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SHORT COMMUNICATION

Effect of rainfall and atmospheric temperature on the prevalence of the whitespot syndrome virus in pond-cultured *Penaeus monodon*

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Disease due to the white spot syndrome virus (WSSV) has devastated the shrimp industry worldwide (Corsin, Turnbull, Mohan, Hao & Morgan 2005). However, not all WSSV outbreaks resulted in crop failure (Withyachumnarnkul 1999). Efforts to reduce the incidence of WSSV outbreaks focus on the production of virus-free larvae; management practices such as soil removal and drying, liming and use of disinfectants; and bio-security measures like the lining of ponds and crab fencing (Corsin *et al.* 2005).

An epidemiological survey in Ecuador farms showed an apparent association between lower temperature and increased mortality rate (Rodriguez, Bayot, Amano, Panchana, de Blas, Alday & Calderon 2003). Abrupt fluctuations in temperature and salinity may contribute to the increase in the viral load in the shrimp population of a pond, which resulted in 80% mortality in a farm in Mexico (Peinado-Guevara & Lopez-Meyer 2006). Guan, Yu and Li (2003) reported that survival of WSSV-infected *Marsupenaeus japonicus* was high at 15° and 33 °C, and low at 23° and 28 °C, while the viral concentration was lower at 15° and 33 °C. Furthermore, Yu, Li and Guan (2003) reported that *M. japonicus* are more susceptible to WSSV under salinity stress due to changes in their immune responses. Acute salinity change >4 ppt in 1h could lead to rapid WSSV proliferation and decreased disease-resistance ability of *Fenneropenaeus chinensis*, which makes the shrimp more susceptible to viral infection than they previously were, resulting in a WSSV outbreak (Liu, Yu, Song, Guan, Jian & He 2006). Continuous small salinity adjustments may also result in the same WSSV proliferation as acute salinity changes and has been attributed to the loss of self-adaptive ability after a long period of salinity stress.

Current practices designed to prevent WSSV outbreaks are not effective. Reports indicate that outbreaks may occur only under stressful conditions. The question then is what stressors could have predisposed the shrimp to WSSV. Rainfall could be one such stressor; farmers in Negros Island, Philippines observed that 2 weeks of rain often result in a WSSV outbreak. The present study assessed how natural phenomena such as rainfall and atmospheric temperature affect the prevalence of WSSV in shrimp farms in Negros Island from 2000 to 2007.

Data on the average daily rainfall, atmospheric temperature and rainy days were gathered from the local weather bureau of Negros Island. The number of consecutive months with ≥ 14 raindays (CMR) was determined. Samples were sets of five shrimps *Penaeus monodon* juveniles/adults submitted to the Negros Prawn Producers Marketing Cooperative Inc. (NPPMCI) for WSSV analysis from 2000 to 2007. The NPPMCI is a cooperative founded by shrimp producers in Bacolod City, Negros Occidental, Philippines. The cooperative monitors different physico-chemical parameters of the water, plankton profile and bacterial level of the rearing water and cultured species and the presence of WSSV in shrimp and crab. White spot syndrome virus detection was carried out on the gills of juvenile or adult shrimp. Two methods were used to detect the presence of WSSV: the polymerase chain reaction (PCR) techniques described by de la Peña, Lavilla-Pitogo, Villar, Paner, Sombito and Capulos (2007), from January 2000 to April 2006, and the Shrimp Biotechnology Business Unit (SBBU) kit from May 2006 to December 2007. All tests included positive and negative controls. The SBBU kit is a test kit for the molecular diagnosis of WSSV developed by the SBBU of Mahidol University, Thailand (Withyachumnarnkul 1999). The kit uses the lateral flow dipstick (LFD) method. Briefly, DNA were extracted from the postlarvae or the gills of juvenile and adult shrimp to be tested and amplified using the kit's reagents. The amplified DNA was applied onto the LFD and placed in an upright position into the assay buffer to allow the bands to develop. The bands in the LFD were then interpreted. Results of the WSSV analysis on shrimp from 2000 to 2007 were evaluated. Prevalence was computed by dividing the number of positive samples by the total number of processed samples. The mean prevalence and the average rainfall between years and months were compared using the one-way analysis of variance and Duncan's multiple range test following the statistical program for the social sciences (SPSS v. 12). The relationship between WSSV prevalence and the different factors was analysed by means of Pearson's Correlation for normally distributed data and Spearman for non-normally distributed data, using the same program.

