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Viability of white spot syndrome virus (WSSV) in sediment during sun-drying (drainable pond) and under non-drainable pond conditions indicated by infectivity to shrimp

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ABSTRACT

White spot syndrome virus (WSSV), is a highly virulent rapidly replicating large, enveloped, double-stranded DNA (dsDNA) virus, causing an estimated losses of over US$6 billion since its emergence in 1992. As part of the better management practices (BMPs), ploughing, tilling and sun-drying of shrimp culture ponds are advocated for prevention and control of this disease. Despite this, instances of outbreaks of white spot disease (WSD) recur, causing loss to shrimp farmers. Some studies have indicated that WSSV DNA is reported to persist for over 20 months in sediments as detected by PCR. Since mere detection of virus using PCR methods does not indicate its viable nature and ability to infect, information on its viability outside the host in water and pond sediment is necessary. Hence, in this study, the viability of WSSV in seawater and shrimp pond sediments under experimentally simulated drainable and non-drainable pond conditions was examined by shrimp infectivity experiments. WSSV with an initial viral load of 1000 virions mL^{-1} was found to be viable for a period of 12 days in seawater of 27 ppt salinity, pH of 7.5 at 29–33 °C as revealed by its ability to infect juvenile shrimp, whereas, in shrimp pond sediment (with initial viral load of 211,500 copies g^{-1}), the virus was viable and infective up to 19 days despite sun-drying. In the case of non-drainable conditions, WSSV (753,600 copies g^{-1}) remained infective for a period of 35 days. Although the sediment samples tested nested PCR positive after 19 days of sun-drying and 40 days under water-logged conditions, shrimps did not develop WSD, suggesting that WSSV was not viable. Over a period of time after 21 days under sun-drying and 40 days under non-drainable experimental conditions, due to reduction in viral load, sediments were positive only by nested PCR, and by this time, viability of WSSV was almost lost as revealed by shrimp infectivity. Hence, PCR testing of shrimp farm sediment before starting culture as one of the BMPs may help in ensuring biosecurity from WSSV. The information generated here would help in the improvement of better management practices (BMPs) with regard to pond preparation protocols for shrimp aquaculture.

1. Introduction

White spot syndrome virus (WSSV), is a highly virulent rapidly replicating large, enveloped, double-stranded DNA (dsDNA) virus of shrimp, assigned by the ICTV to its own new genus, *Whispovirus*, and family, Nimaviridae (Fauquet et al., 2005) and has been responsible for estimated losses of over US$6 billion to shrimp aquaculture since its emergence in 1992 till date (Lightner et al., 2012). Continued devastation of shrimp aquaculture due to WSSV can be mainly attributed to its ability to infect and replicate in a wide host range of more than 98 species, its high virulence and mode of its transmission (Escobedo-Bonilla et al., 2008). WSSV can also be vertically transmitted from infected shrimp brooders to post larvae (Lo et al., 1997; Lo and Kou, 1998). While biosecurity protocols can take care of prevention of WSSV spread through this route, horizontal WSSV transmission to healthy shrimp through cannibalism, carriers in aquaculture ecosystem such as mud crabs (Kanchanaphum et al., 1998), polychaete worms (Vijayan et al., 2005), rotifers and their resting eggs (Yan et al., 2004), marine molluscs (OIE, 2012), seabirds (Vanpatten et al., 2004), ephydrid insect larvae (Lo et al., 1996), and *Artemia* (Li et al., 2004; Zhang et al., 2010) or via the water route (Esparza-Leal et al., 2009) remain challenges confronting prevention and control of WSD.

Review of the pond-level risk factors revealed that pond preparation practices were useful at eliminating the virus from the pond (Corsin et al., 2005) and understanding of the key aspects of the biology of WSSV infection have helped in improving disease management solutions that are widely used in shrimp aquaculture (Lo et al., 2005). However, there is still enormous requirement for improvements in

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the management practices and these have to be continuously upgraded in order to minimise disease outbreaks and to improve productivity. One critical question in environmental virology is how long viruses persist in the environment and what concentration is required to cause disease in individuals. This knowledge is essential to establish biosecurity protocols to prevent disease outbreaks and its spread. WSSV DNA has been reported to be detected in pond soil samples for five days at 70 °C and even after 10 months of storage (Natividad et al., 2008). It was also reported that WSSV genome could be detected in seawater environments three months after the outbreak in at least 90% of the outbreak pond water samples and in 36.67% and 46.67% in surrounding canal water samples, and even after 20 months of outbreak (Quang et al., 2008). However, these investigations employed PCR to detect WSSV, and detection of viral DNA would not indicate its viability and ability to infect. WSSV is reported to be infective for at least 40 days at 30 °C in seawater under laboratory conditions (Momoyama et al., 1998) and viable for at least 3–4 days in ponds (Nakano et al., 1998).

While most of the semi-intensive and improved extensive are drainable, in some parts of India such as West Bengal and Kerala, and also in several Southeast Asian countries, traditional extensive shrimp farming practices are in vogue, where water management is effected through tidal means and, such ponds are either non-drainable or drainable only at low tide. Sun-drying of ponds will not be possible in such places. Keeping in view of these conditions, the objective of this work was to understand the duration of viability of WSSV outside the host in shrimp pond water and sediment during sun-drying and under water-logged condition, and this was examined by carrying out infectivity studies of live healthy juvenile shrimp under experimental conditions.

