Prevalence of White Spot Syndrome Virus in Brood stock, Nauplii and Post-larvae of Tiger shrimp (Penaeus monodon Fabricius, 1798) in Bangladesh

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Prevalence of White Spot Syndrome Virus in Brood stock, Nauplii and Post-larvae of Tiger shrimp (*Penaeus monodon* Fabricius, 1798) in Bangladesh

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ABSTRACT

Bangladesh enters into international shrimp market in early 1970s but now it takes second places in the source of foreign currency earning. Now this sector facing a serious problem with a disease named White Spot Syndrome Virus which causes 100% mortality in the shrimp farm within two weeks. In Bangladesh, outbreak of White Spot Syndrome Virus has been a serious problem since 1995. In the present study, it was observed the present situation of White Spot Syndrome Virus prevalence in wild tiger shrimp brood, nauplii and post-larvae in Bangladesh. The result shows that prevalence of White Spot Syndrome Virus in wild tiger shrimp brood was in decreasing trend, but post-larvae during the study period. White Spot Syndrome Virus prevalence in tiger shrimp brood, nauplii and post larvae was found highest in the month of March and April and after that it was decreasing up to July and again increases in the month of August.

Keywords: Prevalence, Polymerase Chain Reaction, Deoxyribonucleic Acid, *Penaeus monodon,* Brood, Nauplii, Post Larvae, White Spot Syndrome Virus

1. INTRODUCTION

Bangladesh's vast coastal belt and conducive environment has led to the rapid expansion of shrimp farming activities. The tiger shrimp *Peneaus monodon* contributes a major share to shrimp production in Bangladesh. . Due to increase in demand and price, shrimp culture started to expand and presently, land under shrimp culture has increased to about 141352.54 ha to 52000 ha estimated at the year 2001-2002 [1]. In Bangladesh, tiger shrimp hatcheries normally start to operate their production from January and continue to August depending upon the demand for postlarvae; although some tiger shrimp hatcheries operate round the year. About 67 tiger shrimp hatcheries operate in Cox's Bazar to supply tiger shrimp post-larvae to the tiger shrimp farmers in Khulna, Satkhira, Bagerhat and Cox's Bazar. According to Shrimp Hatchery Association of Bangladesh (personal communication) the total demand of tiger shrimp post-larvae is about 8000 million and present annual production is about 6000-7000 million where capable to produce 15000 million. The tiger shrimp hatcheries have just produced PL and sale it locally in Cox's Bazar or to Satkhira and Bagerhat market. As they produce huge amount of PL, almost all of the tiger shrimp hatcheries design followed by large scale production system. As production of PL is less than the demand tiger shrimp hatchery owners have no headache about the health assessment of tiger shrimp broods or PLs.

There are more than 1100 viruses of invertebrates have been reported [2]. More than 30 viral diseases are now known to occur in crustaceans, including penaeids. Major groups of viruses reported in crustacean include Reoviridae, Picornaviridae, Parvoviridae, Paramyxoviridae, Rhabdoviridae and Iridoviridae [3]. It is reported that at least 13 viral diseases of cultured penaeid shrimp are now recognized [4, 5]. They include : *Penaeus monodon* type baculovirus (MBV) [6], which includes Plebejus baculovirus (PBV) [7] , Baculovirus penaei (BP) [8], Baculoviral midgut gland necrosis virus (BMNV) [9] , Type C baculovirus of *P. monodon* (TCBV) [10], Haemocyete-infecting baculovirus of *P. monodon* and *P. esculentus* (HB) [4], Infectious hypodermal and haematopoietic necrosis virus (IHHNV) [11], Hepatopancreatic parvo-like virus (HPV) [12], Lymphoidal parvo-like virus (LOPV) [13], Lymphoid organ vacuolization virus (LOW) [14], Reo virus: Reo – 3 [14], Reo virus: Reo – 3 [15], Yellow Head virus (YHV) [16] and White spot diseases virus or Systemic Ectodermal and Mesodermal Baculovirus [17].

