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White Spot Syndrome Virus vulnerability of Tiger shrimp (*Penaeus monodon*) cultured in the coastal ponds of Cox's Bazar region, Bangladesh

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Abstract. Jumbo tiger prawn, *Penaeus monodon* is the world's most popular cultivable species for its fast growth, hardy nature, delicious taste and market demand. During the last three decades shrimp culture has been expanded rapidly. White spot Syndrome Virus (WSSV) is a great treat to this culture expansion. In this research, WSSV free fries were stocked in four coastal ponds with different salinities. The stocking density was kept low and constant. Water parameters, fish growth, and WSSV contamination were recorded fortnightly. The WSSV contamination was primarily detected by using Enbio Shrimp Virus Detection Test Kit, 'Shrimple' and further confirmed by the PCR test. Among the ponds, Pond A, B, and C were in completely controlled environmental condition where as pond D was traditional one that exposed to tidal variations. Physico-chemical parameter varied from as, temperature 29 to 32°C, salinity 0 to 31 ‰, water pH 7.1 to 8.3, dissolved oxygen 3.8 to 6.3 mL L^{-1}, alkalinity 80-122 mg L^{-1}, ammonia 0 to 1.5 mg L^{-1} and transparency 23 to 50 cm. The WSSV was found positive in the pond D just after hundred days, when the average weight of the shrimps was 26.69 g. It is observed that rapid change in the salinity and temperature, poor environmental conditions and uncontrolled exchange of water made the shrimp more vulnerable to the WSSV.
Key Words: White Spot Syndrome Virus, shrimp disease, Tiger shrimp, PCR test.

Introduction. Aquaculture – the art of rearing aquatic plants and animals first developed in China between 3,500 and 4,000 BC (Ling 1974) and later spread on world wide especially in the Asian countries. Aquaculture is now practiced throughout the world due to continuous declination of yields from capture fisheries, also being stimulated by rising price of quality sea food. Aquaculture has been developed into an important export oriented industries nowadays. Among the aquaculture practice, shrimp farming takes the leading in turns of production that is to say for super economic return. More than 300 species of *Penaeid* shrimps and prawns have been recorded world wide, of which 80 are commercially important in terms of culture and capture fisheries (Apud et al 1983). Among the commercially important species, *Penaeus monodon* Fabricus, 1798 is the most popular cultivable species, dominates over the world (Rosenberry 1999), for its fast growth, reaching 26 cm in body length or 250 g in weight (Liao 1977; Motoh 1981), eurihaline, omnivorous and hardy nature (Liao & Huang 1982). Moreover, it has a delicious taste and great market demand.

Shrimp aquaculture production is currently, almost totally restricted to developing countries (1,124,188 MT or 99.4% of total production); it is especially concentrated in the Asian region (911,773 MT or 80.6%; mainly Thailand, China, Vietnam, Indonesia, India, Bangladesh, Philippines, and Malaysia). The Latin America and Caribbean region also produce significant amounts (208,402 MT or 18.4%; mainly Ecuador, Mexico, Brazil, Colombia, Honduras, Venezeula, Nicaragua, Peru, Belize, and Panama). Remaining regions produce small amounts: Oceania (4,470 MT or 0.4%; mainly Australia and New Caledonia), Africa (3,833 MT or 0.3%; mainly Madagascar and South Africa), North America (USA: 2,098 MT or 0.18%), and lastly Europe (161 MT; mainly Spain and Italy) (FAO 2001).

Bangladesh is a small country lying in the sub tropical belt with an area of 144,000 km^2, including a large coastal area (Pramanik 1988). At present the south-eastern (Chittagong) and south western (Khulna) regions is widely covered by shrimp culture operations. It is estimated that out of the 3.6 million hectare coastal lands about 2.5 million hectare are brackish water areas being suitable for shrimp culture, of which about 0.25 million hectare have been projected as very good for coastal aquaculture (Ahmed 1995).

