier animals to the ponds by water filtration and fencing.



P. monodon infected with WSSV

How to prevent the WSD?

There is no treatment for WSD. Therefore, prevention is the only way to avoid the disease. Following practices can help to avoid the disease

- WSSV can persist in wet soil. Pond preparation should be properly done by removing black soil, drying, applying lime etc. Sufficient time of at least 3 to 4 weeks should be provided between the culture cycles to enable the pond soil dry completely.
- Virus carriers such as wild shrimp, crabs, mysids, copepods and other crustaceans must be avoided in the farm.
- Every drop of intake water must be disinfected with 30 ppm calcium hypochlorite.
- PCR tested WSSV free healthy post-larvae only should be stocked in the ponds.
- Strict biosecurity measures should be put in place by providing reservoir ponds, bird and crab fencing, proper sanitation of men, material and machines.
- Best management practice (BMP) must be practiced to maintain good water quality, proper feed usage and good health of shrimps through regular monitoring.
- Stressful conditions such as low water depth, excessive stocking, poor water quality and high temperature should be avoided.



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Emergency harvest of WSSV infected pond

- Usage of appropriate and proven probiotics and immunostimulants may be helpful.
- Shrimp should be tested for WSSV during the culture period. Live and moribund samples collected in ethyl alcohol can be sent to the laboratories for testing.

What should be done in case of WSSV outbreak?

- During WSSV outbreak, feeding may be reduced to maintain water quality in the pond. Liming may be done to maintain the pH above 7.5.
- To avoid the cross contamination during outbreak, surrounding farmers should avoid water exchange and should not use any equipment (nets, tanks, pumps, boat, etc) from affected farms.
- If the mortality rate is increasing rapidly, emergency harvest should be carried out using cast netting to avoid discharge of infected water into the main water source.
- Remove dead animals and bury them away from the ponds.
- Affected pond water should be disinfected by bleaching powder (50 ppm chlorine for 2-3days), followed by rapid aeration for one day.
- Neighboring farmers should be kept well informed about shrimp disease problems, emergency harvesting and the time and date of water discharge.
- The pond water should be treated in an effluent treatment system (ETS) before discharging to a common water source.



Hepatopancreatic Microsporidiosis (HPM)

What is microsporidiosis and EHP?

Hepatopancreatic microsporidiosis (HPM) is caused by *Enterocytozoon hepatopenaei* (abbreviated as EHP). The microsporidian parasite was reported to affect black tiger shrimp *Penaeus monodon* in Thailand in the year 2009. Since then, EHP is widespread in most of the Southeast Asian countries, including India. It is referred as hepatopancreatic microsporidiosis (HPM) since the parasite is confined to the shrimp hepatopancreas (HP).



The economic losses to aquaculture seem to be substantial, mainly due to retarded growth of shrimp and overall reduction in farm production.

Which shrimp species are affected by EHP?

Black tiger shrimp *Penaeus monodon*, white leg shrimp *P. vannamei* and banana shrimp *P. merguiensis* are known to get affected.

What are the Clinical signs of EHP infection?

EHP does not cause mass mortalities. There are no specific clinical signs for EHP infection, but it is often associated with stunted growth and white feces syndrome. Hence EHP infection may be suspected when unusually retarded growth in the absence of other gross signs are observed. Severe infections by EHP can increase the susceptibility to other bacterial infections due to *Vibrio* spp. in shrimp farms and could manifest in mortality.

How EHP is transmitted?

EHP is an intracellular spore-forming parasite. It replicates within the cytoplasm of the tubular epithelial cells of the hepatopancreas. Transmission of the disease mainly occurs by oral route. Polychaete worms can transmit EHP in shrimp hatcheries. Shrimp may also get infected by consuming feed contaminated with faeces and through cannibalism of infected shrimp or even by consuming spores present in the pond water and sediment. Recently it is reported that EHP can be transmitted from broodstock to larvae (vertical transmission)





How to Diagnose EHP?