2. Materials and methods

2.1. WSSV viability in seawater

2.1.1. WSSV stock and quantification

WSSV infected shrimp (confirmed by OIE (2012) WSSV PCR protocols) of about 19–28 g size were obtained in dry ice from a tiger shrimp farm in Bhimavaram located in Andhra Pradesh, India. WSSV was extracted by homogenization of pooled 10 g WSSV infected shrimp tissue (gills and pleopods) in 100 mL TN buffer (20 mM Tris–HCl, 400 mM NaCl, pH 7.4) followed by centrifugation at 5000 *g* for 10 min at 4 °C (repeated twice) and the supernatant was filtered through 400 μm pore size nylon net. This filtrate was subsequently filtered using 0.45 μm syringe filter and used as viral preparation (Xie et al., 2005). Viral DNA was extracted (Xie et al., 2005) and the WSSV copy number of purified viral suspension was estimated using WSSV detection and quantitative real time PCR kit (LabIndia Life Sciences, Gurgaon, India) as per manufacturer's instructions using Applied Biosystems StepOne™ Real-Time PCR system (California, USA). The kit is based on 5′ nuclease assay using TaqMan probes designed for multiplexed amplification of WSSV and decapod β-actin labelled with a FAM and VIC respectively and TAMRA as the quencher dye.

2.1.2. WSSV viability testing in seawater by shrimp infectivity

The duration of WSSV viability was studied in virus-free sterile seawater obtained by microfiltration by tangential flow filtration (TFF) using CFP-2-E-9A (0.2 μm pore size) hollow fibre cartridge, followed by ultrafiltration by use of UFP-100-C-9A (100 KDa) cartridge (Quixstand™ Benchtop system, GE Health Care Bio Sciences Crop, USA). Such sterile seawater was distributed in a series of buckets. Each of the buckets with 10 L sterile seawater was spiked with purified quantified WSSV to a final count of 1000 virus particles mL^{-1} of seawater, covered with 400 μm pore size nylon net and maintained in the wet lab (open to sky) at ambient temperatures ranging from 29 and 33 °C. The pH of seawater was 7.5 and salinity was 27 ppt during the experiment. The viability of WSSV was examined by carrying out periodic infectivity studies in shrimp for a period of up to 18 days. Ten WSSV free juvenile shrimp (*Penaeus monodon*) (batch of shrimp confirmed negative for WSSV by nested PCR using OIE (2012) protocols) weighing 1.9 to 2.2 g were introduced into each bucket on even days, i.e., 0, 2, 4,..., up to 18 days. Formulated pellet feed (CIBA shrimp grow-out formulation) was provided @ 5% biomass in three portions. Shrimp were examined every 8 h for symptoms of white spot disease and mortality, shrimp mortality was plotted using GraphPad Prism Software (version 5.00 for Windows, San Diego California USA), and moribund and dead shrimp in experimental containers were removed and preserved in −70 °C freezer and tested for WSSV by PCR (OIE, 2012).

2.2. WSSV viability in shrimp pond sediment (simulated drainable pond condition)

The viability of WSSV during different days of sun-drying of shrimp pond sediment was examined using sediment collected from a shrimp farm located at Marakanam, about 140 km south of Chennai, India, soon after emergency harvest following WSD outbreak. The sediment was brought to the laboratory in polythene bags in ice and the DNA was isolated from sediment samples using Power soil DNA isolation kit (MO BIO Laboratories, USA) as per manufacturer's instructions, and confirmed WSSV positive by PCR (OIE, 2012). The initial WSSV load in the sediment was estimated by quantitative real time PCR as described above and the WSSV count was 211,500 g^{-1} of sediment. Two kilograms of this WSSV contaminated sediment (≈3 cm sediment layer thickness) was distributed in a series of buckets of 20 L capacity (23 cm dia, 37 cm height), covered with 400 μm pore size net and kept under sunlight for drying. The atmospheric temperature during sun-drying of the sediment ranged from 29 to 36 °C (daily min–max). A fabricated nylon net (400 μm pore size) of the internal shape and size of the bucket was placed inside the buckets (to facilitate picking up moribund and dead shrimp). 20 L of WSSV free seawater was added slowly on different days of sun-drying, i.e., 0, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 days. Just prior to this, sediment was sampled for PCR test and quantification of WSSV on these specific days of sun drying. The level of water along with sediment was ~16–17 cm. 20 WSSV-free juvenile shrimp, *P. monodon* (2.2–2.7 g) were also introduced on these days along with seawater. During the experiment, the seawater temperature in the experimental containers ranged from 30 to 34 °C (daily min–max), pH 7.7–8.0 and the salinity was 23 ppt. Formulated pellet feed (CIBA shrimp grow-out formulation) was provided @ 5% biomass in three portions. Shrimp were examined every 8 h for signs of white spot disease and mortality (Table 1) and moribund and

Table 1
WSSV persistence and viability in sediment subjected to sun-drying as revealed by shrimp infectivity and confirmation by PCR.

