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Seasonal status of white spot syndrome virus in broodstocks, nauplii and postlarvae of black tiger shrimp (*Penaeus monodon*) in Bangladesh

Iqbal MM[1*], Kabir MA[2], Alan CB[3], Mamun MAA[4] and Hossain MM[5]

[1]Department of Fisheries Biology and Genetics, [4]Department of Fish Health Management, [5]Department of Coastal and Marine Fisheries Faculty of Fisheries, Sylhet Agricultural University, Sylhet, Bangladesh; [2]School of Biological Sciences, University Sains Malaysia, Penang-11800, Malaysia; [3]WorldFish Center, Colombia.

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ABSTRACT

Seasonal variation of white spot syndrome virus (WSSV) prevalence in broodstocks shrimp, nauplii and postlarvae were investigated from 2004 to 2006 in Bangladesh by polymerase chain reaction (PCR) assay. There was consistent pattern of fluctuation of WSSV prevalence in broodstocks, nauplii and postlarvae. WSSV fluctuation pattern in three stages were more or less similar for each year. Average lowest prevalence in broodstock was 0% in September and highest was 90% in May-June during the monsoon season in Bangladesh. The WSSV fluctuation patterns for nauplius correlated with broodstock patterns with average lowest prevalence of 0% in September compared to the highest of 40% in July. Similarly, average lowest prevalence of WSSV in postlarvae was 0% in December and highest was 14% in July. It was evident from this study that WSSV prevalence in broodstock, nauplius and postlarvae remained high during the monsoon season and low during the winter season when the water current, salinity and tidal flashing remained more or less stable in the Bay of Bengal in Bangladesh.

Key words: Bangladesh, broodstocks, nauplii, postlarvae, seasonal, shrimp, WSSV.

INTRODUCTION

White spot syndrome (WSS) caused by WSS DNA virus has seriously affected farmed penaeid shrimp. Susceptible species includes *Penaeus. monodon, P. vannamei, P. stylirostris, P. japonicus, P. chinensis, P indicus, P. merguiensis, P. setiferus, and P. penicillatus* [1,2,3]. This virus has also been detected from non penaeid shrimps, crabs, lobsters etc. [4,5,6,7]. The disease is worldwide spread and is being fought in both the western and the eastern hemispheres, especially in Southeast Asia, North America and Latin America [8,9]. WSSV is an extremely virulent to cultured shrimp, which causes 100% mortality within few days from the onset of symptoms and are notoriously hard to predict [10,11,12]. Hence, it is regarded as a C-1 category pathogen [13]. This virus causes catastrophic economic losses on shrimp farms. Its presence is considered a potential threat to shrimp industry. The disease typically occurs in juvenile shrimp but sometimes manifests itself in later adult stages.

The first occurrence of WSSV was in Chinese Taipei and Chinese mainland between 1991 and 1992 [10]. Subsequently, the virus spread to Japan in 1993 through importation of prawns from China [14]. Korea was first experienced with this disease in 1993 [15]. Then the disease spread to Thailand in 1994 [16], Malaysia in 1994 [17], India in 1994 [18], Bangladesh in 1994 [19] and Philippines in 1999 [20]. WSSV has spread widely in the world not only in Asian countries, but also in the USA [21], Central America and South America [22].

This disease is thought to be transmitted vertically from broodstock to the offspring in the hatchery and horizontally through water or infected hosts to the healthy animals in the farm. Wild black tiger broodstock shrimp are being caught live by the fishermen as an adjunct to normal fishing activities from the main fishing grounds of the Bay of Bengal (Fig. 1). They subsequently communicate with the brokers who collect the captured shrimp for distribution to shrimp hatchery operators.

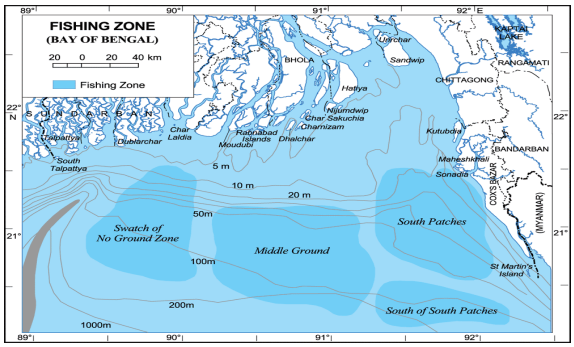

Fig. 1. Four major fishing grounds of Bangladesh in the Bay of Bengal

In Bangladesh, hatcheries are normally started to operate their production from January and continue to August depending upon the demand for postlarvae; although some hatcheries operate round

*Corresponding author: mmiqbal5@yahoo.com

the year. In the beginning of the year the broodstock seems to be apparently healthy and good number of postlarvae can be produced. But with the time especially during the monsoon period from July to September, the broodstock received from the trawlers appear weak and often pale in color which tends to result in poorer postlarvae quality and less in number per weight of broodstock. These times are believed to be more susceptible to various diseases in black tiger shrimp.