The EHP can be diagnosed by demonstration of spores in fecal sample and in hepatopancreas by microscopic examination. But microscopic demonstration remains successful only in severely affected cases and often undetectable in the early infection. The molecular diagnostic technique like PCR is however faster, easier



Detection of EHP by PCR

Prevention and Control of Hepatopancreatic Microsporidiosis (HPM)

Only EHP free seed should be stocked in the ponds. Once spores are in ponds it is very difficult to eradicate the disease. Hence farmers should adhere to strict biosecurity protocols and adopt better management practices (BMPs). Pond preparation should be carried out properly by drying and disinfection after every harvest to ensure that the EHP spores along with the carriers are destroyed. Treatment of pond sediments by application of CaO (quick lime) @ 6 ton/ha has been recommended. and far more accurate to detect the EHP in feces, postlarvae and hepatopancreatic tissue. Recently, CIBA has developed a loop mediated isothermal amplification (LAMP) test for EHP diagnosis. The LAMP test is equally sensitive and as effective as PCR and can be performed in field conditions as it does not require costly equipment like PCR. The preferred samples for disease diagnosis are postlarvae, fresh shrimp and faecal samples.



Detection of EHP by LAMP

The use of higher dose of lime is essential as spores will get killed only by raising the pH of soil to 12 or more. It is advised to plow the CaO into the dry pond sediment (10-12 cm) and then moisten the sediment to activate the lime. NACA also suggested >15 ppm KMnO₄ or >40 ppm chlorine to inactivate spores in soil. The pond should be left for one week for drying before filling. Farmers are also advised to stock only PCR tested seeds in ponds with good plankton/ bloom. In hatcheries, live feed such as polychaetes must be tested by PCR to ensure absence of EHP. Broodstock must also be ensured to be free from EHP by PCR.



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Acute hepatopancreatic necrosis disease (AHPND)

Acute hepatopancreatic necrosis disease (AHPND) earlier known as early mortality syndrome (EMS) is the most important non-viral disease threat to the shrimp industry. The AHPND is usually characterized by the mass mortality during the first 35 days of culture. The disease is caused by a particular strain of *Vibrio parahaemolyticus* carrying *pirA* gene and *pirB* toxim genes.

Does India have threat of AHPND?

The disease was first reported in China (2009). Soon, it created havoc among shrimp farming community in many East-Asian countries like Viet Nam, Malaysia and Thialand. In 2017, the disease has also been reported from Bangladesh. Till date the disease has not been reported from India. However considering its catastrophic nature, a high level surveillance and alert is required by both scientific organisations as well as farmers.

What is the causative agent of AHPND?

The AHPND is caused by a strain of *Vibrio parahaemolyticus* carrying *pirAB* toxin (Photorabdus insect related toxin) on its plasmid. The *pirAB* gene synthesizes Pir-A and Pir-B insecticidal toxins. Both Pir-A and Pir-B toxins are essential for causing AHPND. The recent reports suggest that few strains of other closely related bacterial species such as *V. campbellii* and *V. owenssii* also carry *pirAB* toxin plasmid and may cause AHPND.

Which species of shrimp are affected

Both, black tiger shrimp (*P. monodon*) and American white leg shrimp (*P. vannamei*) are susceptible for AHPND infection.

What are the symptoms of AHPND/EMS?

- Unusually high mortality occurs within about first 35 days of shrimp grow-out culture
- Moribund shrimp sink to bottom of the ponds
- Affected shrimp often have soft shells and partially full or empty gut
- Hepatopancreas (HP) often appears pale to whitish due to loss of pigment.
- The HP of shrimp is significantly shrunken, small or discoloured
- The HP does not squash easily between thumb and finger
- Sometimes black spots or streaks within the HP may be visible.



TCBS plates showing bacterial growth





AHPND affected shrimp and histopathology of hepatopancreas, Courtesy : Loc Tran

Diagnosis of AHPND

Apart from clinical signs, the disease is diagnosed by histopathological examination of hepatopancreas which reveals atrophy, discoloration and growth of bacteria in the hepatopancreas. The confirmatory diagnosis is done by PCR using recently developed AP4 primer methods which target Pir-A and Pir-B toxin gene.

How to Prevent AHPND/EMS?

