Short Paper

Relationship between white spot syndrome virus (WSSV) loads and characterizations of water quality in *Litopenaeus vannamei* culture ponds during the tropical storm

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Summary

An *in-situ* experiment was conducted to investigate the effect of tropical storm on the white spot syndrome virus (WSSV) loads in *Litopenaeus vannamei* rearing ponds. White spot syndrome virus loads, heterotrophic bacteria, *Vibrio* and water quality (including temperature, dissolved oxygen (DO), salinity, pH, NH_4-N, and NO_2-N) were continually monitored through one tropical storm. The WSSV loads decreased when tropical storm made landfall, and substantially increased when typhoon passed. The variation of WSSV loads was correlated with DO, temperature, heterotrophic bacteria count, and ammonia-N concentrations. These results suggested that maintaining high level DO and promoting heterotrophic bacteria growth in the shrimp ponds might prevent the diseases' outbreak after the landfall of tropical storm.

Key words: Relationship, Tropical storm, Water quality, WSSV

Introduction

Over the past 40 years, shrimp farming has become the most profitable sector in global aquaculture industries. With the rapid expansion and intensive culture of shrimp farming, incidence of diseases caused by various infectious agents also increased in cultured shrimp populations, causing economic losses to commercial shrimp aquaculture. Particularly viral diseases, which have become the major bottleneck for shrimp industry expansion during recent years. Among various viral diseases, white spot syndrome virus (WSSV) has been considered as the most serious threat to shrimp aquaculture industries since it was found in 1992 (Lightner, 1996). Within 3 to 10days, high mortalities (up to 100%) are usually observed when WSSV occurs in shrimp culture system (Lightner, 1996). Although WSSV has been intensively studied in the past two decades, and several detecting protocols have been well established (Meng *et al*., 2010), the solution for virus treatments is still not available. Till now, good management in practice has been considered as the only way to prevent viral outbreaks (Sánchez-Martínez *et al*., 1997).

Tropical storm is characterized by a low-pressure center and numerous thunderstorms that produce strong winds and heavy rain. Under certain circumstances, tropical depression and typhoon are also being considered as a part of tropical storm. In China, the majority of tropical storms form between June and November, and Guangdong province is the most affected province. The main destructive impacts of the tropical storm include heavy rain, strong wind, large storm surges at landfall, and tornadoes. Tropical storms represent threats to aquaculture facilities, particularly those operating offshore, in bays or in coastal lagoons. Larger waves, salinity changes, oxygen decreases, structural damage, and facility damage could cause the most serious damages to aquaculture systems (Barange and Perry, 2009). After a tropical storm, diseases such as WSSV are likely to explode in the shrimp ponds. Although this phenomenon has been frequently reported by farmers, till now, the actual mechanisms are still uncertain, and whether the tropical storm plays an important role in the relationship between WSSV loads and water quality characterizations in shrimp culture ponds is worth studying.

Materials and Methods

The present experiment was carried out in three ponds at a commercial farm of Shanwei, Guangdong, China. The testing ponds were 0.22 hectare each, and HDPE lined (high density polyethylene). In each pond, aeration was produced by four paddlewheel aerators (each 1.5 kw), two jet aerators (each 1.5 kw), and air diffusers. *Litopenaeus vannamei* post larvae were stocked in each pond at a density of 2.7×10^7 on July 31, 2010. The operations in these three ponds were considered to be the same as each other, except water exchange that varied slightly.

The tropical storm began to affect the farm on 18th Sept., 2010, and landed on the night of 19th Sept., 2010 with rainstorm. Sample collections were conducted in the testing ponds between 15th and 29th Sept., 2010 over the entire tropical storm period. At each sampling day, 15 live shrimp were randomly sampled from each pond. Then, the gills were dissected and preserved for the WSSV detection by real time PCR assay.

