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Note

Variation in biotic and abiotic factors associated with white spot syndrome virus (WSSV) outbreak in shrimp culture ponds

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ABSTRACT

Shrimp production in India has gradually increased since 2009 with the introduction of *Penaeus vannamei*. Most coastal states in India have been important contributors to *P. vannamei* production. However, among the many challenges faced in sustainable shrimp farming, prevention of viral diseases and pond water quality management remain major concerns. In this study, 27 shrimp ponds located in Kalpakkam and Elavur regions of Tamil Nadu were monitored to characterise the pond water parameters including pH, salinity, temperature, alkalinity, ammonia, nitrite, hardness, dissolved oxygen (DO) and *Vibrio* spp. load. Sudden outbreaks of white spot syndrome virus (WSSV) were observed in some ponds which were found to be associated with variations in abiotic parameters. Parameters such as pH, DO and nitrite were observed to be within the permissible range, while temperature, salinity, *Vibrio* spp. load and ammonia were relatively higher in certain ponds. The influence of these abiotic factors triggering WSSV outbreak were investigated in this study.

Keywords: Aquaculture, *Penaeus vannamei, Vibrio* spp., Water parameters, White spot syndrome virus

Shrimp farming in India is an important livelihood and economic activity. India alone exports 373866 t of shrimp annually generating estimated revenue of 3096.68 million USD (MPEDA. 2016). Among the penaeid shrimps, *Penaeus vannamei* is a dominant species for aquaculture in India. This happened after a major policy decision was taken by the Govt. of India facilitating import of specific pathogen free (SPF) *P. vannamei* for domestic culture in India. Due to the availability of SPF seeds, scope for stocking at higher density, greater tolerance to salinity and rapid weight gain, many farms in India shifted to *P. vannamei* farming (Kumaran *et al*., 2017). Tamil Nadu (TN), the second largest shrimp producing state in India has approximately 7615 ha of *P. vannamei* cultivation producing 44453 t, with around 14 million people dependent on this activity and *P. vannamei* farming in TN has grown over 14.88% than the preceding year (MPEDA, 2016).

Among the many factors considered influencing shrimp growth, water quality is one of the most important criteria in addition to feeding, pollutants, diseases, soil quality, nutrients and use of extensive chemicals (Kautsky *et al*., 2000). Pond physicochemical characteristics affect shrimp growth, health and abundance (Penmetsa *et al*., 2013). In intensive and semi-intensive ponds, excessive water contaminants are observed mainly due to unconsumed feed and waste produced from cultured shrimps. Moreover, the presence of suspended solids, total ammonia, reactive orthophosphate, high stocking density, lack of proper aeration and excessive feeding also adversely affect the quality of pond water (Hopkins *et al*., 1993). Variations in water quality parameters lead to stress in the cultured shrimp stock (Bagarinao and Flores, 1995) which can predispose shrimp to opportunistic infection or exacerbate other outbreaks (Ananda Raja *et al.,* 2012a).

It has been estimated that 60% of shrimp diseases are caused by viral pathogens and 20% by bacterial infestations (Flegel, 2012). The increasing level of stress directly attributes to diminished immune response and susceptibility to bacterial and viral infections (Le Moullac and Haffner, 2000; Tendencia *et al*., 2010; Ananda Raja *et al.,* 2017b). Among many viruses affecting shrimp, white spot syndrome virus (WSSV) has been known to be highly lethal, causing mortality in a very short period of time (Ananda Raja *et al.,* 2017a). The spread of WSSV infection occurs through contaminated water, seabirds, arthropods and other vectors. Shrimps are reported to be more susceptible to WSSV infection under high-stress conditions caused by poor pond management practises, water quality, food and other factors (Sanchez-Martinez *et al*., 2007). Significant economic loss also occurs as a result of outbreaks of *Vibrio* spp., which can become pathogenic under poor pond management practices (Gopal *et al*., 2005; Ananda Raja *et al.,* 2017a). Therefore, it is important to identify these microbial and viral infections at an earlier stage to maintain a healthy stock. Hence in the present study, water quality parameters from 27 semi-intensive shrimp ponds were analysed to comprehend the interrelationship between pond management practices, water quality and shrimp health.