- Follow strict principles of pond preparation (drying, spraying lime, plowing, etc.). This will help to kill all the bacterial and viral pathogens from previous culture.
- Follow strict biosecurity measures. Use reservoir ponds, bird fencing etc. avoid use of water from common water body
- Test the larvae for AHPND/EMS by PCR before stocking ponds
- Stock larger sized shrimps in the culture ponds after nursery rearing of post larvae
- Avoid high stocking density.
- Monitor the ponds regularly, particularly during the early days after stocking
- Provide optimal quantity of feed, avoid excessive feeding
- Use of probiotic bacteria containing *Bacillus* and *Lactobacillus* during pond preparation and culture period may be helpful.
- Using biofloc technology in shrimp culture appears to be useful in preventing AHPND/EMS outbreak





Dried Pond bottom



Biofloc

- Co-culture of tilapia and shrimp or culture with tilapia induced green water would help reduce incidence of this bacterial disease
- Closed re-circulatory systems or zero water exchange practice will help in avoiding contamination

Farmers may consult CIBA to confirm any new disease

Farmers may contact CIBA when they come across symptoms similar to AHPND in grow-out ponds for detailed investigation and confirmation. Samples of affected shrimp showing signs of disease only suitably preserved would be useful for investigation. Dead and frozen samples cannot be processed. Since AHPND is not reported in India so far, it is necessary that EMS like cases require to be investigated thoroughly. On confirmation as positive AHPND, the pond water should be disinfected by chlorination within the pond. The treated water should only be discharged after proper disinfection and deactivation of the disinfectant.

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Infectious Myonecrosis (IMN)

What is Infectious myonecrosis?

Infectious myonecrosis (IMN) is an emerging viral disease in shrimp aquaculture industry. caused by Infectious myonecrosis virus (IMNV). The disease was first recorded in Pacific white shrimp, *Penaeus vannamei* in Brazil in 2002 and then in 2006 in Indonesia including Java island. The disease causes significant economic losses to aquaculture due to associated mortalities in *P. vannamei* in grow out ponds. The estimated loss caused by IMNV infection exceeded \$100 million from 2002 to 2006 in Brazil and \$1 million by 2010 in Indonesia. Recently, occurrence of IMN in *P. vannamei* has been recorded in India in some shrimp farms.

What is the causative agent of IMN?

Infectious myonecrosis (IMN) is caused by a virus called infectious myonecrosis virus (IMNV). IMNV is a double stranded RNA virus and closely related to the family Totiviridae.

What are the symptoms of IMN?

Affected shrimp become sluggish, show disoriented swimming behaviour on the surface of water, with abrupt drop in feeding rate. Whitish and reddish necrotic areas can be seen in the distal abdominal segments and tail fan and shrimp may show cooked appearance. FCR of affected populations may increase. Mortalities can be instantaneously high and continue for several days. Generally mortalities range from 40 to 70% in cultivated *P. vannamei*. Clinical signs may suddenly appear following stressful events such as sudden changes in temperature or salinity. Sometimes disease may progress to a chronic phase with persistent low-level mortalities.



White necrotic areas in the distal abdominal segments of diseased shrimp



Extensive whitish necrosis appearing like cooked shrimp with reddish distal segments and tail fans.





RT –PCR Screening of shrimp samples for IMNV



A Coagulative necrosis of muscle fibres, B Lymphoid organ spheroids Courtesy : Agus Sunarto

How IMN is diagnosed?

IMN is diagnosed using a nested RT-PCR protocol. IMN can be confirmed by histopathology. The principal target tissues for IMNV include the striated muscles, connective tissues, haemocytes, and the lymphoid organ parenchymal cells. Characteristic lesions of IMNV are myonecrosis with coagulative necrosis of striated (skeletal) muscle fibres often with marked oedema among affected muscle fibres and significant hypertrophy of the (LO) caused by accumulations of lymphoid organ spheroids (LOS).

How IMN is Transmitted?

IMN is horizontally transmitted through cannibalism. Vertical transmission especially from female broodstock to progeny is also likely to occur. *Artemia franciscana,* bivalves and polychaete worms may act as vectors or carriers for IMNV.

How IMN can be prevented /controlled?

Being a viral disease, there is no treatment for IMNV. Prevention is the only way to circumvent the disease.

Following practices help to avoid the disease

■ Use of IMNV-free brood stock is an effective prevention measure to minimize IMNV propagation in *P. vannamei* farming. Stock post larvae (PL) of at least PL15 stage. Select healthy PL using stress tests and make sure that the PL are negative for the IMN virus by RT-PCR.