Still now there is no monthly status of WSSV in the broodstock of black tiger shrimp in the Bay of Bengal of Bangladesh. But data on WSSV monthly variations in the broodstocks would be important to develop strategies for health management. So, the objective of the present study was to investigate the monthly status of WSSV in the broodstock, nauplius and postlarvae of black tiger shrimp in Bangladesh.

MATERIALS AND METHODS

Shrimp Quality Support Project (SQSP) funded by USAID and implemented by WorldFish Center providing PCR screening facilities for WSSV of black tiger shrimp in Bangladesh, which is the only running laboratory in the country. For this study, data were collected from the PCR laboratory for over 3 years (2004 to 2006) for broodstock, nauplius and postlarvae from some of 10 hatcheries located in Cox's Bazar. Samples were collected from the respected hatcheries whenever they were available.

Sample collection and DNA extraction

Postspawn broods, nauplii and postlarvae samples were randomly collected from different hatcheries in Cox's Bazar over 3 years period from 2004 to 2006. A tip of pleopod was excised from each brood with a red-hot sterile forceps and scissor aseptically and put on eppendorf tube. Their nuplii were collected between 24 to 36 hrs after spawning. Subsequently their postlarvae were collected at PL7/8 stages. DNA was extracted according to the protocol of IQ 2000 WSSV detection and prevention system. Briefly, 20-30 mg samples were homogenized with lysis buffer provided in the IQ 2000 WSSV detection system kit. After 5 min of incubation at 100^{o}C and brief cool down in ice flakes samples were centrifuged at 1500X g for 10 min. After collecting the liquid phage DNA was precipitated by absolute ethanol. Finally, the pellet was dissolved in sterile distilled water, used as PCR template.

PCR and gel analysis

PCR was performed using the method described in the protocol of IQ 2000 WSSV detection and prevention system. Briefly, 2 µl of each samples were added to 8 µl of the PCR reaction mixtures containing 7.5 µl 1st PCR pre mix and 0.5µl of *Taq* DNA polymerase. The reaction was then carried out for 2 sets of PCR profiles in an automated thermocycler (Applied Biosystems, GeneAmp PCR system 9700). Five cycles comprising denaturation (94^{o}C 30 seconds), annealing (62^{o}C 30 seconds) and polymerization (72^{o}C 30 seconds) in the 1st set and 15 cycles comprising denaturation (94^{o}C 15 seconds), annealing (62^{o}C 15 seconds) and polymerization (72^{o}C 20 seconds) in the 2nd set. Then 15 µl of nested PCR reaction mixture containing 14 µl of nested pre mixture and 1 µl of *Taq* DNA polymerase was added to the 1st PCR product. Nested PCR was done for 25 cycles comprising denaturation (94^{o}C 20 seconds), annealing (62^{o}C 20seconds) and polymerization (72^{o}C 30 seconds). The PCR amplification products were separated electrophoretically by loading 10 µl in 1.5% agarorose gel with 1 X TAE (Tris-Acetate-EDTA) buffer. The gel was staining using ethidium bromide solution (1 µg/ml) for 10 min and after brief washing with distilled water the bands were visualized on a UV transilluminator. A marker provided in the IQ 2000 WSSV detection and prevention system was parallel used with the samples.

RESULTS AND DISCUSSION

Agarose gel analysis of various stages of WSSV infection is shown in Fig 2. The WSSV positive data presented here in the study were severe, moderate, light or very light positive. Three years monthly status of WSSV in the broodstock shrimp, nauplius and postlarvae are illustrated in Fig 3, 4 & 5. In general, the monthly proportion for three years of WSSV-positive broodstock, nauplius and postlarvae were more or less varied.