The data were gathered from 27 shrimp ponds in 13 farms at two different locations. The study areas comprised 20 ponds located in Kalpakkam region (12.5238° N; 80.1568° E) and 7 ponds located in Elavur region (13.4628° N; 80.1191° E) in Tamil Nadu. Sampling was done periodically for one winter crop in Kalpakkam (October - December 2016) and one summer crop in Elavur (March - June 2017). Pond management practices, shrimp health and pond water characteristics were regularly monitored and recorded.

The shrimp farms in Kalpakkam area ranged from 0.3 - 1.0 ha, while in Elavur region, pond sizes were about 0.2 - 0.4 ha. All the selected ponds cultured *P. vannamei* shrimps. The stocking density of each pond varied with respect to pond size (Table 4). PCR tested WSSV negative post-larvae (PL) were stocked in all ponds and fed with commercial pellet feed until harvest.

In Kalpakkam, shrimp farming was practised with regular water exchange while in Elavur, shrimps were acclimatised for zero water exchange.

Water samples were collected in sterile plastic containers from below the pond surface during the culture period. Temperature, salinity (refractometer RHS 10ATC), dissolved oxygen (DO) and pH (EcoTestr pH1) were measured at the pond site. Shrimp samples were collected in sterile polyethylene bags and transported to the laboratory at 4°C for further analyses.

Physico-chemical properties of water *viz*., temperature, salinity, pH, DO, alkalinity, hardness, ammonia, nitrite, *vibrio* load as well as WSSV infection were monitored for the crop until harvest in shrimp farming ponds of Elavur and Kalpakkam. Shrimp samples were preserved in -80°C freezer for further analysis. Water alkalinity was estimated by titration with strong acid until specific pH values were attained (MColortest™ Alkalinity test, Merck) and a colourimetric test was used to determine the nitrite level (MColortest™ Nitrite test kit, Merck). Detection of ammonium ions and dissolved ammonia was calculated based on colourimetric test and sliding comparator (Ammonium test kit, Merck). Colourimetric test strips were used to measure the total hardness in pond water samples (Total hardness test, Merck). DO concentrations were estimated based on titration pipette and colour test using standard kit (MColortest oxygen test, Merck). *Vibrio* spp. load was estimated on thiosuphate citrate bile salt sucrose agar (TCBS) agar (HiMedia, Mumbai) plates (Biswas *et al.,* 2012). The green and yellow colonies developed were counted manually. The gill tissues of preserved shrimp samples were dissected using sterile forceps and scissors and processed for DNA extraction (QIAamp DNA Mini Kit, QIAGEN). Detection of WSSV was done by PCR using WSSV specific primers (Durand *et al*., 1996).

The temperature was found to be in the range of 30 to 32°C for summer crop and 28 to 31°C for winter crop and significant variations were not observed between the two crops (Table 1, 2, 3 and 4). Kalpakkam ponds had salinity between 3 to 25‰ whereas Elavur ponds had salinity ranging between 10 to 24‰. The estimated pH value ranged from 7.1 to 8.9 in Kalpakkam shrimp ponds and 7.1 to 8.3 in Elavur ponds. In this study, pH was not observed to vary substantially. From the analysis of the semi-intensive shrimp ponds, DO concentration was in the range of 3 to 12 mg l^{-1}. Data from our shrimp ponds in Kalpakkam recorded alkalinity in the range of 100 to 267 mg l^{-1} whereas shrimp ponds in Elavur region was in the range of 160 to 350 mg l^{-1}. Hardness was estimated to be greater than 450 mg l^{-1} $CaCO_3$ equivalents from all these pond water samples. The absolute hardness value could not be determined in this study as the pond hardness was above the maximum range of 450 mg l^{-1} supported by the kit used (Total hardness test, Merck). Ammonia was found to be in the range of 0.01 - 1.0 mg l^{-1} and the ponds that tested positive for WSD were observed to have higher concentrations of ammonia (0.5 to 1 mg l^{-1}). Eleven out of twenty seven ponds had high ammonia (1 mg l^{-1}) levels. The concentration of nitrite in Kalpakkam shrimp ponds were 0.05 to 1.00 mg l^{-1} whereas in Elavur shrimp ponds the values were 0.01 to 1.00 mg l^{-1}. *Vibrio* spp. load from these 27 shrimp ponds ranged between 10^3 to 10^6 cfu ml^{-1}.