Genomic DNA of gills was isolated from gills using the genomic DNA rapid extraction kit (Tiangen, China) following the protocol of the manufacturer, and preserved at -20°C for the WSSV detection by real time PCR assay. DNA quantity, purity and integrity were verified spectrophotometrically (A_{260}/A_{280}) and by electrophoresis on 1.0% agarose gels. The detection system of WSSV used in this study was consistent with the study conducted by Durand and Lightner (2002) (Table 1). The RT-PCR was carried out in a total volume of 20 µL, 10 µL 2 × *TaqMan* Universal PCR Master Mix (Takara), forward and reverse primers 0.5 µL (10 µmol/L), respectively, *TaqMan* probes 0.5 µL (5 µmol/L), DNA 2 µL, and ddH_2O 6.5 µL. The PCR program was 95°C for 30 s, then 40 cycles of 95°C for 5 s, 55°C for 15 s and 72°C for 30 s. PCR and data analyses were performed on an Eppendorf Mastercycler Realplex Real Time PCR System. A series of dilutions from the WSSV recombinant plasmid (the virus load known) was prepared as standards for quantification.

Water quality parameters, such as water temperature, dissolved oxygen (DO), pH and salinity were monitored at 09:00 and 5:00 on a daily basis. NH_4-N, NO_2-N, heterotrophic bacteria and *Vibrio* were measured according to the procedures of the National Specification for Marine Monitoring (SOA, 2007) (Table 2). Heterotrophic bacteria and *Vibrio* spp. load in each pond were determined using the spread plate count method according to the procedures of the National Specification for Marine Monitoring (SOA, 2007) (Table 2). The water sample was diluted within a 10-time doubling dilution and then transferred to Marine Agar (Difco 2216E) Chromogenic Medium and *Vibrio* spp. thiosulfate citrate bilesalt sucrose (TCBS) Chromogenic Medium (Qingdao Hope Bio-Technology Co. Ltd., Qingdao City, China) and incubated at 33°C. After 48 h incubation, the bacterial colony forming units (heterotrophic bacteria, *Vibrio* spp.) were counted according to the method described by Anand *et al*. (2013).

Results

Tropical storm is a main factor that influences aquaculture which always appears in summer, causing serious damage to shrimp aquaculture. After tropical storm, shrimp aquaculture environment including various physical, chemical and biological factors will occur, such abrupt changes has deleterious effects on shrimps. In this study, Table 3 and Fig. 1 summarize daily water physiochemical factors, measured over the entire study. All daily readings in the present study were within the accepted range for commercial production of juvenile shrimp. Significant drops were observed in DO and temperature when tropical storm arrived, which might be due to the heavy rain during the tropical storm causing planktonic algaes photosynthesis to weaken. On 20th Sept., 2010, the DO was significantly lower in morning in pond 6 (2.92 mg L^{-1}) and 8 (3.65 mg L^{-1}) than before tropical storm (4-5 mg L^{-1}). The temperature fell 4.3°C in pond 4, 4.5°C in pond 6 and 4.2°C in pond 8 within 3 days. No significant changes were found in pH and salinity ($P>0.05$).

Ammonia can affect the immune response, growth and molting, oxygen consumption and ammonia excretion of crustaceans. Ammonia-N, the principal end

Table 1: The sequences of white spot syndrome virus (WSSV) primers and *TaqMan* probes (Durand and Lightner, 2002)

Primers	Sequences of forward and reverse primers
Upstream primers	5´-TGGTCCCGTCCTCATCTCAG-3´
Downstream primers	5´-GCTGCCTTGCCGGAAATTA-3´
TaqMan probes	5´-AGCCATGAAGAATGCCGTCTATCACACA-3´

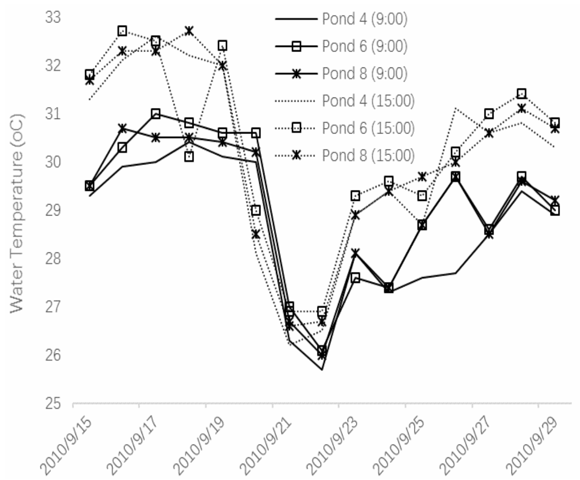
Table 2: Water quality parameters analyzed and monitoring method