Sun-drying (no. of days)	WSSV count in sediment (no. g^{-1})	PCR detection	
		Sediment	Shrimp
Control	ND	−	−
5	211,500	+	+
7	127,400	+	+
11	39,600	+	+
13	25,400	+	+
15	9820	+	+
17	7980	+	+[a]
19	4420	+	+[a]
21	ND	+[a]	−

ND: not detectable.
[a] Nested PCR positive.

dead shrimp in experimental containers were removed and preserved in −70 °C freezer and tested for WSSV by PCR (OIE, 2012).

2.3. WSSV viability in experimentally simulated non-drainable conditions

The viability of WSSV in the experimentally simulated non-drainable pond conditions was examined using sediment from a shrimp farm located at Kalpakkam, 80 km south of Chennai, India, collected immediately after emergency harvest following an outbreak of WSD. The sediment was brought to the laboratory in polythene bags in ice and used for this experiment. The DNA from sediment was extracted as described earlier, confirmed WSSV positive by PCR (OIE, 2012) and the initial WSSV load in the sediment was estimated by quantitative RT-PCR and the WSSV count was 753,600 g^{-1} of sediment. Two kg of sediment (≈3 cm sediment layer thickness) was distributed in a series of buckets of 20 L capacity (23 cm dia, 37 cm height), 20 L WSSV free seawater was carefully added, covered with 400 μm pore size net and maintained in the wet laboratory. The water level was ~16–17 cm including the sediment layer. To these experimental buckets with WSSV contaminated sediment, 20 WSSV-free shrimp, *P. monodon* (size: 2.3 to 2.8 g) were introduced up to 50 days at every 5-day interval, i.e., 5th, 10th, 15th, up to 50 days. Just prior to introduction of shrimp, sediment was sampled for PCR testing and quantification of WSSV, as described earlier. During the experiment, the seawater temperature in the experimental containers ranged from 30 to 34 °C (daily min–max), pH 7.7–8.0 and the salinity was 23 ppt. Formulated pellet feed (CIBA) was provided @ 5% biomass in three portions. Shrimp were examined for signs of white spot disease and mortality (Table 2) and moribund and dead shrimp in experimental containers were removed and preserved in −70 °C freezer and tested for WSSV by PCR (OIE, 2012).

3. Results

3.1. WSSV viability in seawater

WSSV infection in shrimp introduced in experimental containers on different days was confirmed by PCR. Shrimp introduced in the experimental containers on specific days up to 12 days were found to be negative by first step PCR and detected to be positive only by nested PCR as revealed by amplification of 941 bp product (Fig. 1). WSSV (with initial viral load of 1000 particles mL^{-1}) was found to be viable and infective for up to 12 days and produced disease and complete mortality in three to seven days after introduction of shrimp in experimental containers (Fig. 2). No mortality of shrimp was observed in experimental containers in which shrimp were introduced after 14th day onwards and up to 18 days, suggesting that the virus was not viable thereafter in seawater.

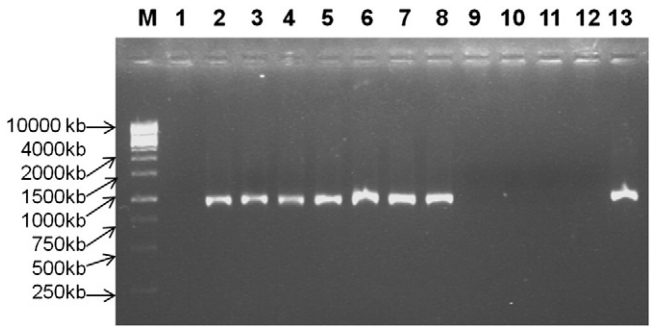

Fig. 1. Viability of WSSV in seawater as revealed by infectivity of shrimp (nested WSSV PCR): Lane M: mol. wt. marker, lane 1: control (shrimp in WSSV free water), lane 2: 0 day, lane 3: 2 days, lane 4: 4 days, lane 5: 6 days, lane 6: 8 days, lane 7: 10 days, lane 8: 12 days, lane 9: 14 days, lane 10: 16 days, lane 11: 18 days, lane 12: negative control, and lane 13: positive control.

3.2. WSSV viability in shrimp pond sediment (simulated drainable pond condition)

WSSV infection was evident in shrimp introduced up to 19 days of sun-drying, as revealed by PCR (Fig. 3). Shrimp introduced in containers up to 15 days of sun-drying of sediment tested positive by first step PCR, while shrimps introduced on 17 days and 19 days of sun-drying tested positive only by nested PCR (Fig. 3 and Table 1). Shrimp developed signs of WSD and complete shrimp mortality occurred in five to seven days (Fig. 4). Shrimp introduced into containers after 21 days of sun drying did not develop WSD as revealed by negative PCR reaction, suggesting that the virus was not viable after 19 days of sun drying. The sediment samples tested first step WSSV positive up to 15 days of sun-drying as revealed by amplification of 1447 bp amplicon and from 17th day onwards, up to 25 days, the sediment samples tested positive only by nested PCR (Fig. 5), indicating reduction in the viral load in the sediment. The WSSV count in the sediment reduced from 211,500 g^{-1}on day 5 to 4420 g^{-1} of sediment on 19 days of sun-drying (Table 1).