This study from 27 semi-intensive *P. vannamei* culture ponds summarises the interrelation of pond water quality, shrimp health and diseases that are observed in two locations in TN. The most common concern in maintaining water quality involve temperature, pH, salinity, ammonia, nitrite, DO and hardness. Periodic monitoring of pond water parameters is important during the growing season prior to harvest (Cohen *et al*., 2005). In a recent study from Pokkali shrimp farms in the state of Kerala in India, which follow a system of cultivating paddy and shrimp

Table 1.Water quality parameters recorded in shrimp ponds in Kalpakkam during first sampling

Farm / Pond	Pond size (ha)	Days of culture (DOC)	pH	Salinity (‰)	Alkalinity (mg l^{-1})	Temperature (°C)	Total Ammonia (mg l^{-1})	Nitrite (mg l^{-1})	Vibrio load (CFU ml^{-1})
Farm A (Pond1)	0.61	74	7.3	17	267.12	28	0.6	0.10	2.6×10^{5}
Farm A (Pond2)	0.28	46	7.5	09	191.52	28	1.0	0.12	4.8×10^{5}
Farm B (Pond1)	0.46	50	7.5	18	126.00	29	1.0	0.20	3.3×10^{6}
Farm B (Pond2)	0.49	56	7.4	25	166.32	29	0.6	0.21	4.8×10^{6}
Farm B (Pond3)	0.61	44	7.7	19	131.04	29	1.0	0.23	1.1×10^{6}
Farm C (Pond1)	0.61	54	8.3	08	110.88	29	1.0	0.05	$>6.5 \times 10^{6}$
Farm C (Pond2)	1.01	61	8.1	11	141.12	29	1.0	0.05	$>6.5 \times 10^{6}$
Farm C (Pond3)	1.01	61	8.6	09	110.88	29	1.0	1.00	4.06×10^{6}
Farm D (Pond1)	0.28	41	8.3	06	166.32	29	0.6	0.13	2.1×10^{6}
Farm D (Pond2)	0.28	41	8.3	06	161.28	29	1.0	0.16	6.2×10^{5}

*Hardness from farm A, B, C, D were above 450 mg l^{-1} $CaCo_3$

Table 2. Water quality parameters recorded in shrimp ponds in Kalpakkam during second sampling