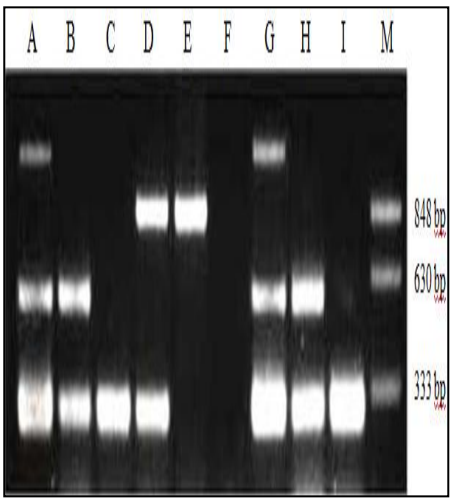

Fig. 2. Gel electrophoresis analysis of various stages of WSSV infection with negative control and standard solution [Lane A: Sample with severe WSSV infection, Lane B: Sample with moderate WSSV infection, Lane C: Sample with light WSSV infection, Lane D: Sample with very light WSSV infection, Lane E: WSSV negative sample, Lane F: Negative control (ddH_2O), Lane G: WSSV (+ve) standard, 2000 copies/reaction, Lane H: WSSV (+ve) standard, 200 copies/reaction, Lane I: WSSV (+ve) standard, 20 copies/reaction, Lane M: Molecular weight marker].

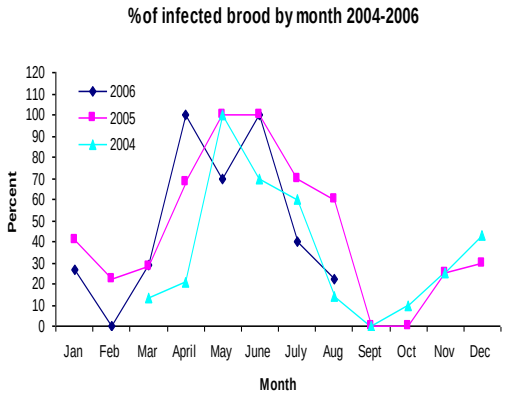

Fig. 3. Three years monthly status of WSSV-positive broodstock

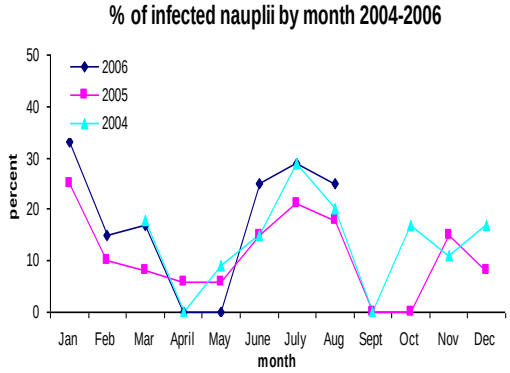

Fig. 4. Three years monthly status of WSSV-positive nauplius

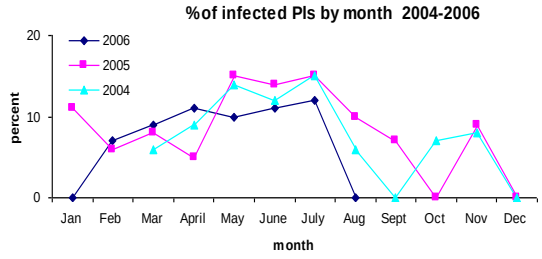

Fig. 5. Three years monthly status of WSSV-positive postlarvae

But seasonal patterns of WSSV prevalence for each stage (broodstock, nauplius and postlarvae) were similar. Monthly variation in three years average prevalence of WSSV in the broodstock is shown in Fig 6. The lowest and highest average prevalence for the broodstock were found 0 and 90 percent, respectively. The prevalence in the broodstock gradually increased from February and reached to its peak in the months of May and June.

It is not known why the prevalence of WSSV infection was high during May and June. However, these months in Bangladesh generally covers the monsoon season with frequent storms and heavy rainfall in the Bay of Bengal in Bangladesh, where the fishermen capture the wild broodstock shrimps. Strong seawater currents forced the broodstock to aggregate in certain 'safe' areas, causing population congestion and therefore increasing the chance of horizontal transmission of WSSV into shrimp [23].

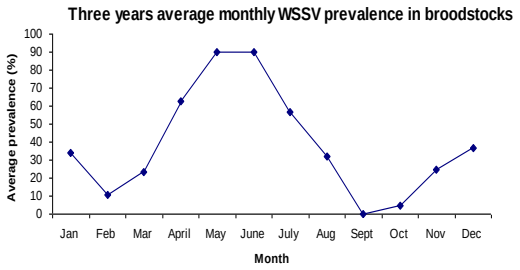

Fig. 6. Monthly variation in three years average prevalence of WSSV in the broodstock

