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The Effect of Temperature and Ph on WSSV Infection in Cultured Marine Shrimp *Penaeus monodon* (Fabricius)

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Abstract: White spot syndrome virus (WSSV) has been one of the major disease problems in shrimp culture around the world. The present study was conducted in shrimp farms to know the actual cause of WSSV and its eradication from the culture ponds. The first experimental farm is located on the Northern bank of Uppaanar Estuary in Thennampattinam village and the second experimental farm is located on the southern bank of Kavari back water in Vanagiri village. The pH recorded during the culture period in the farm 1 was from 7.7 to 9.5 and farm 2 it was from 7.6 to 9.7. The lowest temperature (23°C) was recorded in the month of December and the maximum was recorded (32°C) in the month of October from the farm 1. In the farm 2, the lowest temperature was observed (22°C) in the month of December where as it was higher (32°C) in the month of October. The dissolved oxygen was ranging between 3.0 to 4.0 ppm in the farm 1 and it was 3.1 to 4.2 in farm 2. Maximum population of yellow colony was recorded in the month of November and maximum of green colony was recorded in the month of December from the farm 1. However, in farm 2, the maximum yellow colony was recorded in the month of October and maximum green colony was recorded the month of December. Despite the fact that our present study clearly shows that lower temperature and high pH influence WSSV infection in cultured shrimps of *P. monodon*.

Key words: WSSV · *Penaeus monodon* · Virus · pH · Temperature · Dissolved oxygen

INTRODUCTION

Infectious diseases are a major constrain to shrimp aquaculture production in many countries. The rapid increase in culture areas since the 1980s facilitated spread and outbreaks of high number of pathogens, viruses in particular. Since its emergence in 1992 [1] white spot syndrome virus (WSSV) has been one of the major disease problems in shrimp culture around the world [2,3,4]. In culture penaeid shrimp, WSSV infections can cause a cumulative mortality up to 100% within 3-4 days [5]. Infected shrimp show lethargic behavior, loss of appetite, reddish discoloration and white spots in the exoskeleton composed of calcified deposits [1]. WSSV not only infects all shrimp species, but also a wide range of other decapod crustaceans [6]. Reports have described both acute and chronic WSSV infections which caused different rates of mortality in shrimp ponds [7] and under experimental conditions [8,9]. The disease by white spot viruses is a major concern in shrimp farms. So studies on WSSV are need of the hour to know the real cause of the disease. Most of the previous studies concern with WSSV is conducted only laboratory level that to in small scale. But in the present study was conducted in outdoor especially in large scale farms to know the actual cause of WSSV and its eradication from the culture ponds.

MATERIALS AND METHODS

Location of Experimental Farms: The first experimental farm is located on the Northern bank of Uppaanar Estuary in Thennampattinam village. The farm is situated about 16 km away from Sirkali. The southern side of the farm is elevated to a height of 3.5 m from Uppaanar estuary. The experiment was conducted from 25th October 2007 to 20th December 2007. The total area covered is 2.0 ha of which water spread area are about 1.5 ha. Totally two ponds are there, one pond size is 0.7 ha another pond size is 0 .8ha.

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The second experimental farm is located on the southern bank of Kavari back water in Vanagiri village. The farm is situated about 22 km away from Sirkali and 4 km away from Poombukar. The northern side of the farm is elevated to a height of 4.0 m from Kavari back water. The experiment was conducted from 1st October 2007 to 12th December 2007. The total area covered is 1.2 ha of which water spread area are about 1.7 ha. Totally two ponds are there, one pond size is 0.5 ha another pond size is 0 .7ha.

Culture Pond: The ponds are rectangular in shape and semi intensive type with stocking densities of 10 post larvae/m^2. The depth of pond was 1.2 m and pond bed slope 30 cm from inlet point towards outlet. Monk type outlet was constructed and it was opposite to the inlet. The dimension of the sluice was 2 m long, 0.7 m width and 2 m height. The shutter was made out of wooden planks, whereas the filter is made up of nylon mesh fitted in wooden frames. All ponds have a common drainage canal and the drainage canal is excavated on the eastern side of the ponds. The depth of drainage canal is constructed 2 feet below the culture pond bottom to facilitate easy flow of water from the individual ponds. The width of drainage canal is about 80 cm. Two numbers of paddle wheel aerators of 1 hp (Team) Taiwan made was provided per pond. Aerators placed 5 m from the dike, about 30-40 m distance from each other. They were used to create the water current for the accumulation of black soil and waste in the center of the pond and also to increase the dissolved oxygen in the water column.