Farm/Pond	Pond size (ha)	Days of culture (DOC)	pH	Salinity (‰)	Alkalinity (mg l^{-1})	Temperature (°C)	Total ammonia (mg l^{-1})	Nitrite (mg l^{-1})	Vibrio load (CFU ml^{-1})	PCR test for WSSV	Remarks
Farm A (Pond1)	0.61	-	-	-	-	-	-	-	-	Positive	Harvested due to WSSV outbreak in nearby pond
Farm A (Pond2)	0.28	-	-	-	-	-	-	-	-	Positive	Harvested due to WSSV outbreak in nearby pond
Farm B (Pond1)	0.46	62	7.2	20	226.80	30	1.0	0.05	$>6.5 \times 10^{6}$	Negative	
Farm B (Pond2)	0.49	68	7.1	20	105.84	30	0.6	0.05	$>6.5 \times 10^{6}$	-	
Farm B (Pond3)	0.61	56	7.1	23	105.84	30	1.0	0.1	$>6.5 \times 10^{6}$	-	
Farm C (Pond 1)	0.61	66	7.2	8.0	176.40	30	0.6	0.05	$>6.5 \times 10^{6}$	Negative	
Farm C (Pond 2)	1.01	73	7.4	9.0	151.20	30	1.0	0.15	$>6.5 \times 10^{6}$	Negative	
Farm C (Pond 3)	1.01	73	7.3	9.0	196.56	30	1.0	1.0	$>6.5 \times 10^{6}$	Negative	
Farm D (Pond 1)	0.28	53	7.7	7.5	226.80	30	1.0	0.05	$>6.5 \times 10^{6}$	Negative	
Farm D (Pond 2)	0.28	53	7.9	6.0	221.76	30	0.6	0.05	6.2×10^{5}	Negative	
Farm D (Pond 3)	0.61	45	8.0	7.0	201.60	30	0.2	0.05	1.5×10^{6}	Negative	
Farm D (Pond 4)	0.28	45	8.0	5.0	236.88	30	0.6	0.25	1.3×10^{6}	-	
Farm D (Pond 5)	0.46	45	7.6	5.0	151.20	30	0.4	0.15	2.02×10^{6}	-	
Farm E (Pond 1)	0.49	7	8.8	3.0	161.28	30	0.05	0.13	5.2×10^{6}	Negative	
Farm E (Pond 2)	0.61	7	8.9	3.0	100.80	30	0.05	0.16	1.0×10^{6}	-	

*Hardness from farm A, B, C, D were above 450 mg l^{-1} $CaCo_3$

Table 3. Water quality parameters recorded in shrimp ponds in Kalpakkam during third sampling

Farm/ Pond	Pond size (ha)	Days of culture (DOC)	pH	Salinity (‰)	Alkalinity (mg l^{-1})	Temperature (°C)	Total ammonia (mg l^{-1})	Nitrite (mg l^{-1})	Vibrio load (CFU ml^{-1})	PCR test for WSSV	Remarks
Farm D (Pond4)	0.28	60	8.1	4	252.00	31	0.50	0.24	>6.5 x 10^6	Positive	
Farm D (Pond5)	0.46	60	7.8	5	231.84	31	0.20	0.14	>6.5 x 10^6	Negative	
Farm E (Pond 1)	0.49	23	8.7	5	100.80	31	0.14	0.14	>6.5 x 10^6	Positive	High mortality rate
Farm E (Pond 2)	0.61	23	8.9	5	105.84	31	0.20	0.20	8.0 x 10^3	Negative	High mortality rate
Farm F (Pond 1)	0.61	25	8.9	5	231.84	31	0.20	0.20	>6.5 x 10^6	Negative	
Farm F (Pond 2)	0.61	25	8.7	5	126.00	31	0.18	0.18	>6.5 x 10^6	-	
Farm F (Pond 3)	1.01	25	7.8	6	141.12	31	0.24	0.24	1.1 x 10^5	-	
Farm F (Pond 4)	1.01	25	8.7	5	226.80	31	0.17	0.17	>6.5 x 10^6	-	
Farm G (Pond 1)	0.61	85	8.6	4	221.76	31	0.05	0.05	>6.5 x 10^6	Negative	High mortality rate

*Hardness from farm D, E, F and G were above 450 mg l^{-1} $CaCo_3$; All the ponds in farm A, B, C as well as ponds 1, 2 and 3 in farm D were subjected to harvest due to WSSV outbreak in nearby ponds. Mortality rate of shrimps in farm D and F could not be observed due to emergency harvest