- Tilling and restocking of affected farms with IMNV-free stocks of *P. vannamei* help in preventing its recurrence.
- Adopt strict biosecurity measures by providing reservoir ponds, bird and crab fencing, proper sanitation of men, material and machines.
- Implement best management practices (BMP) to maintain good water quality, proper feed usages and good health of shrimps through regular monitoring.

Farmers may consult CIBA to confirm any new diseases

IMNV was detected in farming areas in AP and TN during the years 2017 and 2018 while disease investigations were carried out by ICAR-CIBA. Farmers may contact CIBA when they come across symptoms similar to IMNV in rearing ponds for detailed investigation and confirmation. Samples of affected shrimp showing signs of disease must be suitably preserved for investigation. Dead shrimp samples cannot be processed. Live and moribund samples collected in RNA later can be sent for IMNV testing. It is necessary that IMNV like new diseases require to be investigated in depth with intensive surveillance. On confirmation of a positive IMNV case, the pond water should be disinfected by chlorination within the pond. The treated water should only be discharged after proper deactivation of the disinfectant.



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Management of white feces syndrome (WFS) in *P. vannamei* farms

What is White feces Syndrome (WFS)?

White feces syndrome (WFS) has recently been recognized as a serious concern for shrimp aquaculture in major shrimp farming nations. The syndrome is named as WFS, since the affected farmed shrimps excrete white faecal strings. According to the study carried out at ICAR-CIBA, the WFS has been found to be significantly associated with the microsporidian *Enterocytozoon hepatopenaei* (EHP) in *P. vannamei* grow-out farms. It has been reported that the Thai production loss due to WFS was estimated to be about 10–15% in 2010. In India since 2015, 17% of shrimp farms in the east coast were affected with WFS. The disease can cause moderate to severe economic loss due to reduced productivity compared to the normal ponds.



What are the Clinical signs of WFS?

In affected shrimp farms, WFS is evident with whitish faecal strings floating on the pond surface. White faecal threads could also be found in the feeding trays. Shrimp affected with WFS excrete white faecal strings and show white/golden brown intestine. Feed consumption in the affected ponds is significantly reduced.



WFS usually become evident after 30-40 days of culture. Ponds affected with WFS show white faecal strings floating on the pond surface for a period of 10 days to 45 days or more, elevated FCR, growth reduction, size variation, loose shell and daily mortalities. Loose shell affected shrimps are less active and found sluggishly swimming at the surface of pond water.



How to Diagnose WFS ?

Apart from clinical signs, the disease is diagnosed by squash preparation and histopathological examination of hepatopancreas. Squash preparation of WFS affected hepatopancreas reveals non-motile vermiform bodies/ ATM structures (aggregated transformed microvilli structures) in the lumen of the tubules of the hepatopancreas (HP). Smears of affected HP stained with Eosin clearly show ATM structures inside HP tubule lumen. Histological sections of affected HP stained with hematoxylin and eosin (H & E) reveal the cross section of ATM structures in the HP tubule lumen and dilated HP tubules, sloughed HP epithelial cells, accompanied by severe necrosis.



What is the causative agent of WFS?

The occurrence of WFS has been reported to be associated with gregarine worms, ATM structures, vibriosis, *Enterocytozoon hepatopenaei*, bacteria such as *Candidatus, Bacilloplasma* and *Phascolarcto*, blue green algae and fungi. It was later found that Gregarines worms had no role in WFS. No vibrio spp was found to be predominant among the WFS affected shrimp according to the metagenomic studies conducted at CIBA. One study reported that WFS was due to aggregated transformed sloughed of (ATM) microvilli structures in HP tubule. EHP is significantly found to be associated with WFS affected shrimps according to investigations at ICAR-CIBA.





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Management of WFS Occurrence of WFS can be reduced by good management practices and proactive biosecurity measures in the farm. During the WFS affected period, feed can be reduced in shrimp ponds. White faecal threads containing dense EHP spores should be removed from the affected ponds on daily basis. Since shrimp hepatopancreas show regeneration ability, medications/additives (e.g taurine) enhancing the epithelial cell proliferation may be used. Since EHP is highly prevalent in WFS affected shrimps, effective measures recommended against EHP may be followed. Pond preparation should be carried out as per best management practices (BMPs) by drying and disinfection after every harvest to ensure that the EHP spores along with the carriers are destroyed. Treatment of pond sediments by application of CaO (quick lime) @ 6 tons per ha has been recommended. The use of higher dose of lime is essential as spores will get killed only by raising the pH of soil 12 or more. It is advised to plough the CaO into the dry pond sediment (10-12 cm) and then moisten the sediment to activate the lime. NACA also suggested >15 ppm $KMnO_4$ or >40 ppm chlorine to inactivate spores in soil. Then pond should be left for one week for drying before filling. Farmers are also advised to stock only PCR tested EHPfree seeds in ponds with good plankton/bloom. In hatcheries, all live feed must be tested by PCR to ensure absence of EHP.



