Central Institute of Brackishwater Aquaculture
(Indian Council of Agricultural Research)
75, Santhome High Road, RA Puram, Chennai-28. India

CIBA e-Publication Series No.25

Ensuring WSSV Free Sediment and Water for Prevention of White Spot Disease

The white spot syndrome virus (WSSV), the causative agent of white spot disease (WSD) of shrimp is an extremely virulent and most important cause of economic loss to the aquaculture sector. WSSV is widespread in shrimp aquaculture ecosystems globally and is transmitted vertically from infected broodstock to larvae and horizontally by ingestion of infected organisms. While WSD transmission vertically is being prevented by screening out infected broodstock, its horizontal transmission in grow out farms is a serious challenge. At present there is no treatment available to prevent the unrestrained occurrence and spread of the disease. Better management practices (BMPs) have helped alleviate this problem to a great extent, by minimising risks of its transmission through carrier organisms such as mud crabs, *Artemia*, rotifer eggs, molluscs, polychaete worms, insect larvae and seabirds etc. However, concerns of WSD transmission through contaminated water and pond sediment remain unaddressed. Pond preparation practices have proved to be useful in eliminating the virus from the pond and reducing the risk of disease outbreaks. This advisory is an outcome of research carried out at CIBA and an improvement over the existing information with regard to pond preparation protocols for prevention of mortality of shrimp due to WSD in freshly stocked shrimp ponds.

Research carried out at CIBA has indicated that white spot disease can be transmitted by WSSV contaminated inadequately dried pond sediment, resulting in mass mortality of shrimp. The WSD affected ponds can leave high WSSV load in sediment (of the order of 10^6 g^{-1} WSSV particles) and water (of the order of 10^2 ml^{-1} WSSV particles). Although WSSV cannot replicate outside its hosts and carrier organisms, it appears to retain infectivity for quite some time. CIBA's work on the duration of viability of WSSV in pond sediments has revealed that under experimentally simulated drainable pond conditions, the virus remains viable and infective up to 19 days in the sediment despite sun-drying, as indicated by shrimp infectivity experiments. Under actual field conditions, in the pond sediments, WSSV was found to be infective up to 26 days post emergency harvest due to WSD. Under experimental conditions with 10^3 WSSV

Ref: Satheesh Kumar *et al.* (2013). Aquaculture 402-403: 119-126.
This information was generated from a research project funded by the Department of Biotechnology, Govt of India.

particles ml^{-1}, it has been found that WSSV was infective to juvenile shrimp for 12 days in the absence of any carrier organisms. Hence testing pond sediment and reservoir water for WSSV by PCR and understanding its presence/ absence status prior to stocking ponds can help in deciding on starting fresh culture and thereby preventing WSD in freshly stocked ponds.

As part of the BMPs during pond preparation for shrimp culture, pond sediment testing (for pH and organic matter content), leaching (for acidic sediments), tilling, pond drying, control of undesirable fauna (such as finfish, crustaceans, molluscs, amphibians, reptiles, etc.) using physical and chemical methods, liming, fertilisation etc. have been in vogue. Despite these protocols, WSD outbreaks do occur during early days of stocking as often reported recently by shrimp farmers. Such WSD outbreaks are possibly due to inadequate pond preparation, wherein the WSSV continues to remain viable and infective. CIBA's work has indicated that drying pond sediment for at least three weeks can help in prevention of WSD. If the WSD affected ponds after harvest are disinfected, the drying period can be a little less. However, the viability of WSSV in pond sediment depends upon several factors, and the most important factor being moisture content in the soil. The work carried out at CIBA has revealed that the virus can remain infective in sediment with moisture content of 1.3 to 1.5%. Hence, thorough pond drying must be carried out and sediment to be tested for WSSV by PCR as standard operating procedures (SOPs) before commencing new crop. Similarly testing water before being used for aquaculture will also help in prevention of WSD. CIBA has developed methods to concentrate viruses from water and test for any shrimp pathogens using a technique called tangential flow filtration (TFF). Water in the reservoir, after appropriate treatment ensuring removal of undesirable fauna using physical and chemical methods should be tested for WSSV, in order to obtain freedom from WSD in one's farm.

BMPs for prevention of WSD

Pathogen exclusion or biosecurity is the only means of prevention of WSD at present. 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 (2012). Following practice will help in ensuring biosecurity from WSSV.

1. Source water: Ideally farms must have reservoirs of adequate requirement of seawater for operation of aquaculture. Filter source water first through coarse screens to remove larger aquatic animals and debris and then pump into a supply/settling canal. Then, pass

Ref: Satheesh Kumar *et al*. (2013). Aquaculture 402-403: 119-126.
This information was generated from a research project funded by the Department of Biotechnology, Govt of India.

the water 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. Chlorinate water in the reservoir with sufficient 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. Vigorously aerate reservoir at least 48 h for de-chlorination 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 hypochloride 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.

Once the pond is ready to start fresh culture after having completed pond preparation following the above BMPs, pond sediment may be tested for WSSV by PCR to ascertain biosecurity from WSSV. Following are some useful aspects on pond sediment PCR testing.

How to collect pond sediment for WSSV testing?

Collect about half-a-kilo of sub surface sediment sample after scraping off about top 4-6 inches of sediment from four to five points especially in the wet patches within the pond

Ref: Satheesh Kumar *et al.* (2013). Aquaculture 402-403: 119-126.
This information was generated from a research project funded by the Department of Biotechnology, Govt of India.

including the area near the sluice gate. Pool all the samples, mix thoroughly and send about half-a kg of sediment to the laboratory in zip-lock bag taking care to avoid air within the cover.

Should the sediment from all ponds in the farm be tested for WSSV?

If there was an incidence of WSD during the previous crop, it is advisable to carry out this test on all affected ponds. Testing sediment from an unaffected pond in the same farm located far from an affected pond may not be necessary. However, ponds adjacent to the WSD affected ones should be tested since possibilities of contamination of adjacent ponds are quite high.

Where to get the sediment tested for WSSV?

Pond sediment may be tested for WSSV at CIBA or a reputed laboratory that has capacity to carry out this test. Nucleic acid extraction from sediment is crucial because of PCR inhibitors.

How much time is required for testing sediment for WSSV?

For testing presence of WSSV in shrimp pond sediment, 7-8 hours would be required.

Based on the test results how to decide on starting new crop?

If the sediment is first step PCR positive for WSSV (OIE 2012), do not stock ponds. Disinfect the pond and dry the pond thoroughly and get the testing done again.

If the sediment is nested PCR positive for WSSV, farmers may stock the pond after about 4-5 days, since the viral load is likely to reduce further to non-infective levels.

S.V. Alavandi, M. Muralidhar, P. Ravichandran and A.G. Ponniah

Central Institute of Brackishwater Aquaculture (ICAR),
75 Santhome High Road, R.A.Puram, Chennai 600 028. India
Email:director@ciba.res.in

March, 2014

Ref: Satheesh Kumar *et al.* (2013). Aquaculture 402-403: 119-126.
This information was generated from a research project funded by the Department of Biotechnology, Govt of India.