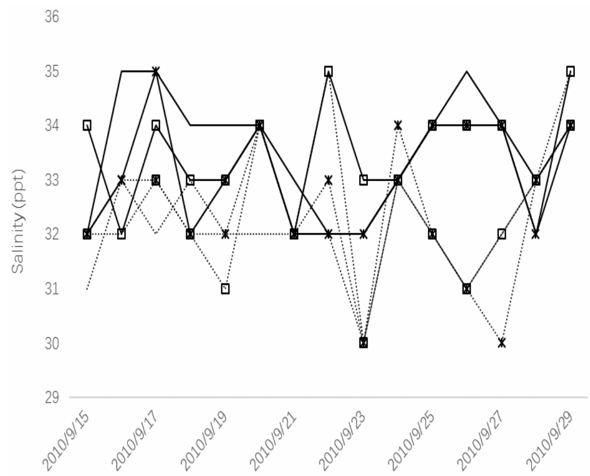
Parameter	Method
Water temperature	YSI model 550 (YSI, USA)
Dissolved oxygen	YSI model 550 (YSI, USA)
Salinity	CDT1-WYT-II (Beijing Midwest Group, Beijing)
pH	PHB-3 (Shanghai San-Xin Instrumentation Inc., Shanghai)
NH_4-N	Indophenol blue spectrophotometric method (SOA, 2007)
NO_2-N	N-(1-naphthyl)-ethylenediamine dihydrochloride spectrophotometric method (SOA, 2007)
Heterotrophic bacteria	Spread plate method on mrine aar (Difco 2216) (SOA, 2007)
Vibrio	Spread plate method on thiosulfate-citrate-bilesalt-sucrose (TCBS) (SOA, 2007)

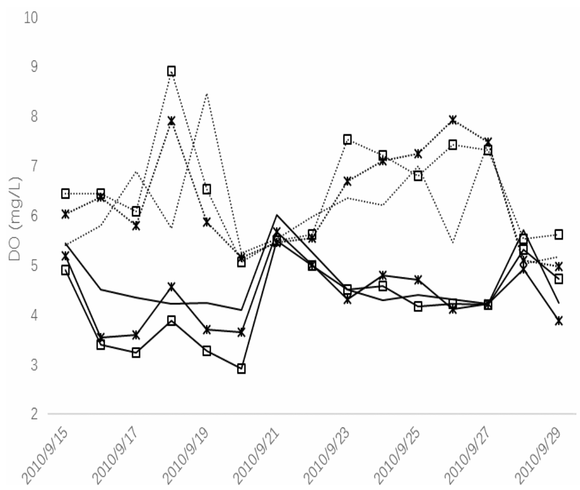
Table 3: Weather condition

Time	Weather						
	2010/9/15	2010/9/16	2010/9/17	2010/9/18	2010/9/19	2010/9/20	2010/9/21
9:00	sunny	sunny	sunny	overcast	light rain	heavy rain	heavy rain
15:00	sunny	sunny	cloudy	overcast	moderate rain	rainstorm	moderate rain

Time	Weather							
	2010/9/22	2010/9/23	2010/9/24	2010/9/25	2010/9/26	2010/9/27	2010/9/28	2010/9/29
9:00	light rain	Mostly Sunny	overcast	sunny	sunny	sunny	sunny	sunny
15:00	overcast	Mostly Sunny	overcast	sunny	sunny	sunny	sunny	sunny







