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WSSV risk factors related to water physico-chemical properties and microflora in semi-intensive *Penaeus monodon* culture ponds in the Philippines

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ABSTRACT

Whitespot syndrome virus, WSSV, is the most important among the shrimp diseases. One of the suggested WSSV risk factors is the occurrence of stress since stressors could compromise the shrimp defence system thus increasing the risk of WSSV infection. Stressors are usually related to the physico-chemical properties of both water and pond bottom. This paper investigates the effect of some biotic and abiotic components of the pond ecosystem on WSSV infection and outbreak. Water physico-chemical properties and microflora of 91 production cycles of 8 semi-intensive shrimp farms were analyzed to determine WSSV risk factors, using factor analysis and logistic regression. Fluctuations of temperature and pH are important risk factors that will result to an infection but not necessarily to an outbreak. Exposure to high salinity and high temperature are important factors for an infection to result to an outbreak. The risk of an infection is reduced when the water temperature is high, salinity fluctuations are small, and percentage of yellow *Vibrio* colonies is greater than the green ones. Further studies are needed to clarify the effects of water depth, water transparency, and various bacterial counts; these factors could be individual or interactive.

1. Introduction

Whitespot syndrome virus, WSSV, is the most important among the shrimp diseases. One of the suggested WSSV risk factors is the occurrence of stress (Chou et al., 1995; Lo et al., 1996; Mohan et al., 2008). Stressors are usually related to the physico-chemical properties of both water and pond bottom. Stressors could compromise the shrimp defence system thus increasing the risk of WSSV infection (Takahashi et al., 1995). Furthermore, some physico-chemical conditions might stimulate the rapid replication of WSSV which could subsequently cause the death of the shrimp (Lo and Kou, 1998). In the following paragraphs we give an overview of present knowledge on the effect of water parameters on WSSV infection; starting with evidence on abiotic factors acquired from tank experiments and farm surveys, an intermezzo on effects of bottom soil, and ending with biotic factors in water.

Tank-based studies have reported that fluctuations in salinity and temperature could weaken the shrimp's immune system and affect viral replication. In *Marsupenaeus japonicus*, the immune response becomes weaker as the deviation from the original salinity they were kept in becomes greater (Yu et al., 2003). Acute salinity change of greater than 4 ppt in 1 h, as well as continuous small salinity adjustments, 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 WSSV outbreak (Liu et al., 2006). Furthermore, a decrease in temperature increases the viral load; while hyperthermia could protect *Penaeus vannamei* from WSSV (Rahman et al., 2006b; Reyes et al., 2007; Granja et al., 2003). Guan et al, (2003) reported that viral concentration was lower at 15 °C and 33 °C than at 23–28 °C. WSSV DNA load in WSSV-infected shrimp is reduced at 32 °C (Granja et al., 2006).

A few authors reported farm based WSSV risk factors. Corsin et al. (2001) reported that WSD outbreaks were preceded or coincided with higher pH and un-ionised ammonia in the pond water. This was contradicted by Sahoo et al. (2005), who reported that clinical white spots in shrimps associated with high water pH disappear after molting. Low salinity and low hardness of pond water are stress factors that made shrimp susceptible to *Vibrio* and subsequently to white spot disease (Hettiarachchi et al., 1999). On farms in Ecuador, Rodriguez et al. (2003) showed temperature being associated with mortality. In Mexico abrupt fluctuations in temperature and salinity due to heavy rain were reported to contribute to increased viral loads in the shrimps, which resulted in 80% mortality (Peinado-Guevara and Lopez-Meyer, 2006).

Water microflora also affects WSSV infection. Hettiarachchi et al. (1999) reported that prior exposure to *Vibrios* make shrimp susceptible to WSSV infection. Phuoc et al. (2008) reported accelerated mortality in WSSV-infected *Littopenaeus vannamei* after infection with *V. campbellii*. Dela Peña et al (2003) reported *V. harveyi*

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and WSSV dual infection in *Penaeus monodon* in the Philippines. Planktons such as *Chlorella* sp, *Scrippsiella troichoidea* and rotifer serve as vector in WSSV transmission (Liu et al., 2007; Yan et al., 2007). On the other hand, *Spirulina* could delay the onset of clinical signs but has no effect on the final mortality (Rahman et al., 2006a).

Mohan et al. (2008) reported no direct relationship between the condition of pond sediment and risk of disease outbreaks or production. However, in other studies the occurrence of black and toxic bottom sediments has been shown to adversely affect shrimp health and lead to disease outbreak or poor survival. Sludge removal, ploughing of pond bottom and liming reduces the risk of WSSV and other infections as it reestablishes a healthy pond bottom (Avnimelech and Ritvo, 2003).

Summarising, most papers on the effect of physico-chemical water parameters on WSSV are tank-based experiments and not on *P. monodon*. Papers on WSSV epidemiology in ponds are mostly on farm management practices, which are assessed by the authors in another type of survey (Tendencia et al., forthcoming). Little is known how the pond ecology characterised by both physico-chemical and bacteriological parameters affects WSSV epidemiology. This paper investigates the relation of bacterial and algal counts, bacterial characteristics and water main physico-chemical parameters to WSSV infection and outbreak in pond-cultured *P. monodon* in the Philippines.