Table 4. Water quality parameters recorded in shrimp ponds in Elavur

Farm/ Pond	Pond size (ha)	Stocking density (No.per m^2)	Sampling No.	Daysof culture (DOC)	pH	Salinity (‰)	Alkalinity mg l^{-1}	Temperature (°C)	Ammonia (Mg l^{-1})	Nitrite (mg l^{-1})	Dissolved oxygen (mg l^{-1})	Vibrio load (CFU ml^{-1})	Remarks
Farm A (Pond 1)	0.40	99	1	17	7.9	20	200.16	31.0	0.20	0.01	07	5.4 x 10^5	
Farm A (Pond 2)	0.28	99	1	17	8.1	20	210.17	31.0	0.20	0.01	07	4.2 x 10^5	
Farm B (Pond 1)	0.40	82	1	10	7.6	10	175.14	31.0	0.20	0.01	12	6.0 x 10^4	
Farm C (Pond 1)	0.40	50	1	27	8.3	20	275.22	31.0	0.20	0.01	05	2.2 x 10^5	
Farm D (Pond 1)	0.40	74	1	30	8.0	13	250.20	31.0	0.20	0.01	07	1.8 x 10^5	
Farm E (Pond 1)	0.20	84	1	27	8.1	21	350.28	31.0	0.20	0.01	05	7.8 x 10^5	
Farm F (Pond 1)	0.40	61	1	22	8.3	19	290.23	31.0	0.20	0.01	05	4.8 x 10^5	
Farm A (Pond 1)	0.40	99	2	34	7.6	16	200.16	30.0	0.20	0.01	09	2.4 x 10^6	
Farm A (Pond 2)	0.28	99	2	34	7.9	18	250.20	30.0	0.20	0.01	09	1.9 x 10^6	
Farm B (Pond 1)	0.40	82	2	27	7.6	10	175.14	30.0	0.20	0.01	12	7.2 x 10^5	
Farm C (Pond 1)	0.40	50	2	44	8.3	20	315.25	30.0	0.20	0.05	05	1.5 x 10^6	
Farm D (Pond 1)	0.40	74	2	47	7.9	10	300.24	30.0	0.20	0.05	12	1.6 x 10^6	
Farm E (Pond 1)	0.20	84	2	44	7.9	18	320.26	30.0	0.60	0.50	05	3.2 x 10^6	

Cont.........................