3.3. WSSV viability in sediment under simulated non-drainable pond conditions

Under the non-drainable experimental conditions, the viability of WSSV was found to be up to 35 days as revealed by shrimp infectivity study and produced WSD as confirmed by PCR (Fig. 6) and mortality of shrimp. Shrimps introduced up to 25 days of non-drainable set-up were first step PCR positive, while those introduced on 30th and 35th days were tested positive only by nested PCR, and shrimps introduced after 40 days of non-drainable condition were WSSV-PCR negative (Fig. 6). While shrimp mortality was rapid (within five to six days) in experimental containers that were introduced with shrimp on 5th, 10th, 15th and 20th days, the shrimp mortality was slow (12 to 19 days) in containers that were introduced with shrimp after 25 days (Fig. 7), suggesting reduction in viral load (Table 2). The sediment samples tested first step PCR positive up to 35 days and from 40th day onwards, the sediment sample tested positive only by nested PCR indicating reduction in the viral load in the sediment (Fig. 8). Shrimp introduced into containers after 40 days under water logged condition did not develop WSD as revealed by negative PCR reaction, suggesting that the virus was not viable after 35 days. The WSSV count in the sediment reduced from 753,600 on day 5 to 4650 DNA particles g^{-1} of sediment on 30 days under non-drainable conditions (Table 2).

Table 2
WSSV persistence and viability under non-drainable condition as revealed by shrimp infectivity confirmed by PCR.

Shrimp introduced post number of days of non-drainable condition	WSSV count in sediment g^{-1}	WSSV detection by PCR	
		Sediment	Shrimp
Control	ND	−	−
5 days	753,600	+	+
10 days	629,640	+	+
15 days	616,780	+	+
20 days	181,790	+	+
25 days	139,140	+	+
30 days	4650	+	+[a]
35 days	30	+	+[a]
40 days	25	+[a]	−
45 days	7.2	+[a]	−
50 days	13	+[a]	−

ND: not detectable.
[a] Nested PCR positive.

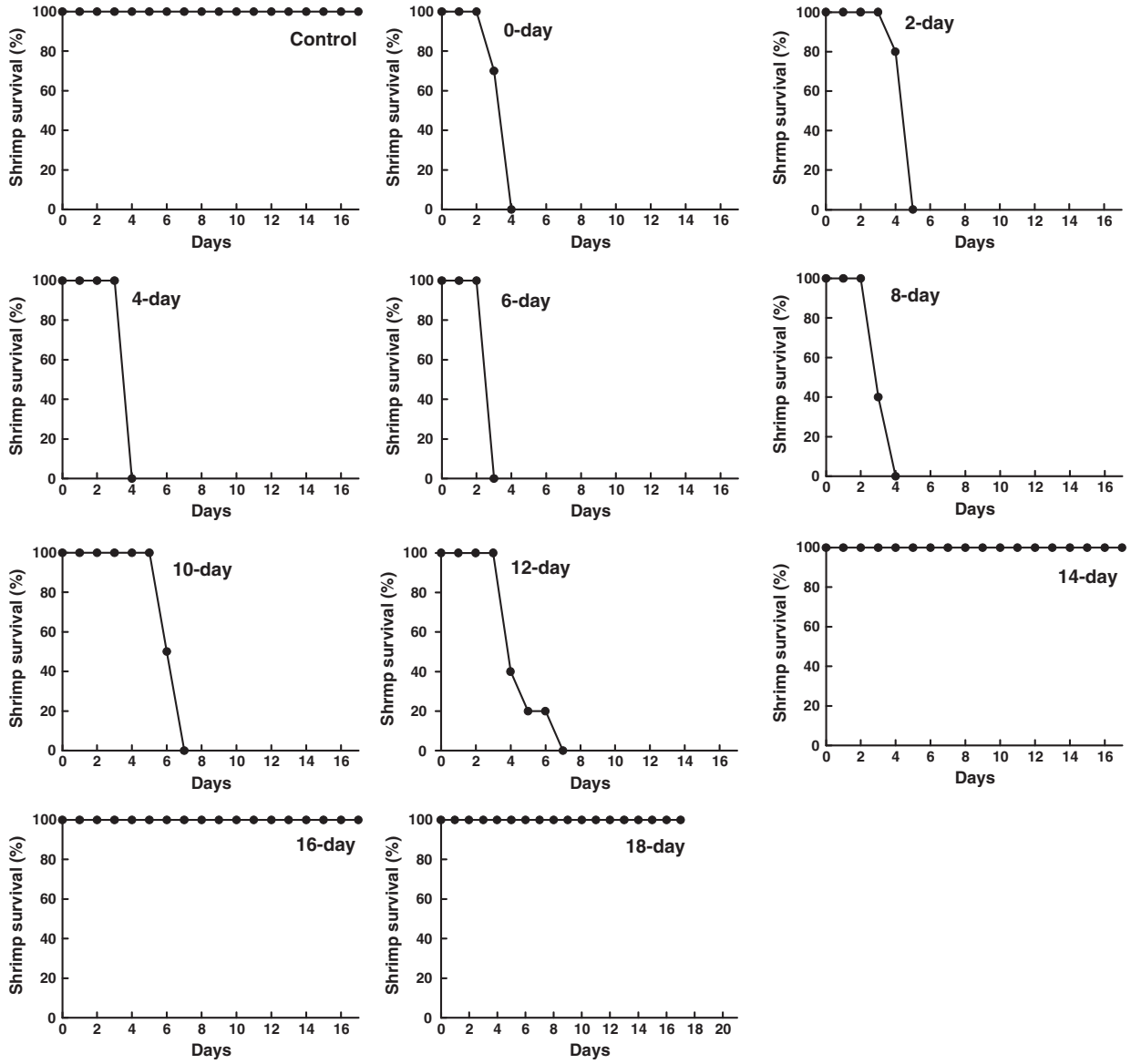

Fig. 2. Shrimp mortality pattern due to WSSV infection and WSD, an indicator of decline in WSSV viability in seawater.