Another possibility is that broodstock catchers are preferred to trawl in shallow (e.g. 10 to 20 m depth) water during monsoon season because rough sea may not permit them to trawl from the deep sea (e.g. 50 to 70 m depth). Such inshore broodstock populations might contain large numbers of WSSV infected broodstock. Population dynamics (e.g. mortality, growth, reproduction and movement) of wild shrimp are regulated by environmental and ecological factors. Changes in environmental factors such as temperature or salinity may adversely affect in wild shrimp population. When changes in environmental factors occur in combination, interactions among stressors may be extremely important. Exposure to extremes of a single factor may be tolerated; however combinations may be adverse or even lethal. Under these conditions, wild shrimp exposure to WSSV may predispose the host to infection. Viruses and their hosts interact in complex ways within the marine environment. WSSV interacts with shrimp depending upon environmental condition and physiology of the host. How natural factors influence this interaction is essentially unknown, particularly because the antiviral immunity of the shrimp is poorly understood. Greatest mortality from WSSV occurrence in shrimp happened just during season changes or when it was observed noticeable changes in temperature [24]. Predictive factors to be considered in determining the prevalence of WSSV in shrimp include rainfall, water current, salinity, tidal flushing etc.

Warmer temperatures, changing water current including heavy rainfall, especially those prevailing during the monsoon season, favor the multiplication and survival of WSSV in the shrimp. Most of the shrimp affected by WSSV found from this study occurred in the monsoon season. On the other hand, prevalence of WSSV in broodstock shrimp is low during the winter season when the salinity in the sea is apparently stable and high as well as tidal flushing and sea storms are absent. Rapid decrease in salinity, mainly caused by rain, may trigger virus infection, or generate the appearance of gross clinical signs when

the virus was already present, but shrimp were asymptomatic [25, 26]. It is therefore possible that environmental factors such as rainfall, sea water current and salinity levels known to promote the multiplication of WSSV in the broodstock shrimp in Bangladesh.

Three years average prevalence for WSSV in nauplius is shown in Fig 7. Incidences for WSSV in the nauplius were varied from 0 to 40%. WSSV prevalence in nauplius was increased from February and remained high in July, which reflect the status of WSSV in the wild broodstock shrimp. Since WSSV is a DNA virus and can infect oocytes and follicle cells in the connective tissue in the ovary, it has been suggested that the virus can be transmitted vertically from broodstock to the offspring [27, 28].

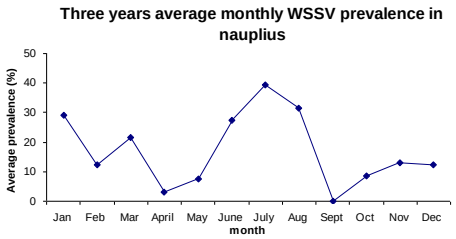

Fig. 7. Monthly variation in three years average prevalence of WSSV in the nauplius

Although the patterns are similar but the present study revealed that WSSV prevalence in the broodstock was in peak estimating 90% during May-June period. On the other hand, highest prevalence in nauplius was 40% in July. It is generally believed that this virus sticks to the outside of the shrimp eggs. Hatchery technicians are generally washed nauplius with different disinfectants before transferred to the larval rearing tank (LRT). Effective washing through various disinfectants might be successfully prevented its transmission from positive broodstock to their nauplius. It was found that sodium hypochlorite and isodine of halogenous disinfectants were useful in the protection or prevention of WSS viral disease [29]. Hence prevalence of WSSV in the nauplius was relatively low compared to the wild broodstock shrimp.

Table 1 represents the three years average WSSV prevalence in the postlarvae. The lowest prevalence was found 0% in December and the highest was 14% in July. WSSV prevalence in the postlarvae was increased slowly from January to April (6% to 8%) and then rapidly from April to July (8% to 14%). It is revealed from this study (Table 1) that WSSV prevalence in the postlarvae remained high during the rainy season, when the broodstock as well as their nauplius were highly susceptible to WSSV.

This investigation looked into the seasonal status of WSSV in broodstock, nauplius and postlarvae of the black tiger shrimp in Bangladesh. It was evident from this study that WSSV prevalence was high in all stages during the monsoon season. Even though the reason for this outbreak is unclear, rainfall, seawater currents, tidal flushing and salinity fluctuations appear to be crucial factors for triggering the multiplication of this virus into shrimp. Apparently healthy broodstock as well as healthy postlarvae batches are found relatively more before and after monsoon season in Bangladesh.

Table 1. Three years average WSSV prevalence in postlarvae

	WSSV prevalence (%) in postlarvae			
Month	**2006**	**2005**	**2004**	**Average**
Jan	0	11	Nd*	6
Feb	7	6	Nd	7
Mar	9	8	6	8
April	11	5	9	8
May	10	15	14	13
June	11	14	12	12
July	12	15	15	14
Aug	0	10	6	5
Sept	Nd	7	0	4
Oct	Nd	0	7	4
Nov	Nd	9	8	9
Dec	Nd	0	0	0

*Nd: Not done

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