Farm/ Pond	Pond size (ha)	Stocking density (No. per m^2)	Sampling No.	Daysof culture (DOC)	pH	Salinity (‰)	Alkalinity mg l^{-1}	Temperature (°C)	Ammonia (Mg l^{-1})	Nitrite (mg l^{-1})	Dissolved oxygen (mg l^{-1})	Vibrio load (CFU ml^{-1})	Remarks
Farm F (Pond 1)	0.40	61	2	39	7.9	20	250.20	30.0	0.01	0.01	09	1.8 x 10^5	
Farm A (Pond 1)	0.40	99	3	50	7.8	15	225.18	32.0	1.00	0.50	05	2.5 x 10^6	
Farm A (Pond 2)	0.28	99	3	50	7.8	15	290.23	32.0	1.00	0.50	05	2.4 x 10^6	
Farm B (Pond 1)	0.40	82	3	43	7.7	10	160.13	32.0	0.40	0.25	07	1.8 x 10^6	
Farm C (Pond 1)	0.40	50	3	60	7.6	15	210.17	32.0	0.60	0.50	05	6.5 x 10^6	
Farm D (Pond 1)	0.40	74	3	63	7.2	12	350.28	32.0	0.60	1.00	07	1.7 x 10^6	
Farm E (Pond 1)	0.20	84	3	60	7.8	15	290.23	32.0	0.40	0.50	07	3.2 x 10^6	
Farm F (Pond 1)	0.40	61	3	57	7.6	16	260.21	32.0	0.01	0.01	05	1.1 x 10^6	
Farm A (Pond 1)	0.40	99	4	67	7.7	19	225.18	31.5	1.00	1.00	05	1.6 x 10^6	
Farm A (Pond 2)	0.28	99	4	67	7.9	19	325.26	31.5	1.00	1.00	03	1.4 x 10^6	
Farm B (Pond 1)	0.40	82	4	60	7.8	10	150.12	31.5	0.50	0.25	07	4.2 x 10^5	
Farm C (Pond 1)	0.40	50	4	77	7.1	22	300.24	31.5	2.00	0.50	03	1.6 x 10^6	
Farm D (Pond 1)	0.40	74	4	80	-	-	-	-	-	-	-	-	Harvested
Farm E (Pond 1)	0.20	84	4	77	7.3	20	275.22	31.5	1.00	1.00	03	1.6 x 10^6	
Farm F (Pond 1)	0.40	61	4	74	8.0	20	250.20	31.5	0.01	0.01	05	4.4 x 10^5	
Farm A (Pond 1)	0.40	99	5	81	7.1	20	215.17	30.0	1.00	1.00	05	9.8 x 10^5	
Farm A (Pond 2)	0.28	99	5	81	7.1	20	280.22	30.0	0.60	1.00	05	1.2 x 10^6	
Farm B (Pond 1)	0.40	82	5	74	-	-	-	-	-	-	-	-	Harvested
Farm C (Pond 1)	0.40	50	5	91	-	-	-	-	-	-	-	-	Harvested
Farm E (Pond 1)	0.20	84	5	91	7.8	24	240.19	30.0	0.60	1.00	05	1.7 x 10^6	
Farm F (Pond 1)	0.40	61	5	88	8.0	22	280.22	30.0	0.50	0.50	05	7.0 x 10^5	
Farm A (Pond 1)	0.40	99	6	102	-	-	-	-	-	-	-	-	Harvested
Farm A (Pond 2)	0.28	99	6	102	-	-	-	-	-	-	-	-	Harvested
Farm E (Pond 1)	0.20	84	6	112	-	-	-	-	-	-	-	-	Harvested
Farm F (Pond 1)	0.40	61	6	109	-	-	-	-	-	-	-	-	Harvested

*Hardness from all farms were above 450 mg l^{-1} $CaCo_3$

alternately; high fluctuation in the concentration of DO was observed due to the presence of heavy algal blooms and lack of water quality management practices (Ajin *et al*., 2016). Water quality management practices are universally recommended to maintain healthier culture (Wyk and Scarpa, 1999).

A study done in *P. vannamei,* outlined high susceptibility to *V. alginolyticus* infection consequent to an increase in water temperature from 27 to 34°C (Cheng *et al*., 2005). This parameter has been reported by many researchers and has been found to be inter-related with other physicochemical characteristics (Cheng *et al.,* 2005; Moser *et al.*, 2012). In shrimp, low water temperature will directly affect metabolic rate and thereby decrease the rate of ammonia accumulation (Kir *et al*., 2004). Wyban *et al*. (1995) reported that the optimal temperature for *P. vannamei* growth is 27°C. In the present study, the average temperature was 30°C, which was well within the range of 26 to 33°C reported by Wickins and Lee (2008) for *P. vannamei* culture. Ferreira *et al.* (2011) reported that 24°C is the optimum temperature for *P. vannamei* farming in Brazil.

Although, shrimps are tolerant to euryhaline conditions, the optimal salinity for best growth, range between 15 to 25‰ (Baliao, 2000). Low salinity often leads to undesirable changes in the environment (Wahab *et al*., 2003). Low salinities were observed in few ponds in Kalpakkam, which might be a factor contributing to shrimp stress. Increased mortality rate in shrimps were observed in farms E and G of Kalpakkam, which had lower salinity values than other ponds (Table 2 and 3). Shrimp ponds D4 and D5 which had the onset of WSSV outbreak were also found to have low salinity levels. Variations in salinity is a major factor affecting shrimp immune response and combined with variations in temperature and pH, can influence WSSV outbreaks (Tendencia *et al*., 2011). Ramos-Carreno *et al*. (2014) reported that *P. vannamei* was less susceptible to WSSV at intermediate salinities of 15 - 28‰. Our findings of low salinity affecting shrimp ponds and triggering WSSV outbreak, agree with these reports.