From the early 70's shrimp culture started in the south-eastern region of Bangladesh (Farmer 1989) but during the last three decades shrimp culture has been expanding rapidly in both horizontal and vertical terms in the coastal area of Bangladesh. The intensity of rearing was increasing day by day. Semi-intensive culture system got popularity in the coastal region with higher yield (Mahmood 1987). The conversion to semi-intensive culture was too high that fry scarcity became severe, as there were only few hatcheries in the country. The demand of fry encouraged the business people to import shrimp-fry from abroad. The import was started in 1992 and the main exporters are Thailand and Taiwan (Ahmed 1995). During the year 1994-1995 mass mortality of cultured tiger shrimp, *P. monodon* occurred in the semi-intensive farms of Cox's Bazar for the first time in this country by a viral disease name WSSV. The semi-intensive culture system completely collapsed with in couple of years. In the first crop of 1994, out of cultured twenty-one farms, seventeen were affected and the loss was 50% and in the second crop, out of twenty-one farms, fifteen did limited culture and the loss was 100% (Ahmed 1994). Even after taking all possible preventive measures in the affected farms, again, the outbreak of similar disease caused considerable loss in 1995.

WSSV was first reported in farmed *Penaeus japonicus* from Japan in 1992-93, but was thought to have been imported with live infected PL from Mainland China. WSSV then spread rapidly throughout most of the shrimp growing regions of Asia, later to the other part of the world (Figure 1), probably through infected broodstock and PL *P. monodon.* The mode of transmission of WSSV around Asia was believed to be through exports of live PL and brood-stock. WSSV, as with most viral diseases, is not thought to be truly vertically transmitted, because disinfection of water supplies and the washing and/or disinfection of the eggs and nauplii are successful in preventing its transmission from positive broodstock to their larvae.

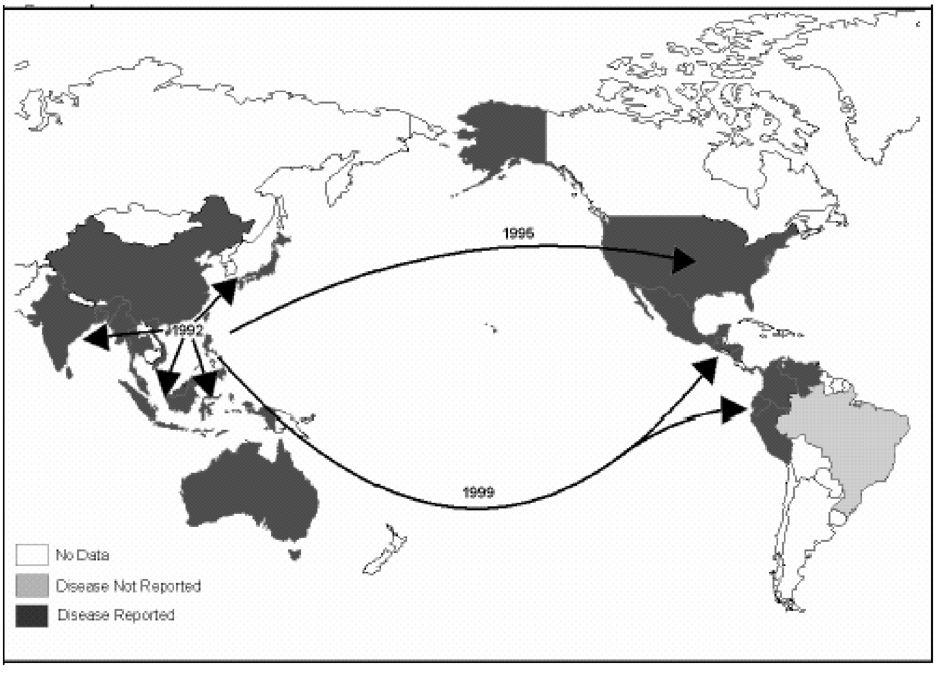


Figure 1. Spread and distribution of White Spot Syndrome Virus (CSIRO 2002).