Initially all the pond of the present study was allowed to dry and crack to increase the capacity of oxidation of hydrogen sulphide and to eliminate the fish eggs, crab larvae and other predators. Then pond bottom was scrapped 2 to 4 cm by using a tractor blade to avoid topsoil. Then the pond bottom was ploughed horizontally and vertically a depth of 30 cm to remove the obnoxious gases, oxygenate the bottom soil, discoloration of the black soil to remove the hydrogen sulphide odour and to increase the fertility. The soil pH was recorded in the ponds with the help of cone type pH meter. The average pH was calculated from the collected data and required amount of lime was applied to neutralize the acid soil condition and increases the availability of nutrient.

Water Culture: The initial water levels in all ponds were maintained at 70 cm level. Required amount of organic fertilizers such as rice bran; groundnut oil cake, dry cow dung and yeast were soaked over night and applied the extract to all the ponds. The same procedure was continued for three days. After three days the water color turned to light green. Then water level was raised to 100 cm of the ponds and also added urea and super phosphate to improve the primary production. Fertilization enhanced the optimal algal bloom in the ponds and the transparency in the ponds ranged from 33 to 36 cm. During the culture period lime was used to maintain the pH and algal bloom and chain dragging was done daily before stocking of seeds.

The *P. monodon* (PL16 pass the PCR test and stress test) seeds were purchased from venture hatchery, Marakanam and stocked in Farm 1. However, it was purchased from Raj hatchery and stocked in Farm 2. The seeds after purchase were transported in oxygenated double-layered polythene bags with crushed ice packs between inner and outer covers of the bag and packed in a carton. The seeds were brought to the farm site and bags were kept in the pond water for some time to adjust the temperature. Then the pond water was added slowly into the seed bag to adjust the salinity and pH. Subsequently the seeds were released slowly in to the ponds. The stocking density per pond was 12 m^2.

Water Quality Management: The water level was measured by using a standard scale with cm marking. The water salinity, pH, temperature, dissolved oxygen and transparency were measured by using a hand refractometer, pH pen, thermometer and dissolved oxygen meter and secchi disc, respectively. During the first 3-4 weeks of culture, water exchange is not required. Water was exchanged five days once or depends upon the water and shrimp quality. The purpose of water exchange is to maintaining water quality and also to stimulate molting of the shrimp, resulting in acceleration of growth and production.

Feed Management: Feed management plays a major role in the shrimp culture. The commercial feed was used (Farm: 1 and farm: 2) during the entire cycle, distributed manually by using the boat. During the first month after stocking, feeding rates were based on estimated survival and feeding tables and distributed four times per day. After 30th DOC, daily rations were adjusted using feed trays and increased to five times per day there after.

Monitoring of Growth: Cast net was used to measure the growth rate of shrimps. The first sampling was taken after 40th day of culture and number of individuals and the average body weights were recorded in each sampling. Sampling was regularly performed every ten days until harvest.

Table 1: Composition of Dobell marine agar medium

Composition	Amount (g)
Peptone	5.0
Yeast extract	1.0
K2 HPO4	0.5
Feso4	Trace
Agar	15
50% seawater	1000ml
pH	7.2

Microbiological Analysis: For microbial analysis, the water and sediment samples were collected separately from different parts of the ponds in sterile conical flask and were mixed to make a single sample. This procedure was repeated for every pond and the final samples were brought to the laboratory immediately and were analyzed for microbial counts. It was then transferred to a sterile conical flask (150-ml) containing 99ml of sterile diluents and serial dilution was performed to get 10^1, 10^2, 10^3, 10^4 and 10^5 suspension samples. For enumeration of Total Heterotrophic Bacteria (THB), Zobell marine agar medium (Hi-media, Mumbai) was used (Table 1). For enumeration of *Vibrio* spp TCBS media was obtained from Hi-media, Mumbai.