Another major factor for successful shrimp farming is optimum pH. Pond water with pH in the range 6.5-9.0 is considered optimal for aquaculture production (Carbajal-Hernandez *et al*., 2012). High pH has been identified commonly in many shrimp ponds and is caused due to the removal of carbon dioxide for phytoplankton growth (Penmetsa *et al*., 2013). The estimated pH value in our study ranged between 7.1 to 8.9 and was within the permissible range reported for shrimp farming.

Hypoxia increases the susceptibility of cultured shrimps to imbibe WSSV infection (Lehmann *et al.,* 2016). The tolerance to hypoxia was reported to vary between species but the effects start to appear when oxygen drops below 2 mg l^{-1} (Diaz, 2001). The concentration of DO has been directly associated with the presence of algal bloom in ponds. Hypoxia reduces metabolic rate which results in mortality in fish (Shang *et al*., 2006) and shrimp (Wu *et al*., 2002). The immune resistance can also be impacted under hypoxic conditions, which can lead to disease outbreak (Direkbusarakom and Danayadol, 1998). The current study revealed that all ponds had DO concentrations greater than 2 mg l^{-1}. A previous study conducted in central India showed a variation of 3.0 to 8.3 mg l^{-1} DO level in shrimp ponds (Mishra *et al*., 2008). In the present study, DO was found to be within the permissible range.

Alkalinity, another important parameter signifies the total sodium bicarbonates and carbonates present in the pond water. Apart from other parameters, alkalinity and hardness are relatively stable but can alter with time (Wurts and Durborow, 1992). A recent study suggested that increased alkalinity levels protect the crustaceans from trace metal toxicity and the optimal level for alkalinity is 100 mg l^{-1} (Boyd *et al*., 2016). Calcium deposition on exoskeleton followed by slow growth in shrimps is reported when the pH is above 8.3 and alkalinity above 150 mg l^{-1} (Chanratchakool, 2003). Pond alkalinity tends to correlate with pH, hardness and carbon dioxide concentration. Data from our shrimp ponds in India recorded alkalinity in the range of 100 to 350 mg l^{-1}. In this study, it was observed that, WSSV positive ponds as well as non-infected ponds had alkalinity value greater than 150 mg l^{-1}. The desired total alkalinity level for most of aquaculture species are reported to be within 50-150 mg l^{-1} but not less than 20 mg l^{-1} (Wurts, 2002). Based on these reports, the alkalinity levels in our study recorded an increase by 200 mg l^{-1}, however these variations did not affect successful shrimp harvest.

Water hardness is an important factor in determining the pond water quality (Boyd *et al*., 2016). The proportion of divalent ions, as calcium and magnesium are major contributors to water hardness (Wurts and Durborow, 1992). Whilst Wyk *et al*. (1999) have reported optimal level to be greater than 150 mg l^{-1}, Ferreira *et al*. (2011) reported 1000 mg l^{-1} $CaCO_3$ equivalents. From our study, hardness quality was found to be uniformly high in all the shrimp ponds.

Optimal amount of ammonia and nitrite content in shrimp ponds are necessary to harvest a healthy crop. Accumulation of ammonia leads to toxicity and causes adverse side effects including slow growth, increased oxygen consumption and shrimp mortality (Chen and Kou, 1992). Increased levels of ammonia in water have been shown to affect the immunity of *P. vannamei* leading to susceptibility to *V. alginolyticus* infection (Liu and Chen, 2004). Other studies from India estimated ammonia level to be in the range of 0.02 to 2 mg l^{-1} among the southern region (Chakravarty *et al*., 2016) and 0.001 to 0.01 mg l^{-1} among the northern regions (Mudassir *et al*., 2016).