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Soil and water quality management for shrimp farming

The success of aquaculture can be assured by selecting a suitable site with good quality soil and water. It is essential to understand the pond soil and water characteristics and their optimum requirements to increase the productivity of the ponds.

Soil requirements

The soils with moderately heavy texture (sandy clay, sandy clay loam and clay loam), electrical conductivity value of 4 dS m⁻¹ or more, pH ranging between 6.5 and 7.5, organic carbon content of 1.5 to 2% and calcium carbonate content of more than 5% are the best suited for shrimp aquaculture.

Water requirements

Water quality and quantity determine the success or failure of an aquaculture operation. An annual water budget should be calculated for a potential farm site so that the supply is adequate for existing and future needs. Water should be free from pesticides and heavy metals. Maintenance of good water quality is essential for both survival and optimum growth of animals. Water treatment is an important step for the maintenance of good water quality at later stage.



Pond preparation

Pond drying: Following the harvest of a crop, the deposits of organic debris in the pond bottom should be removed, or treated, ploughed, tilled and levelled. All parts of ponds should be thoroughly sun dried for at least three weeks for microbial decomposition of soil organic matter and mineralization of organic nutrients.

Investigations on duration of drying period revealed that farms practicing 3, 5, and 10 days of drying were affected with white spot, running mortality syndrome (RMS) and white gut diseases and had to be prematurely harvested within 60-70 days of stocking whereas the farms that adopted a drying period of 30 to 45 days had successful harvest. Drying pond sediment for at least three to four weeks can help in prevention of WSD.

After emergency harvest due to white spot disease (WSD): Do not discharge water from WSD affected ponds. Remove aeration devices and implements and disinfect by evenly distributing calcium hypochlorite



to provide a minimum final free chlorine concentration of 10 ppm. Allow the system to stand for a minimum of 24 to 48 hours at this minimal chlorine concentration.

CIBA's work on the duration of viability of WSSV in WSD affected pond sediments has revealed that the virus remained viable and infective up to 19 days in the sediment despite sun-drying under experimentally simulated pond drying conditions, and up to 26 days post emergency harvest under actual field conditions.

Lime application has to be done based on pH of the soil and the type of lime material available. The lime requirement depends on the percent effective calcium carbonate (PECC) value, determined by multiplying the neutralisation efficiency (calcium carbonate equivalent



value) and fineness of lime material. Based on PECC, the quantity of agricultural lime, dolomite and quick lime required to raise the pH from 6 - 6.5 to 7 varied from 5.5 to 2.8, 5.7 to 2.8 and 4.6 to 2.3 tons/ha, respectively. In soils with chronically low pH it may be beneficial to apply half the total dosage before slight tilling in order to neutralize the underlying soil layers.

Water source: Filter water first through coarse screens to remove larger aquatic animals and debris and then pump into a supply/settling canal for allowing the suspended particles in the water to settle. Then, pass the water through a series of progressively finer screens (150–250 μ m mesh size) before introducing into the reservoir.

Chlorinate water in the reservoir with sufficient chlorine (10 ppm) to kill any potential vectors or carriers in the source water. 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 10 ppm. However, it is advised to calculate the actual dose based on the chlorine demand of water and actual chlorine content in bleaching powder. Vigorously aerate reservoir at least 48 h for de-chlorination to remove residual chlorine.