Recently, disease outbreaks have caused mass mortalities among cultured penaeid shrimps worldwide, especially in Asian countries [18]. White spot viral disease has caused high mortalities and severe damage to the shrimp culture industry in China [19], Thailand [17], Japan [20], Taiwan [21], Indonesia and India [22]. The causative agent, white spot syndrome virus (WSSV) is a bacilliform, double-stranded DNA virus [21, 5]. The entire genome sequence of the virus has recently been reported. It is a 292.976 kb long, circular, double-stranded DNA representing an entirely new virus family name 'Nimaviridae' has been proposed [23]. WSSV has a wide host range, affects almost all species of cultured shrimp [24] and is extremely dangerous because of its ability to cause mass mortalities in culture ponds. Hence, it is regarded as a C-1 category pathogen [25] There is evidence of wssv infection in the reproductive tissue of male and female *Penaeus monodon* broodstock [26, 27] and the post-larvae (PL) from infected *Penaeus monodon* broodstock have been found to be infected with WSSV [28]. Hence *Penaeus monodon* PL are considered as a major entry route for WSSV into culture ponds [29-31].

Wang *et al.* [32] classified the white spot viral infection into two types. Type I is an acute infection that causes high mortality within two weeks in species such as *P. monodon, P. indicus* and *P. penicillatus,* as described by Chou et al. [33] and Nakano *et al*. [34]. Type II is latent and the individuals harboring this specific virus remain alive as in the case of *Macrobrachium* sp., wild crabs and wild lobsters (and also do not exhibit any disease symptoms) as described by Peng *et al*. [35].

In Bangladesh, an outbreak of WSSV of cultured black tiger shrimp occurred in semi-intensive farms in Cox's Bazar the southeastern part in 1994 [36] and quickly spread to Khulna the southwestern part of the country. Since then shrimp farming industry in the country has been seriously affected by WSSV resulting in sustainable loss in every year. Shrimp productions from a unit area have also been declined since its outbreak of WSSV in Bangladesh. According to the Department of Fisheries (DoF), Bangladesh suffered a 44.3% production loss in 1996, leading to a reduction in foreign income of 42.3% from shrimp exports [37]. The total shrimp farming area in Bangladesh is about 160,000 ha [38] and most of the farms are traditional extensive typically yields 120kg/ha/yr [39]. WSSV 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 [26, 40]. Wild black tiger broodstock shrimp are being caught by the industrial trawlers as an adjunct to normal fishing activities mainly for export market from the four major fishing grounds of the Bay of Bengal. They subsequently communicate with the brokers who collect the captured shrimp for distribution to shrimp hatchery operators.

But farmers are suffering with unhealthy tiger shrimp PL mainly with WSSV. WSSV is severely affected tiger shrimp ghers with 100% mortality within two weeks. There is no way to cure the WSSV affected farm without taking preventive measure. Transmission of WSSV can be done in two ways. One is vertically i.e. from mother to offspring and another is horizontally i.e. from environment. So the farmers have to use WSSV free PL and WSSV free environment.

The present study was done to know the present status of WSSV prevalence in broods, nauplii and post-larvae of *P. monodon* and to determine whether there were any consistent fluctuations in prevalence of WSSV with season that might help to develop proper strategies for tiger shrimp health management in the country.

2. MATERIAL AND METHODS

The study was conducted in Cox's Bazar as about all Bagda hatchery situated in Cox's Bazar. The study was conducted from January, 2008 to August, 2010. Six hatcheries were selected for the study. Samples of brood tiger shrimp (a tip of pleopod, after spawning), nauplii, shrimp larvae (Early PL, Mid PL and PL-15) were collected from six hatcheries. DNA was extracted according to the protocol of IQ 2000 WSSV detection and prevention system. For WSSV detection nested PCR method was used following the PCR protocol of protocol of IQ 2000 WSSV detection and prevention system at the laboratory of Bangladesh Fisheries Research Institute, Marine Fisheries & Technology station, Cox's Bazar.