This virus is now the most serious threat facing the shrimp farming industry in Asia (since 1992) and Latin America (since 1999). It is an extremely virulent pathogen with a large number of host species (Flegel 1997; Lightner & Redman 1998). This disease is probably the major cause of direct losses of up to 1 thousand million US$ per year since 1994 in Asia. Similarly, in Latin America, losses due to WSSV have been substantial.

Disease is a major issue in semi-intensive shrimp farming system because of growth retardation. Physical deformities, physiological malfunction and mortalities of the growing stock, it is also a great threat of economic loss for the farmers. There is a common belief that animals reared in artificial culture conditions experience stress in the culture period and become more susceptible to disease. Shrimps are poikilothermic gill breathing aquatic invertebrates and thus are very easily affected by environmental changes. In complex environmental conditions of culture ponds they easily get diseased, usually disease initiates when the dynamics of equilibrium between the shrimps and the pathogens collapse and shifts in favor of the disease, primarily due to the stress caused by the environmental degradation.

Material and Method. Studies were carried out at Samridhi Multipurpose Aquaculture Facility and Research Center (SMAFRC), located in the southeastern part of Bangladesh in the district of Cox's Bazar (Figure 2). Bordered on the south – west by the Bay of Bengal, the district of Cox's Bazar constitutes about 29,131 ha devoted to shrimp culture (Hossain & Lin 2001). There are many private and government owned brackish water aquaculture project around the north-west side of the city. The climate of the area is similar to that of other areas bordering the Bay with certain regional variations.