The number of shrimp samples analysed fluctuated and the annual prevalence of WSSV tended to increase with time (Table 1). The annual prevalence of WSSV-positive shrimp was significantly higher from 2004 to 2006 than in 2000–2001, with immediate values not significantly different from those either in 2003 and 2007. The lowest annual prevalence was observed in 2001, when the observed lowest monthly temperature was the highest (21.1 °C), and the number of consecutive months with at least 14 rain days was the lowest (4 months). Higher WSSV prevalence was observed in November, December, January and February, when the observed average minimum atmospheric temperature was generally lower (Table 2). Correlations between the prevalence of WSSV-positive adult shrimp with the monthly rainfall ($\sigma = -0.25$; $P<0.05$) and the number of rain days ($\sigma = -0.24$; $P<0.05$) are weakly negative, and somewhat stronger with minimum atmospheric temperature ($\sigma = -0.41$; $P<0.01$). The average minimum temperature is correlated with the number of rain days ($\sigma = 0.68$; $P<0.01$) and monthly rainfall ($\sigma = 0.46$; $P<0.01$).

The negative correlation of WSSV prevalence with decreased temperature is in consonance with the results obtained by Liu *et al.* (2006) and Guan *et al.* (2003). Rahman, Escobedo-Bonilla, Corteel, Dantas-Lima, Wille, Alday Sanz, Pensaert, Sorgeloos and Nauwynck (2006) and Reyes, Salazar and Granja (2007) reported that a decrease in temperature weakens the immune response of the shrimp, thereby making them susceptible to WSSV infection. Furthermore, the same authors mentioned that a decrease in temperature also increases viral replication and load.

Table 1 Total number of samples, prevalence (%) of the white spot sydrome virus (WSSV) in juvenile/adult *Penaeus monodon*, number of months wherein WSSV was observed (NMW), number of rain days, number of consecutive months with ≥ 14 raindays (CMR), observed lowest monthly atmospheric temperature (°C), and the mean of minimum temperature (°C) observed in Negros Island, Philippines, from 2000 to 2007

Year	# of samples	Annual prevalence	NMW	Total rain days	CMR	Lowest monthly temperature (°C)	Mean minimum temperature (°C)
2000	77	6^{ab}	2	234	8	20.9	22.4
2001	33	0^{a}	0	190	4	21.1	22.5
2002	70	10^{abc}	4	160	5	20.1	22.0
2003	107	20^{abcd}	4	175	7	17	22.0
2004	179	32^{cd}	6	181	7	16.2	22.0
2005	303	34^{d}	8	192	8	17	21.95
2006	963	36^{d}	12	198	6	18.9	22.0
2007	302	28^{bcd}	9	194	8	17.5	22.27

Superscript value a,b,c,d represent $P>0.05$.

Table 2 Monthly mean prevalence of white spot syndrome virus (WSSV) shrimp, percentage WSSV outbreaks, average rainfall, number of rain days, average minimum atmospheric temperature and observed lowest temperature in Negros Island, Philippines, from 2000 to 2007