Nitrite toxicity in *P. vannamei* has been extensively studied in terms of LD_{50}, growth and survival (Lin and Chen, 2003), immunity, gut health and anti-oxidant capacity (Guo *et al.,* 2013; Duan *et al.,* 2018), as well as in disease resistance and related molecular mechanisms (Tseng and Chen, 2004; Guo *et al.,* 2016) and are considered to cause chronic toxicity in shrimps. In our study of winter crop, initially nitrite concentrations were uniformly low in all ponds; however as the days of culture increased to 50 days, the nitrite concentration increased. This shows that accumulation of nitrite was continuous throughout the culture period; however, successful crops were harvested. Enhanced nitrite accumulation can lead to elevated levels of reactive oxygen species (ROS), DNA damage, cell apoptosis, oxidative stress and reductions in total haemocyte counts (THC) (Xian *et al*., 2011). Continuous imbalance in nitrite concentration is known to inhibit anti-oxidant enzymes needed for shrimp metabolism and growth (Roques *et al*., 2015). Tseng and Chen (2004) have also highlighted that water nitrite level caused lower immunity in *P. vannamei* triggering *V. alginolyticus* infection. Periodical removal of sludge from pond bottom has been recommended to diminish the level of total ammonia nitrogen (TAN) in water (Ajin *et al*., 2016). In general, it is recommended that the concentration of pond nitrite should remain below 2 mg l^{-1} (Romano and Zeng, 2013). The data from farms in Kalpakkam and Elavur revealed that nitrite concentration reaching to 1 mg l^{-1} levels did not considerably affect the ponds where successful shrimp harvest was carried out.

Vibriosis causes mass mortalities in both hatcheries and grow-out systems leading to high economic loss (Ananda Raja *et al.,* 2017a, b). Many shrimp diseases like tail necrosis, loose shell syndrome (LSS), red disease, cuticular wounds, septic hepatopancreatitis and white gut disease (WGD) occur due to infections caused by *Vibrio* spp. (Ray *et al*., 2017). The infestations of these bacterial species are wide-spread and to maintain healthy stock, periodical assessments of *vibrio* load in pond water are necessary. Ferreira *et al*. (2011) reported *Vibrio* load level of 10 cfu ml^{-1} whereas the *Vibrio* load from the farms in the study area was relatively high. However, in our study, a few ponds with high *Vibrio* content tested negative for WSSV and did not result in WSSV outbreak.

WSSV has been known as a serious threat for shrimp cultivation (Thitamadee *et al*., 2016). Low water quality causes stress and lowers the shrimp immune system and can be the cause for outbreaks of lethal viruses like WSSV (Kautsky *et al*., 2000). Increase in viral load have been associated with pond water physicochemical characteristics including DO, ammonia, temperature and bacterial count (Zhang *et al*., 2016). In *P. monodon,* WSSV outbreaks were triggered by fluctuation in temperature coupled with *Vibrio* spp. load and low salinity (Tendencia *et al*., 2011; Ananda Raja *et al.,* 2017a, b). Increased evidence of WSSV outbreak with extreme salinity conditions has been reported as well (Liu *et al*., 2006; Ramos-Carreno *et al*., 2014).

On screening the shrimps, four out of twenty ponds (20%) in Kalpakkam area were positive for WSD while ponds located in south Elavur region were not affected until harvest (Table 1, 2, 3 and 4). During this study, it was observed that ponds that were located adjacent to the infected ponds were also immediately harvested in an attempt to pre-empt the further spread of disease. For example, pond B1 in Kalpakkam tested negative for WSSV; however, an emergency harvest was carried out following a disease outbreak in nearby farms. In analysing the Elavur shrimp farming ponds, few parameters like ammonia, nitrite and *Vibrio* load were found to be higher than the optimal value, yet successful crop was harvested

In conclusion, optimal water physicochemical characteristics and water quality maintenance, are important for shrimp health and for sustainable shrimp production, routine monitoring of shrimp ponds and adoption of pond management practices are important.

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