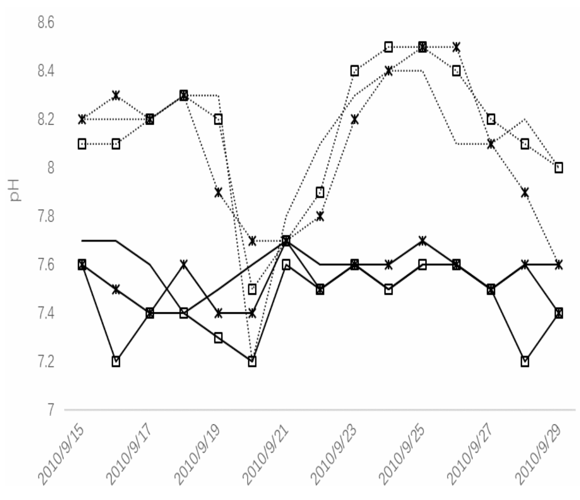


Fig. 1: Variations of water temperature, DO, pH, and salinity during sample period in 3 ponds

product of protein catabolism, is one of the important environmental toxic factors in pond. In this study, both ammonia-N and NO_2-N showed temporal variation in the testing ponds through the tropical storm period (Fig. 2). Ammonia-N levels fluctuated substantially, between 0.066 and 0.236 mg L^{-1}, and reached a peak when tropical storm arrived. Through the entire study period, a slight increase of NO_2-N was observed in all the testing ponds when tropical storm arrived. However, the development trends of NO_2-N varied between the testing ponds. The concentration of NO_2-N in pond 4 and 6 increased with tropic storm and dropped slightly after the tropical storm passed, while a substantial increment in pond 8 was observed. These facts suggested that tropical storm could affect the variation of ammonium-N and NO_2-N levels in shrimp ponds, which might be because the decline of DO and water temperature disturbed the metabolism of nitrobacterias and blockedthe nitrogen cycle, causing accumulation of ammonia-N and NO_2-N.

The developments of heterotrophic bacteria in three ponds showed similar development trends as ammonia-N over the tropical storm period (Fig. 2). The concentrations of heterotrophic bacteria reached a peak when tropical storm arrived, and dropped after the tropic storm passed. A slight increase of *Vibrio* was detected in pond 4 and 8 when tropical storm arrived, while there was a substantial increase in pond 6. These results indicated that when the tropical storm made landfall, the changes of the water quality environment likely stimulated the breeding of pathogenic microorganisms, thereby causing