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Soil and water management

- In order to understand the condition of the pond bottom, soil pH, organic matter and redox-potential (E_h) for oxidized/reduced pond bottom condition have to be monitored regularly. The E_h of pond sediment should not exceed -200 mV.
- The water parameters that should be monitored routinely in ponds during culture period are temperature, pH, salinity, dissolved oxygen and transparency.
- The pH should be in optimum level of 7.5 to 8.5 and should not vary more than 0.5 in a day.
- Variations in salinity not exceeding 5 ppt in a day will help in reducing stress on the shrimp.
- The optimum range of transparency measured using secchi-disc is 25-35 cm.
- Total Ammonia Nitrogen (TAN) and nitrite N concentration should not be more than 1 and 0.5 ppm, respectively.
- Any detectable concentration of hydrogen sulphide is considered undesirable.
- Periodical exchange of chlorine treated water from reservoir as and when required will help in maintaining the water quality in optimal range. The use of aerators results in mixing of water at surface and bottom and breakdowns the DO and thermal stratification.
- Use of inputs without proven efficiency should be strictly avoided.
- The discharge water from the shrimp ponds has to be allowed into a treatment system pond before letting it into the environment so that the suspended solids may settle at the bottom.



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Responsible use of farm inputs in brackishwater aquaculture

Brackishwater aquaculture in India has been rapidly expanding, contributing significantly to the National economic development. For enhanced production and income, aquaculture is being continuously intensified and diversified. These initiatives have been contributing to increased incidences of disease. Diseases in aquaculture can be due to infectious agents, or poor farm management related environmental causes. One should note that viral diseases such as white spot disease (WSD) and parasitic diseases like hepatopancreatic microsporidiosis (HPM) will not respond to treatment with any antibiotic or chemical interventions. No definite infectious agents have been identified as causative agent of stunted growth, white faeces syndrome, running mortality syndrome or white muscle syndrome.

Considering these on farm health and productivity issues, use of medicines/drugs/chemicals without scientific knowledge would most likely aggravate disease conditions and drastically affect farm produce. It must be also noted that the efficacy of drugs and chemicals drastically reduces in saline waters and many of these substances persist in pond sediments for long periods of time. Indiscriminate use of antibiotics leads to development of antibiotic resistance among the microflora. The ecosystem harbouring a number of bacteria of public health importance and human infections with these bacteria would become untreatable and pose threat to public health. Further, use of antimicrobials also results in residue issues in aquaculture produce, which would be viewed seriously by the importing countries, affecting



foreign exchange revenues the to country. Alternatives to the use of antimicrobial agents include good husbandry, adequate feed composition and use of probiotics, biocontrol agents and disinfectants and movement restrictions.

General principles to be followed while using inputs in shrimp aquaculture operations

- Major disease issues in aquaculture can be prevented by following best management practices (BMPs). Medicines/drugs/chemicals should only be applied with expert advice.
- Providing good environment by water exchange generally resolve problems in the aquaculture ponds except for infectious diseases caused by viral agents or HPM.
- Identify cause of the problem before initiating any treatment and all information such as the nature of the disease and the range of therapeutic options available should be considered.



- Bio-control agents, probiotics, immunostimulants, vaccines and disinfectants are the best alternatives to anti-microbial substances.
- Use Medicines/drugs/chemicals for targeted treatment only after proper diagnosis.
- Government approved medicines/drugs/chemicals are only be used if necessary. It must be noted that no antibiotics are approved for use in aquaculture in India.
- Qualified technician should supervise application of medicines/drugs/chemicals.
- Strictly follow instructions on dose and schedule of drug application given by the manufacturer and the technician.
- During the disease conditions there is a possibility of reduced feed consumption, hence the dose should be calculated accordingly to avoid under dosing.
- Quantity of drug should be calculated based on the biomass and feeding rate.
- Administer medicines/drugs targeting the animals orally as feed top dressing or as immersion treatment.
 While preparing medicated feed by top dressing, homogeneity of the drug should be ensured.
- Good quality binder should be used for feed top dressing to avoid leaching of drug into water.
- Since affected shrimp/fish are not active and lose appetite, use of feed attractants in binder is advisable.
- All the drugs/medicines and the medicated feed should be stored in clean and dry place as suggested by the manufacturer with restricted access.
- The products should never be used after the expiry date under any circumstances.
- All interventions should be recorded, so that alternative options can be explored in cases of failure, if any.