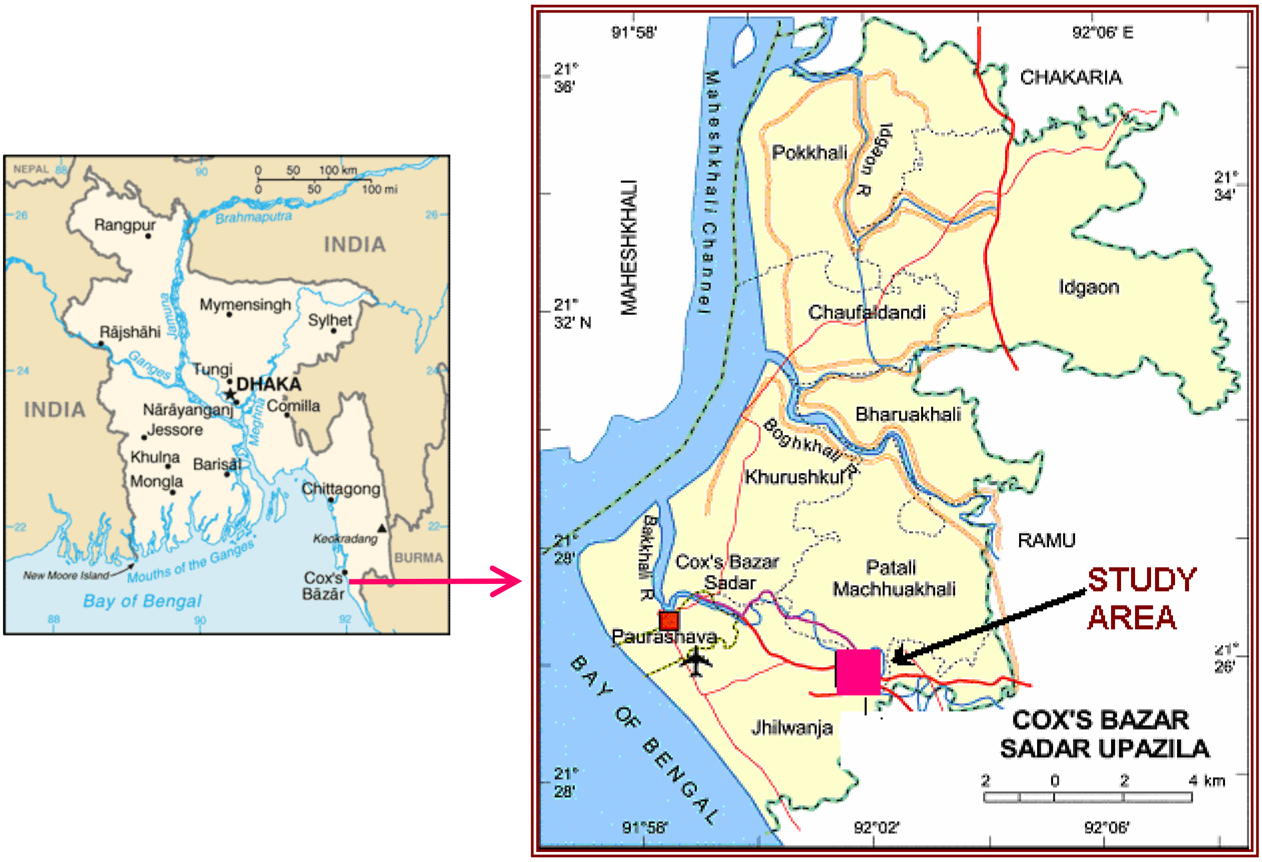


Figure 2. Location of the study area.

The temperature in this region varies from 18 to 35 ºC; the humidity remains high (70%-90%) almost through the year. Average annual rainfall varies in between 350 cm to 500 cm. The salinity of the water of Bankkhali River ranges from 0 to 32‰. The soil of the area is silt–clayey mixed with sandy loam having good water retention capacity. The pH value of the area varies from slightly acidic to slightly alkaline. The tidal amplitude along the coast is quite high. The land elevation of the farm site from the mean lower low water level (MLLW) is about 2 meter while the tidal amplitudes during spring tides are 3.0 to 3.3 m, voicing suitability for the construction of tide fed ponds.

The present studies on the vulnerability of WSSV on *P. monodon* at different water environments through some modification in low density/existing culture systems was carried out in 4 earthen ponds at Cox's Bazar, Bangladesh. The areas of the ponds A, B, C and D were 1240 m^2, 128 m^2, 135 m^2, and 560 m^2 respectively. Pond A and D were saline with water exchange by tidal fluctuation from Bankkhali River. The inlet of pond A was fitted over nylon screens (mesh size 350 micron) to prevent the entry of pest species and escapes of shrimps, where as the pond D was fitted over bamboo pens with nylon net (mesh size 0.5 cm) to prevent the entry of big size fishes. Pond B was moderate saline water with water exchange by pumping and pond C was completely fresh water with no water exchange. Pond A, B and C have controlled environmental conditions where as pond D has uncontrolled or traditional pond environments.

Ponds were dried and renovated with respect to dykes, depth, slope, bottom elevation, supply and drainage facilities. Soil pH was assessed prior to application of treatments. These ponds were limed (agricultural lime, $CaCO_3$) at a rate of 800-1,200 kg ha^{-1}. Then inorganic fertilizer was applied (100-200kg ha^{-1}; Urea:TSP 2:1) followed by organic fertilizer (cow dung, containing 50% moisture) at a rate of 4-5 ton ha^{-1}. Ponds were filled with water up to 1.2 meter. After 3-5 days when the color of the water turned green, WSSV free PL (confirmed by PCR Test) were purchased from the local hatchery and stocked at a rate of 3 pl m^{-2} early in the morning.

In case of pond B and C no water exchange was done. Only new water was added with same quality if pond water level was reduced due to evaporation. In pond A, about 40- 50% of water was exchanged daily during spring tides of full and new moon and the water level was maintained between 1.0 and 1.2 m. In case of pond D, water was exchanged naturally and uncontrolled with tidal fluctuation in spring tides times, but a blockage was used to retain water after the last spring tide.

Through out the culture period no supplementary feed was given. To maintain sufficient natural feeds, subsequent to each water exchange inorganic fertilizers were applied at a reduced rate (Urea 35 kg ha^{-1} and T.S.P. 20 kg ha^{-1}). Sometimes organic manure was also provided at a rate of 2 ton ha^{-1}. Major water quality parameters like water temperature, water pH, salinity, dissolved oxygen, alkalinity, ammonia, and Secchi depth were recorded fortnightly with great care and accuracy.