4. Discussion

Despite the fact that WSSV persists in sediments for over 20 months (Natividad et al., 2008; Quang et al., 2008) the information on WSSV viability in shrimp pond sediment and water in terms of its infectivity remain major data gaps. To limit the spread of WSSV and issues concerning biosecurity requirements, the primary uncertainties are the presence, viability and infectivity of viruses in aquaculture ecosystem. In order to establish biosecurity measures aimed at prevention of spread of infection, it is essential to have knowledge on the duration of survival of the virus in sea water, with and without its intermediary hosts. In the present study, the mode of infection was aimed at simulating natural mode of infection of shrimp by WSSV present in the water column and in the pond sediments usually found in

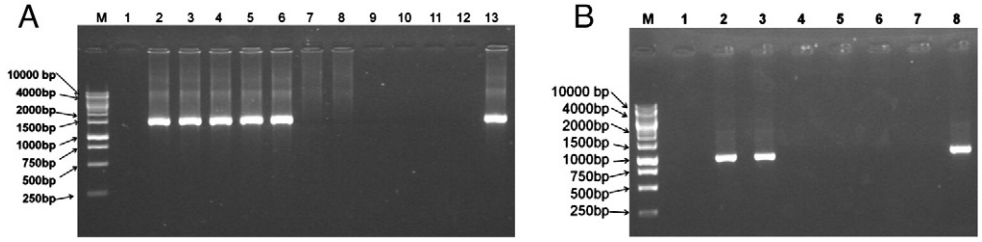

Fig. 3. Viability of WSSV in sediment subjected to sun-drying as indicated by shrimp infectivity experiment confirmed by PCR (*A*: first step; *B*: nested). *A*: Lane M: mol. wt. marker, lane 1: control (WSSV-free sediment), lane 2: 5 days, lane 3: 7 days, lane 4: 11 days, lane 5: 13 days, lane 6: 15 days, lane 7: 17 days, lane 8: 19 days, lane 9: 21 days, lane 10: 23 days, lane 11: 25 days, lane 12: negative control, and lane 13: positive control. *B*: Lane M: mol. wt. marker, lane 1: control, lane 2: 17 days, lane 3: 19 days, lane 4:21 days, lane 5: 23 days, Lane 6: 25 days, Lane 7: negative control and lane 8: positive control.