	WSSV		Rainfall		Temperature (°C)	
Month	Monthly prevalence*	Percentage outbreaks†	Number of days	Average (mm)	Average minimum	Observed lowest
January	39^{de}	75^{abcd}	7^{a}	33^{a}	20.7^{a}	19.6
February	34^{cde}	84^{cd}	9^{a}	64^{ab}	20.7^{a}	19.7
March	13^{abcd}	61^{abcd}	10^{a}	76^{ab}	21.5^{b}	20.1
April	17^{abcd}	42^{abcd}	9^{a}	52^{ab}	22.4^{cd}	21.5
May	10^{abc}	48^{abcd}	18^{bc}	262^{abc}	23.3^{g}	22.0
June	1^{a}	25^{abc}	22c	711^{d}	23.0^{fg}	22.2
July	2^{a}	16^{a}	22^{c}	497^{cd}	22.9^{efg}	22.2
August	6^{ab}	21^{ab}	22^{c}	509^{cd}	22.8^{defg}	22.5
September	5^{ab}	39^{abcd}	21^{c}	377^{abcd}	22.7^{def}	22.1
October	31^{bcde}	89^{d}	23^{c}	393^{bcd}	22.5^{cde}	22.0
November	35^{cde}	79^{bcd}	16^{b}	218^{abc}	22.1^{c}	21.6
December	56^{e}	88^{d}	15^{b}	166^{abc}	21.6^{b}	20.7

*Computed by dividing the number of WSSV-positive samples by the total number of processed samples.
†Computed by dividing the number of WSSV-positive samples that resulted in an outbreak by the total number of WSSV-positive samples.
Values with the same superscripts are not significantly different ($P > 0.05$).

The effect of 14 days of continuous rain on WSSV prevalence observed by farmers and the data on the number of consecutive months with >14 rain days in the present study are in accordance with the observation of Karunasagar, Otta and Karunasagar (1997) in the West Coast of India, wherein a WSSV outbreak occurred when the monsoon was at its peak, salinity near 0 ppt, with heavy surface runoff that may carry contaminants and turbidity in natural waters. Furthermore, farmers in Kalimantan, Indonesia, associate poor shrimp harvest with high water turbidity due to rain (personal communication).

Rainfall affects water temperature and salinity through various processes. An increase in rainfall could dilute the pond water, which would result in lower salinity and cooler water (Abney & Rakocinski 2004). Water temperature is negatively related to rainfall, but on the other hand, is positively related to air temperature (Nargis & Pramanik 2008). Thermal pollution due to casual runoffs from upland during rains increases the temperature of surface water (Jones, Hunt & Hunt 2007). These runoffs do not modify the usual thermal stratification in tropical regions. Clouds during the rainy season reduce cooling during the night and the higher average minimum water temperature also prevents ponds from cooling. This higher temperature of pond water explains the weak negative correlation between rainfall and WSSV occurrence, which is the dominant trend. However, after prolonged rains the thermal pollution of runoff water has an inverse effect and cools down water and thus disturbs thermal stratification (Jones *et al.* 2007). Together with the negative relation between rain and pond water, this might explain the effect of 14 continuous rain days.

Most farmers in the Philippines do not stock during the cold season because it is during these months that they experience most WSSV-related problems. They prefer stocking during the warm months, although they experience problems after 14 days of heavy rains. This may be related to the fact that *Vibrios* are dominant during the warm season (Ortigosa, Esteve & Pujalte 1989), making them more susceptible to secondary infection by other pathogens like the WSSV (Phuoc, Corteel, Nauwynck, Pensaert, Alday-Sanz, Van den Broeck, Sorgeloos & Bossier 2008).

This study confirmed that low atmospheric temperature is a hazard factor in the development of WSSV in cultured shrimp *Penaeus monodon*. We explained that rainfall on subsequent days is also a hazard factor while the dominant trend is a negative correlation between rainfall and WSSV. In general, the average temperatures during the rainy season are higher and thus the water temperature; however, temperatures of runoff water and of air are affected by several continuous rain days, thus a effecting pond water temperature. Continuous exposure to a temperature lower than the optimum level for shrimp culture acts as a stressor (Baliao & Tookwinas 2002).

Although the recent literature and practice demonstrate that pond lining (P. Sorgeloos pers. comm.) and increased bio-security reduces WSSV occurrence and outbreaks (Mohan, Corsin & Padiyar 2004), our analysis shows that WSSV will remain a risk in outdoor shrimp culture. Cost-effective measures to mitigate the effect of low atmospheric temperature and other natural phenomena need to be explored.

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