3. RESULTS

From the data of PCR test, it was found that in case of tiger shrimp brood highest WSSV prevalence was found 100% in the month of February, 2008 and lowest WSSV prevalence was found 25% in the month of June, 2008. In 2009, highest WSSV prevalence in brood was found 83% in the month of April and lowest was found 15% in the month of February. In 2010, highest WSSV prevalence in brood was found 70% in the month of July and lowest was found 11% in the month of February. (Fig.1)

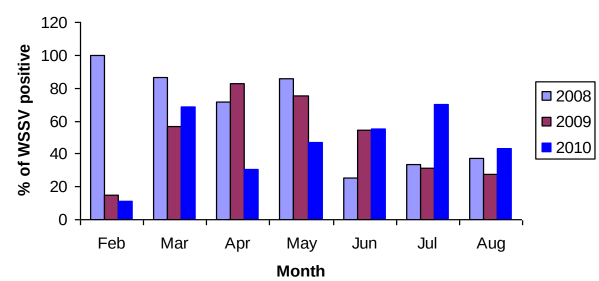

Fig. 1 Three years monthly status of WSSV-positive brood stock

Table 1: Year wise status of WSSV in brood stock

Year	Total no. of sample	WSSV negative	WSSV positive	% WSSV positive
2008	258	71	187	72
2009	338	146	192	57
2010	358	157	201	56

From the data of nauplii, it was found that highest WSSV prevalence was 33% in the months of April and August, 2008 and lowest was 10% in the month of May, 2008. In 2009, highest WSSV prevalence in nauplii was found 33% in the month of June and lowest was found 4% in the month of July. In 2010, highest WSSV prevalence in nauplii was found 45% in the month of March and lowest was found 8% in the month of July. (Fig. 2)

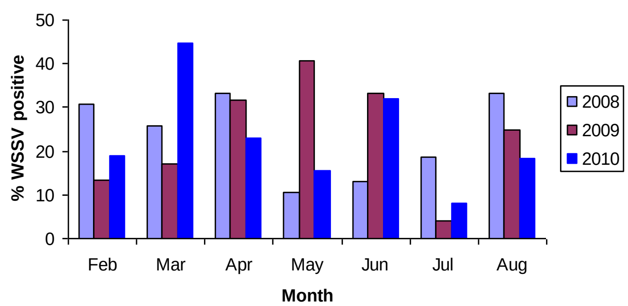

Fig. 2 Three years monthly status of WSSV-positive nauplii

From the data of PL, it was found that highest WSSV prevalence was 45% in the month of March, 2008 and lowest was 0% in the month of June, July and August, 2008. In 2009, prevalence of WSSV in PL was found 14% only in the month of June. In 2010, highest WSSV prevalence was found 21% in the month of August and lowest was 0% in the month of February and May. (Fig: 3)

Table 2: Year wise status of WSSV in nauplii

Year	Total no. of sample	WSSV negative	WSSV positive	% WSSV positive
2008	291	228	63	22
2009	328	245	83	25
2010	243	177	66	27

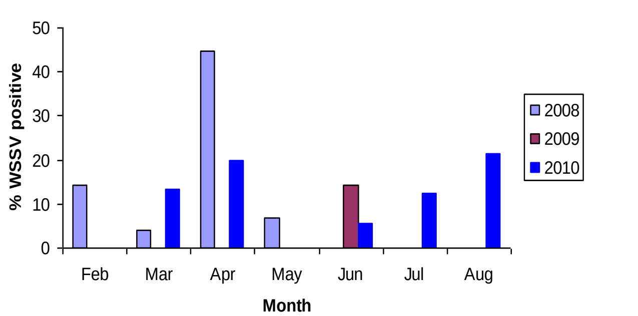

Fig. 3 Three years monthly status of WSSV-positive brood stock

From the three years average data it was found that highest WSSV prevalence in brood was 71% in the month of March and lowest was 25% in the month of February. In the case of nauplii, highest WSSV prevalence was found 30% in the month of March and April and lowest was 9% in the month of July. In the case of PL, highest WSSV prevalence was found 34% in the month of April and lowest was found 3% in the month of May and July. (Fig: 4)

Table 3: Year wise status of WSSV in PL

Year	Total no. of sample	WSSV negative	WSSV positive	% WSSV positive
2008	156	138	18	12
2009	104	101	3	3
2010	109	100	9	8

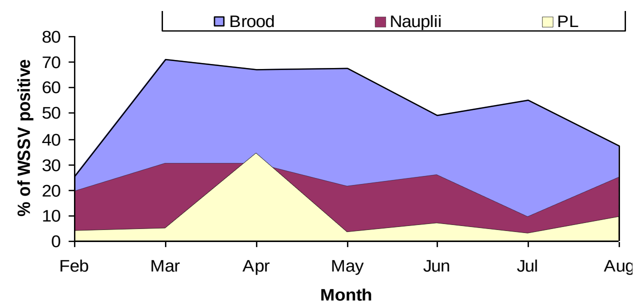

Fig. 4 Monthly variation in three years average prevalence of WSSV in the brood stock nauplii and PL