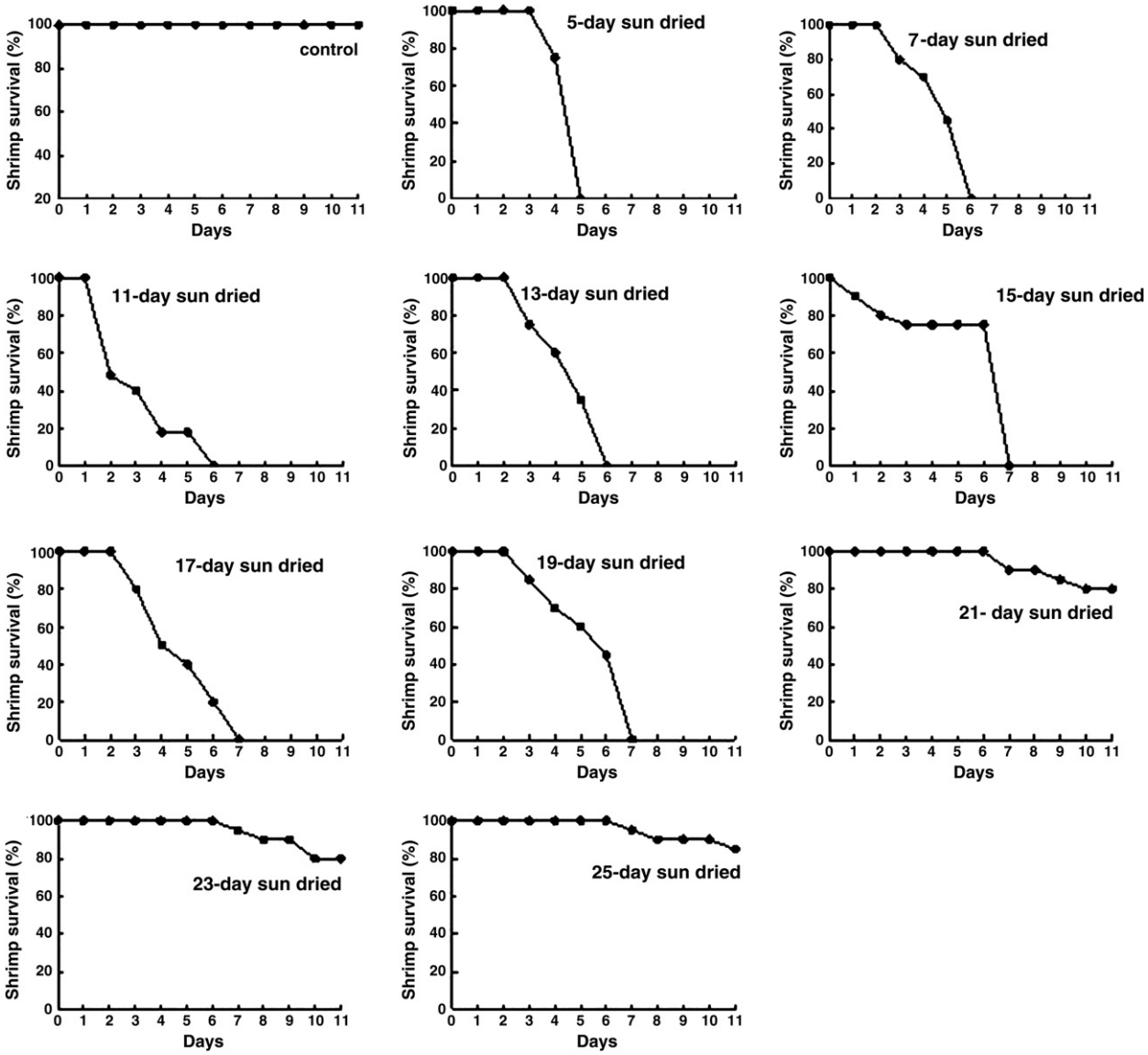

Fig. 4. Shrimp mortality pattern due to WSSV infection and WSD, an indicator of decline in WSSV viability in shrimp pond sediment during various days of sun-drying.

aquaculture facilities, except that no intermediate hosts were present, since it was a closed experimental set-up.

The present studies indicated that WSSV was viable and remained infective in seawater of 27 ppt, pH 7.5 at 30–32 °C (with initial viral load of 1000 virions mL^{-1}) for a period of 12 days to juvenile shrimp of about 1.9 to 2.2 g. In an earlier study, it was reported that the survival period of PRDV (equivalent of WSSV) in sea water reduced with the increase in temperature, and lost infectivity within 40 days at 30 °C, 50 days at 25 °C, 100 days at 20 °C and 130 days at 15 °C respectively (Momoyama et al., 1998). But these authors examined WSSV survival by testing its infectivity through intramuscular injection. They also reported that baculovirus penaei (BP) and baculovirus midgut gland necrosis virus (BMNV) survival in seawater was much less, which lost infectivity in 14 days at 22 °C and 7 days at 25 °C respectively.

In this study it was also observed that the ponds with heavy shrimp mortality due to WSD could leave high WSSV load in the pond sediments (>211,500 copies g^{-1}) which can survive and remain infective

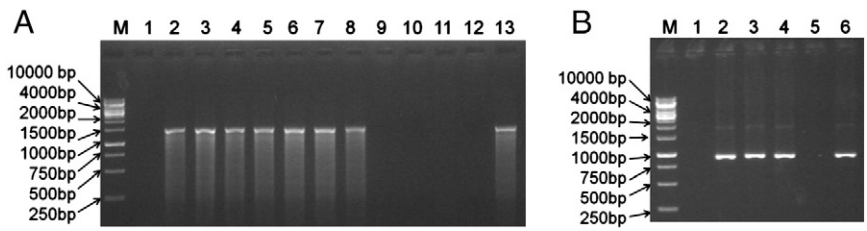

Fig. 5. Detection of WSSV DNA by PCR in sediment samples subjected to sun-drying (*A*: first step; *B*: nested): *A*: Lane M: mol. wt. marker, lane 1: control (WSSV negative sediment), lane 2: 5 days, lane 3: 7 days, lane 4: 11 days, lane 5; 13 Days, lane 6: 15 days, lane 7: 17 days, lane 8: 19 days, lane 9: 21 days, lane 10: 23 days, lane 11: 25 days, lane 12: negative control, and lane 13: positive control. *B*: Lane M: mol. wt. marker, lane 1: control, lane 2: 21 days, lane 3: 23 days, lane 4: 25 days, lane 5: WSSV negative control, lane 6: WSSV positive control.

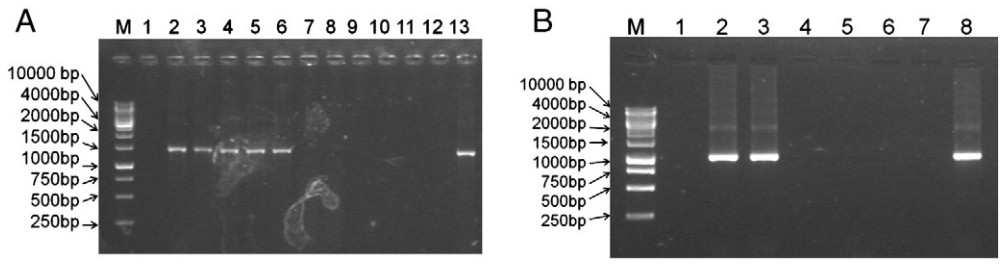

Fig. 6. WSSV viability in non-drainable experimental conditions as revealed by WSSV infection of shrimp, confirmed by PCR (*A*: first step, *B*: nested). *A*: Lane M: mol. wt. marker, lane 1: control, lane 2: 5 days, lane 3: 10 days, lane 4: 15 days, lane 5: 20 days, lane 6: 25 days, lane 7: 30 days, lane 8: 35 days, lane 9: 40 days, lane 10: 45 days, lane 11: 50 days, lane 12: negative control, and lane 13: positive control. *B*: Lane M: mol. wt. marker, lane 1: control, lane 2: 30 days, lane 3: 35 days, lane 4: 40 days, lane 5: 45 days, lane 6: 50 days, lane 7: negative control, and lane 8: positive control.