2. Materials and methods

2.1. Pond characteristics

Ponds used in the study are *P. monodon* ponds in Negros Island, Philippines. Pond size ranged from 0.2 to 0.8 ha. Ponds were stocked with WSSV PCR negative post larvae (PL) 13–20 at an average density of 10–16 ind/m^2. Shrimp were fed with commercial pellets from stocking until harvest. In some cases, shrimp culture was aborted after 19 days of culture (DOC), in other cases shrimp were harvested at DOC 184; next to market demand the day of harvest depended on the health status of the shrimp.

2.2. Data gathering

Data were gathered from 75 ponds in 8 farms over 100 production cycles. Data from 2 production cycles were taken from 21 of the 75 ponds, and 3 production cycles from 2 ponds. To avoid power reduction due to missing values, only 91 of these production cycles were used in the analysis. Forty-eight culture periods did not have WSSV infection, 15 had infection but did not result in an outbreak, and 28 had infection that resulted in an outbreak.

Physico-chemical water parameters, namely, temperature, pH, salinity, transparency and water depth were measured twice daily at 08:00 h and 17:00 h from DOC 1 until the culture was aborted or until the shrimp were harvested. Bacterial and algal flora in pond water were counted every three days. The spread plate and dilution techniques were used for total bacterial count (TBC), luminous bacterial count (LBC) and Presumptive *Vibrio* count (PVC). TBC and LBC were plated and counted on nutrient agar; for PVC we used thiosulfate citrate bilesalt sucrose agar (TCBS). All plates were incubated for 24 h at 30 °C. Luminescence for the LBC was observed in a dark room. Green and yellow *Vibrio* colonies were counted separately on TCBS. Algal flora was counted using a haemocytometer.

Shrimp samples ($n = 5$) were analyzed for WSSV detection weekly, when abnormalities in behaviour/appearance and when mortalities were observed using the SBBU kit, a test kit for the molecular diagnosis of WSSV developed by the Shrimp Biotechnology Business Unit (SBBU) of Mahidol University, Thailand (Withyachumnarnkul 1999). Positive and negative controls were included in all tests. WSSV infection was reported when shrimp tested positive for WSSV. An outbreak was reported when mass mortalities were observed in WSSV positive shrimp.

2.3. Data analysis

Averages and daily fluctuations of the observed physico-chemical water parameters were computed, as well as averages of the counts of the microflora. To avoid dilution of the data, the series of repeated measures in the culture period were cut-off to a limited number of days of culture (DOC) before data analysis. For culture periods with outbreak, DOC cut-off was set at 4 days before outbreak and for those with infection not resulting in outbreak at 4 days before infection. The cut-off of 4 days was based on previous observations that 4 days of exposure to multi stressors could lead to WSSV infection (Tendencia et al., unpublished data). To determine the cut-off for culture periods without infection, the median of the DOC at which WSSV outbreaks occurred and the DOC at which highest WSSV incidence was observed were considered (see Results). We used the average of the values observed between these periods to characterise production cycles without incidence.

The data analysis was done in two steps: variable reduction and binary logistic regression in SPSS®17, both complemented with correlation analysis. The number of variables was reduced using factor analysis. Before the second step the number of explanatory variables within a category was reduced by excluding correlated variables; the highest positively and negatively correlated factors were maintained. WSSV risk factors were identified using binary logistic regression's backward stepwise method with a likelihood ratio test. Not all WSSV infection resulted in an outbreak. To determine which factors were important for an outbreak to occur, two analyses were done: one on the entire dataset and one on the cases where infection occurred whether or not resulting in an outbreak. In the entire dataset, WSSV infection whether resulting or not resulting in an outbreak was used as the dependent variable. In the smaller dataset with cases where infection was confirmed, WSSV outbreak was considered the dependent variable. The model which gave a lower negative log likelihood value, a R^2 value closer to one, and a higher percentage correctness in predicting WSSV incidence was retained. We used Spearman correlation to check the direction of the impact by the variables in the model, and the multicollinearity diagnostics from the linear regression analysis to assess if the model parameters are biased.

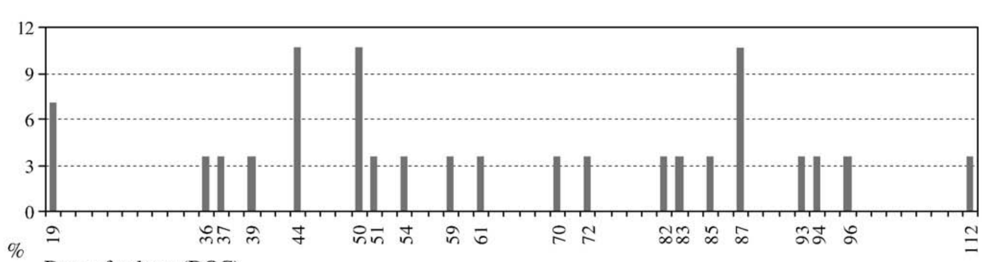

Fig. 1. Frequency distribution of days of culture at which WSSV outbreak is observed in semi-intensive *P. monodon* farms in the Philippines.