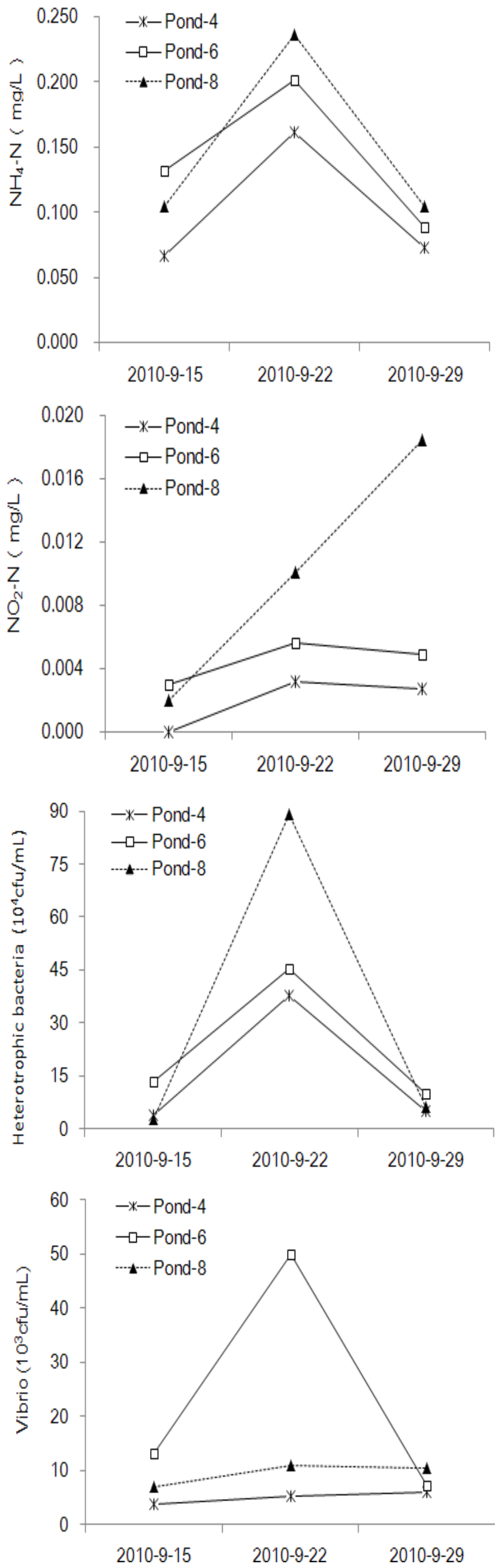


Fig. 2: Variations of ammonia-N, nitrite-N, heterotrophic bacteria, and *Vibrio* concentrations during sample period

vibriosis to occur in shrimp cultivation ponds.

Typhoon has a significant effect on the numbers of copies of WSSV in gills (Fig. 3). Before the typhoon, the viral loads in gill were 4.6×10^4, 2.4×10^4 and 1.1×10^5 copies g^{-1} in three testing ponds (pond 4, pond 6, and pond 8), respectively (Fig. 3). After this, viral loads dropped when typhoon landfall occurred, and the viral loads were 1.4×10^4, 1.9×10^4 and 1.7×10^4 copies g^{-1} in the selected experimental ponds. A substantial increase in viral loads occurred in pond 6 and 8 when typhoon passed a week. The number of copies of WSSV rose to 9.4×10^6 and 3.0×10^7 in these two ponds. There was a slight increase of viral load in pond 4.

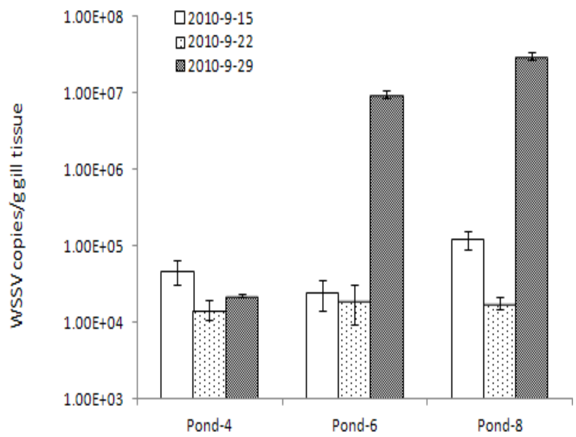


Fig. 3: The numbers of copies of WSSV in gills of shrimp *Litopenaeus vannamei* during sample period

Discussion

Strong evidence supports the hypothesis that the amplification of viral and onset of disease can be induced by environmental or physiochemical stress, such as salinity, temperature, oxygen, hardness, among others (Tsai *et al.*, 1999; Kautsky *et al.*, 2000; Vidal *et al.*, 2001; Guan *et al.*, 2003; Yu *et al.*, 2003; Lotz *et al.*, 2005; Liu *et al.*, 2006; Peinado-Guevara and López-Meyer, 2006; Rahman *et al.*, 2006; Sánchez-Martínez *et al.*, 2007; Ruiz-Velazco *et al.*, 2010; Tendencia and Peña, 2010). Low WSSV copies were observed when typhoon made landfall. During the landfall, low temperature, high heterotrophic bacteria count, and high level ammonia-N were recorded in this study. After typhoon passed a week, significantly high WSSV copies in pond 6 and 8 were observed when the temperature raised, and the heterotrophic bacteria count and ammonia-N level decreased. Previous studies suggested that low temperature, high heterotrophic bacteria count and high oxygen concentration are inhibitory factors for WSSV (Tendencia *et al.*, 2010; Tendencia and Verreth, 2011). Shrimp is likely to suffer diseases outbreak, especially WSSV, after tropical storm passed, the substantial variations of oxygen concentration, temperature, heterotrophic bacteria count and ammonia-N level are likely to trigger the disease outbreak. Since the heterotrophic bacteria in the rearing environment are more efficient in removing nitrogen from the water than plants and autotrophic bacterial (Avnimelech, 2009). Keeping a high level of oxygen concentration and promoting heterotrophic bacteria growth are two important methods to prevent disease outbreak after tropical storm.

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