even up to 19 days despite sun-drying. Studies have shown that viruses are present in polluted estuarine sediment at higher concentration than in the overlying seawater (LaBelle et al., 1980), and that the adsorption to sediment greatly increases virus survival time (Labelle and Gerba, 1980). Hence it is likely that WSSV in shrimp pond sediments would remain viable for longer than in the water column. Since virus association

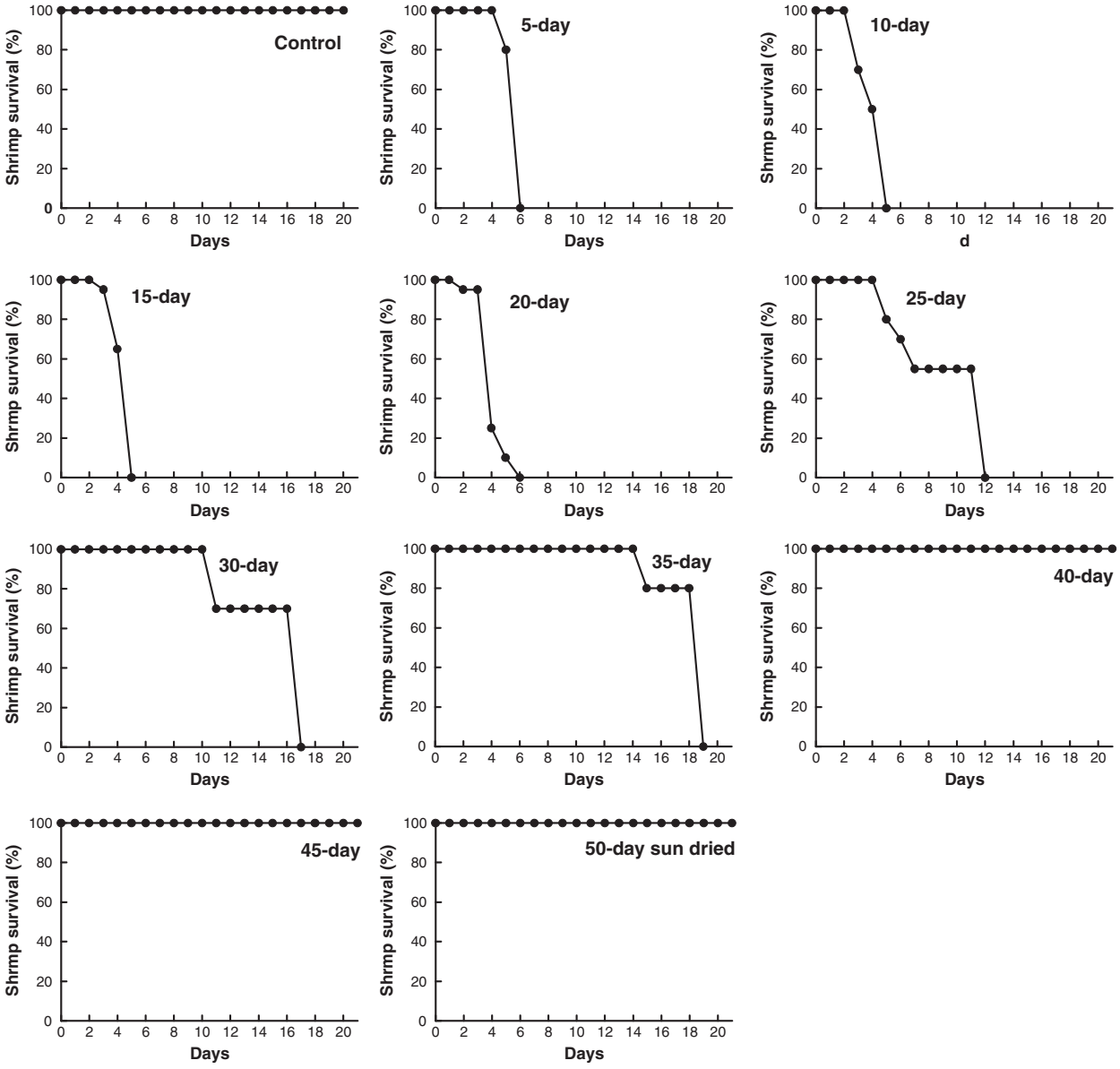

Fig. 7. Shrimp mortality pattern due to WSSV infection and WSD, an indicator of decline in WSSV viability in shrimp pond sediment during various days of non-drainable experimental conditions.