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Responsibility of aquafarm technicians

- Aquafarm technicians should have overall understanding of the aquaculture system so that suitable management strategies are followed to avoid the occurrence of disease and must always avoid use of chemicals and drugs.
- Providing good environment in the pond by water exchange should be the first step in remediating any problem in the farm.
- Should ascertain on farm clinical and environmental conditions and resort to treatment only if necessary.
- Before initiating any treatment, cause of the problem in the farm should be ascertained by an approved laboratory.
- Technician should be aware of the national recommendations or regulations regarding the indications, dose and schedule of the drug to be prescribed. He should have knowledge of contraindications, pharmacodynamics, pharmacokinetics and effectiveness of each drug. He should be also aware of drugs not permitted for use in different culture systems.
- Decision to use the drug/medicine in aquaculture farm should primarily be based on expert knowledge and judgement.
- Drugs/medicines should be procured from authorized sources based on the prescription of competent aqua health professionals.
- Technician should take all precautions while handling the medicine/drug in the farm.
- During the course of treatment, farm should to be monitored regularly to enable revision of the course of treatment, if necessary.
- Technician should systematically maintain records of all inputs used in the farm. Lack of effectiveness of any drug has to be recorded and brought to the notice of the authorities.



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Redox potential, an indicator of aquaculture pond health

Pond environment is crucial in the outcome of aquaculture operations. As the culture period progresses, the pond bottom condition deteriorates due to accumulation of organic matter residues. This leads to high oxygen consumption and the development of reducing (anaerobic) conditions in the pond bottom. When reduced species are building up, the pond condition is termed "REDUCED". This condition leads to the diffusion of toxic reduced compounds from the bottom soil upward into the water column, high sediment oxygen demand, deterioration of the pond environment and adverse effects on shrimp growth.

What are the indicators of pond bottom condition?

Redox potential also called as oxidation-reduction potential (ORP) is an important parameter to measure the relative degree of oxidation and reduction in aquaculture ponds. ORP in the pond soils decreases towards



reduction (more negative side) with progress of culture



period. Though highly variable, ORP values denoted by E_h and quantified in milli volts (mV) are the best used as an indicator to understand the relative status of the soil.

- Organic carbon (OC) content of soil is another index of pond sediment condition. High OC values imply more organic matter accumulation on pond bottom. The organic matter comprises a large fraction of stable, slowly degradable OC and hence, it is not a sensitive indicator. Conventional soil organic carbon determination procedure includes exposing the soil to air drying and analysis has to be done in the laboratory.
- Concentration of soluble iron and manganese (reduced species) is more sensitive indicator of the redox conditions. The soluble forms of the reduced species of ferrous or manganese ions (2⁺) in the pond bottom soils are to be analysed.
- Though OC and reduced element species content can be used as indicators for pond bottom deterioration, estimation of OC takes more time and hence cannot be used as quick indicator to plan management practices for improvement of pond bottom condition. It is possible to evaluate the intensity of the pond anaerobic conditions by measuring the sediment redox potential. Hence, redox potential is considered as one of the important, instant and better indicators that can be used to understand the deterioration of pond bottom sediment.

How to measure redox potential?

Measurement of redox potential of sediment near sluice gate and away from the aerators at any point of time during culture period gives the early indication of pond bottom deterioration. The following protocols are recommended.

ORP can be measured at soil water interface (SWI) near sluice gate and away from the aerators by portable multi parameter analyser with ORP probe.

If probes are not available, the sediment sample at 10-cm depth is to be collected in a polythene bag under air tight condition near sluice gate and away from the aerators. Once the sample is brought out of the pond, immediately ORP has to be measured under air tight condition by using a portable/ bench top redox meter.

In order to minimise the errors of ORP variability, minimum of three sampling places have to be fixed near sluice gate and repeated measurements are to be taken at each sampling place (SWI or 10-cm depth soil in polythene bag) and the average value can be taken as final value.

In intensive shrimp culture ponds, the accumulated black sludge in the pond bottom leads to reducing conditions and negative redox values. CIBA studies have revealed that ORP value of -200 mV or more is not desirable during the culture period as this negative redox potential reduces dissolved oxygen levels, increases metabolites (ammonia and nitrite) concentration and also generates reduced compounds like sulphide, methane etc. This problem can be avoided by following better management practices such as optimum stocking density, providing proper aeration uniformly throughout the pond by placing aerators at right place, chain dragging along the pond, central drainage system and disposal of sludge through heavy duty pumps.





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