4. DISCUSSION

From the result it was found that prevalence of WSSV in brood, nauplii and PL does not match with the result of 2008. In 2008, highest WSSV prevalence was found in the month of February where as in 2009 and 2010, lowest WSSV prevalence was found in February. The main reason may be the sample size of 2008. Another reason may be the environmental factors of 2008. To consider the result of February, 2008, the present situation of WSSV prevalence is slightly changed with the situation of last two years. Present years result shows that highest WSSV prevalence in brood stock found in the month of July. In February, WSSV prevalence in brood stock found lowest. After that it increases in the month of March, then decrease in the month of April and then it gradually increase from May to July and in August it decrease again. If the data was compared with the data of 2008, the situation found that WSSV prevalence in brood increased in the month of March and then decreased in the month of April and again increased in the month of May and then gradually increased from June to August. If the data was compared with the data of 2009, it was found that lowest WSSV prevalence in brood was found in the month of February, then it became to increase from the month of March to April and after that it gradually decreased from the month of May to August. From the overall yearly data of brood, it was found that prevalence of WSSV in brood remarkably decreased from 2008 to 2010. The reason of this may be the availability of quality brood. In 2007 and 2008, crisis of brood observed. For that reason, hatchery owners had no option to check the quality of brood; they bought what they got from the mother catching ship.

In case of nauplii, the last three years feature shows increasing trend i.e. prevalence of WSSV increasing from 2007 to 2010. But from the result of PL, it was found that WSSV prevalence occurred in first three months (February to April) of 2007 and after that it was nil. Prevalence of WSSV in PL was found only in one month (June) in 2009. But In 2010, it appears almost every month. The main reason for this situation may be horizontal transmission of WSSV because of improper management of the hatchery.

From the three years average data, it was found that WSSV prevalence became high in the month of March and April in brood, nauplii and PL. It is unknown conclusively why the prevalence of WSSV infection was high during March and April. However, certain assumptions and inferences may be proffered. These months mainly face sudden storm, high temperature, and sudden rainfall in the Bay of Bengal in Bangladesh. Withyachumnarnkul *et al.* [41] stated that 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. Another possibility is that broodstock catchers prefer to trawl in shallow water (e.g. 10 to 20 m depth) during monsoon season because rough sea may not permit them to trawl in 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, salinity and others 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. Snieszko [42] described that infectious diseases of fishes occur when susceptible fishes are exposed to virulent pathogens under certain environmental stress conditions. So, WSSV may interact 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. Chanratchakool [43] stated that greatest mortality from WSSV occurrence in shrimp happened at the time of seasonal changes or noticeable changes in seawater temperature in the Tumbes located around the northwestern part of Peru. Predictive factors to be considered in determining the prevalence of WSSV in shrimp include rainfall, water current, salinity, tidal flushing etc.

Three years average prevalence for WSSV in nauplii is shown in Fig 4. Incidences for WSSV in the nauplii varied from 9 to 30%. WSSV prevalence in nauplii increased from March and remained high in June, which reflect the status of WSSV in the wild broodstock shrimp. Lo *et al*. [26] and Tsai *et al*. [40] stated that WSSV, a DNA virus generally transmitted vertically from broodstock to the offspring through eggs via nauplii. They also found the virus particles in follicle cells, oogonia, developing oocytes and connective tissue in the broodstock shrimp

An important difference to note is that the prevalence of WSSV in postlarvae was much lower compared to the prevalence in either wild shrimp broodstock shrimp or their nauplii. Hatchery technicians in Bangladesh generally use different concentrations of formalin randomly in the postlarvae tank for controlling various parasitic diseases. This chemical may kill or eliminate weak postlarvae containing WSSV in the LRT. This evidence was justified by Limsuwan [29] who successfully reduced concentrations of WSSV infected postlarvae using formalin and PCR screening. Oseko *et al*. [44] also stated that WSSV could be inactivated by formalin, which thereafter reduced infection rates of healthy shrimp.

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