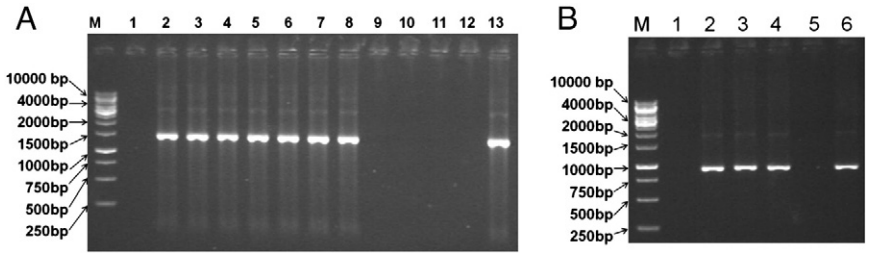

Fig. 8. Detection of WSSV DNA by PCR in sediment samples drawn from non-drainable experimental conditions (*A*: first step, *B*: nested). *A*: Lane M: mol. wt. marker, lane 1: control, lane 2: 5 days, lane 3: 10 days, lane 4: 15 days, lane 5: 20 D, lane 6: 25 days, lane 7: 30 days, lane 8: 35 days, lane 9: 40 days, lane 10: 45 days, lane 11: 50 days, lane 12: negative control, and lane 13: positive control. *B*: Lane M: mol. wt. marker, lane 1: control, lane 2: 40 days, lane 3: 45 days, lane 4: 50 days, lane 5: negative control, lane 6: positive control.

with sediment acts to prolong its survival in the marine environment the better management practices (BMPs) for shrimp farming need to be given a fresh look.

In the case of simulated non-drainable pond conditions, which had heavy WSSV load, remained infective for a period of 35 days (Table 3). However, high initial viral load would have had a bearing on its survival and infectivity of shrimp. In India, the traditional system of coastal aquaculture is practised in approximately 50,000 ha in the low lying brackishwater areas of West Bengal, Kerala, Karnataka, and Goa (Sathiadhas et al., 2009), where sun-drying shrimp ponds would not be often practical, and similar conditions exist in other south Asian nations. Hoa et al. (2011), based on an epidemiological study using VNTR molecular markers found that the transmission of WSSV in improved extensive shrimp farms was due to the recycling of WSSV over time in the same pond, whereas in semi-intensive shrimp farms, transmission of WSSV was mainly from neighbouring ponds through carrier organisms. However, in the present experimental set-up, no extraneous planktonic carrier organisms were involved. Considering the presence of umpteen varieties and numbers of carriers in natural non-drainable shrimp ponds, WSSV could be expected to survive for much prolonged periods.

Numerous interacting biological, physical and chemical factors such as proteolytic microorganisms, soil pH and temperature may affect persistence of baculoviruses in soil (England et al., 1998; Gerba, 2005). Hurst et al. (1980) examined the effect of several environmental factors on the survival of coxsackievirus, echovirus, poliovirus, rotavirus and bacteriophages using cell culture systems and reported that the most important factors that influenced survival of these viruses in soil were soil temperature, degree of virus adsorption to the soil and soil moisture content. Organic material in the pond water and sediment, sunlight (ultraviolet light) can also affect survival of viruses in aquaculture ponds.

In addition to various environmental factors, genetic and biological composition of virus also play vital role in the survival of virus in the environment. Virus type (lipid and non-lipid enveloped) may also affect virus survival and infectivity. Generally, viruses with lipid envelopes tend to survive longer at lower relative humidity (RH), and the non-lipid enveloped viruses such as respiratory adenoviruses and rhinoviruses tend to survive longer in higher RHs (Karim et al., 1985; Cox, 1998). WSSV capsid is known to contain both envelope proteins and lipids (Fauquet et al., 2005; Tsai et al., 2006) and may increase its persistence in the sediments. Pirtle and Beran (1991) reviewed the survival of viruses in the environment and concluded that "viruses pass into the environment from clinically ill or carrier hosts; although they do not replicate outside living animals or people, they are maintained and transported to susceptible hosts", and that, when extant outside the hosts which support their replication, they are the least understood of infectious agents. Viruses are shed from infected hosts into the environment and while outside of their cellular hosts and in the environment they have the potential to survive, persist and be transported by various routes to reach again other susceptible hosts. Hence, it was suggested that the greatest prospects for disease control for the future, lie in environmental measures to halt or reduce transmission of pathogens.

Table 3
Summary of WSSV viability in seawater, in sun-dried sediment and under non-drainable conditions.

Condition	WSSV DNA copies	WSSV survival (days)
Seawater	1000 mL^{-1} of seawater	12
Sun-drying (drainable pond)	211,500 g^{-1} of sediment	19
Water-logged (non-drainable pond)	753,600 g^{-1} of sediment	35

5. Conclusion

Pond preparation practices have proved to be useful in eliminating the virus from the pond and reducing the risk of disease outbreaks (Corsin et al., 2005). In the present study, it has been observed that WSSV could be viable in shrimp pond sediments for as many as 19 days despite sun-drying. In sediments which are nested PCR positive by OIE protocols, WSSV may remain infectious for about two days and possibly may not be infectious thereafter. WSSV may remain viable and infective for a period of 12 days in seawater even in relatively low counts. But in the actual field conditions, the viability and infectivity of WSSV may vary depending on their counts and a number of biotic and abiotic factors. Based on the observations in this study, it can be suggested that PCR testing of shrimp farm sediment prior to stocking water in the ponds for culture as one of the BMPs may help in ensuring biosecurity from WSSV.

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