Isolation and Enumeration Enumeration of the microbes was done by adopting spread plate method. In this method, sterile media were poured into Petri dishes aseptically and allowed to solidify. One milliliter of serially diluted sample was pipette out into sterile Petri dish. It was made spread in the plate first by rotating it in clockwise and then anticlockwise directions for three times and then spread with the help of a 'L'-rod. The plates were incubated in an inverted position at 28±2°C. After the incubation period of 2 to 3 days, the colonies were counted. The plates were examined and the number of colonies per plate. The microbial load in the given sample was calculated using the following formula and it is expressed as Colony Forming Units (CFU) per gram of the sample.

$$\text{Total microbial load in the given sample (CFU/g)} = \frac{\text{Total number of colonies}}{\text{Samples of volume plated (0.1) X Dilution}}$$

RESULTS

The pH recorded during the culture period in the farm 1 was from 7.7 to 9.5 and farm 2 it was from 7.6 to 9.7. The lowest temperature (23°C) was recorded in the month of December and the maximum was recorded (32°C) in the month of October from the farm 1. In the farm 2, the lowest temperature was observed (22°C) in the month of December where as it was higher (32°C) in the month of October. The dissolved oxygen was ranging between 3.0 to 4.0 ppm in the farm 1 and it was 3.1 to 4.2 in farm 2 (Table 2).

Table 2: Average results of farms 1 and 2

	Farm : 1			Farm : 2		
Parameters	Oct	Nov	Dec	Oct	Nov	Dec
pH	7.7-8.0	8.0-8.4	8.8-9.5	7.6-7.8	7.9-8.5	9.0-9.7
Temperature (°C)	30-32	28-31	23-26	30-32	27-30	22-26
Dissolved oxygen (ml/l)	3.8-4.0	3.2-3.7	3.0-3.2	4.0-4.2	3.7-4.1	3.1-3.6

Table 3: Average results of microbial population in farms 1and 2

	Oct		Nov		Dec	
Month	Y	G	Y	G	Y	G
Farm 1	200	60	280	160	200	1250
Farm 2	250	40	200	100	140	1680

Table 4: Harvest details of farms 1and2

Details	Farm 1	Farm 2
Location	Thennampattinam	Vanagiri
Distance	16 KM away from Sirkali	4 KM away from Poombukar and 22 km away from Sirkali
Water source	Uppanar back water	Kavari back water
No.of.ponds	2	2
Pond size	.7 Ha and .8 Ha	.5 Ha and .7 Ha
Stocking density	10 m²	10 m²
Date of stocking	25/10/2007	1/10/2007
Aerator	2 (1 HP)	2 (1 HP)
Hatchery	Venture	Raj
PCR Result	Negative	Negative
Affected/harvested	20/12/2008	12/12/2007
DOC	55	73
ABW	10g	13g

Maximum population of yellow colony was recorded in the month of November and maximum of green colony was recorded in the month of December from the farm 1. However, in farm 2, the maximum yellow colony was recorded in the month of October and maximum green colony was recorded the month of December (Table 3). Due to low temperature and high pH and increasing the green colony in the shrimp's farms was infected and ultimately affected by WSSV. The shrimps in the farm 1 affected by WSSV on 55 th DOC when the shrimps reached the average weight of 10 g. Where as the shrimps in the farm 2 was affected by WSSV on 73 th DOC when the shrimps reached the average weight of 13 g (Table 4).

DISCUSSION

There has been considerable increase in the culture of brackish water shrimp due to its taste, market demand both national and international markets. In order to prevent many problems due to shrimp culture, sustainable shrimp farming is need of the hour. Even though shrimps are bottom dwelling organisms, the depth and volume of water in a pond has certain physical and biological consequences. The volume of water behaves like a buffer, which prevents weather fluctuations from influencing the environment in which shrimp lives. The ideal water depth is between 0.8 to 1.5 m depending upon the stage of culture. It is recommended that a minimum depth of 1 m will be maintained at operational level. In the present study 100cm water level was maintained in farms 1 and 2 up to the end of the culture period. The stocking density between 10 - 20 post larvae /m² is ideal for successful shrimp farms [10]. pH is one of the vital environmental characteristics, which decides the survival and growth of shrimp culture; it also affects the metabolism and other physiological process of shrimps. The optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production [11,12,10]. The pH recorded during the culture period from farm 1 was 7.7 to 9.5 and farm 2 it was 7.6 to 9.7.

Dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation. Oxygen affects the solubility and availability of many nutrients. Low level of dissolved oxygen can cause damages in oxidation state of substances from the oxidized to the reduced form. Lack of dissolved oxygen can be directly harmful to shrimp and cause a substantial increase in the level of toxic metabolites. Low level of oxygen tension hampers metabolic performances in shrimp and can reduce growth and molting and cause mortality [13,14]. The dissolved oxygen recorded during the culture period was ranging between farm 1 was from 3.0 to 4.0 ml/l and farm 2 it was from 3.1 to 4.2 ml/l. The microbial population was evident from the presence of higher load of green colony in the farms 1 and 2. The occurrence of green colony in all ponds was concluded by presence of luminescence in the nighttime and occurrence of dead animals in the check tray. Maximum population of yellow colony was recorded in the month of November and maximum of green colony was recorded in the month of December from Farm 1. In Farm 2, the maximum yellow colony was recorded in the month of October and maximum green colony was recorded in the month of December (Table 3).

Disease is the end result of complex between host, pathogen and environment [15]. Water temperature is considered to be one of the most important environmental factors for shrimp since it influence metabolism, oxygen consumption, feeding rate, growth, moulting, survival and tolerance to toxic metabolites. It is widely assumed that temperature plays an important role in inducing outbreaks of white spot disease. High temperature can reduce mortality in WSSV inoculated shrimp, *P. vannmei* and cray fish, *Procambarus clarki* [16]. And an inhibition of WSSV replication or reduction of viral load has been shown as possible explanation for the reduced mortality [17,18,19]. In the present study also correlated with previous study that lower temperature influences the appearance of WSSV in *P.monodon* of both the farms. Some reports also showed that protection occurs at low temperatures [20,21]. In general; a sudden change of temperature affects the immune system. The optimum range of temperature for the black tiger shrimp is between 25 to 31 °C [22,10]. In the present study the lowest temperature recorded was 23°C and maximum was 32°C in farm 1 and in farm 2, the lowest temperature was 22°C and maximum was 32°C.

In the present study high pH and lower water temperature might be the possible reason for WSSV in shrimps and ultimately the mass mortality was occurred. Many such studies were carried out only in laboratory level [13,23,24,25,26]. But the present study was carried out in outdoor that to in large scale farms. The shrimps infected and affected by WSSV due to low water temperature and high pH that condition is directly increasing green colony. The shrimps affected by WSSV on 55th DOC in farm 1 and the animals reached the average weight of 10 g. It was happened on 73rd DOC in the farm 2 and the animals reached the average weight of 13 g. So it is confirmed that the mass mortalities occurred due to low

water temperature and high pH.

It is generally accepted that invertebrates such as shrimp do not have an adaptive immune response system such as that present in vertebrates. There is increasing awareness that diseases in aquatic populations are often linked to environmental changes or pollution, which depresses the immune system. Although these effects are well documented in cultured shrimp, fish and mollusks, there have been few quantitative studies on the impact of environmental stressors on the immune system of shrimp. Temperature has a direct effect on other environmental parameters such as salinity and oxygenation of the water. In general, a sudden change of temperature affects the shrimp immune system, which can be critical if the timing coincides with the presence of a pathogen. Lower temperature can induce a decrease in the number of circulating hemocytes and their phagocytic capability, as measured by their oxidative metabolism. A temperature increase can increase circulating hemocytes and plasmatic protein, but decrease total hemocytic prophenoloxidase. The decrease of temperature in ponds from 27 to 18°C results in high mortalities. Mortalities are also recorded when temperature increases [13]. In general, the lower temperature reduced rather than stopped viral replication. Despite the fact that our present study clearly shows that lower temperature and high pH influence WSSV infection in cultured shrimp of *P. monodon*.

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