Growth was observed fortnightly by random sampling of 50 indivs/pond. After removing the water by means of bloating paper, the shrimp were weighted individually by an electronic balance. Body length was measured with the help of a fine-headed divider and millimeter scale.

Shrimps were collected fortnightly for WSSV test. Initially En-Bio Shrimp Virus Detection Kit "Shrimple" was used to detect WSSV (Figure 3). WSSV positive results were further confirmed by PCR test. The PCR examination was carried out by the Center for Health and Population Research of International Center for Diarrhoeal Disease Research, Bangladesh (ICCDR'B).

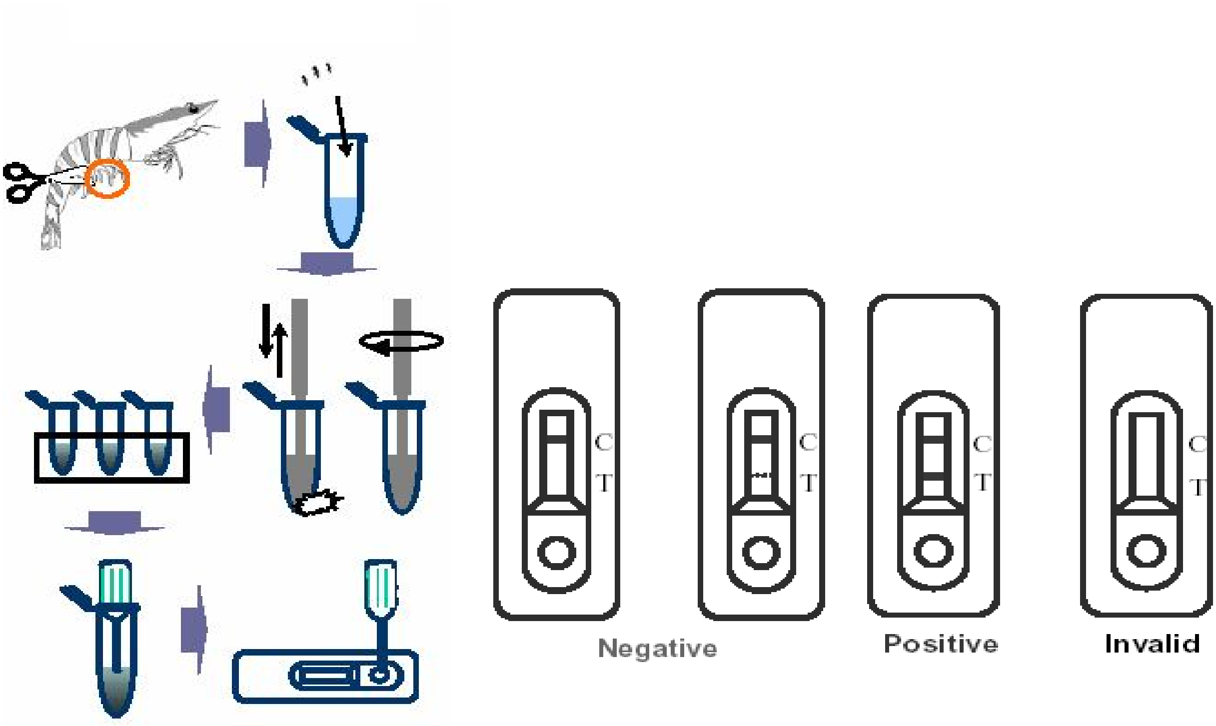


Figure 3. Illustration and interpretation of “Shrimple” Test-Kit.

Results

During the culture period, average surface water temperature was 30.88°C, 30.38°C, 30.06°C and 30.56°C in ponds A, B, C and D respectively. The salinity of pond A, B and D was 22 to 31‰, 3 to 10‰ and 10 to 30‰ respectively, where as, pond C was completely fresh water during the culture period. The water pH and DO of the ponds was found satisfactory. Analysis of water parameters showed no detrimental impact to the water conditions of culture ponds (Table 1).

Fish survival & growth. The survival rates in the ponds were 70.16%, 55.31%, 63.31% and 49.30% for the ponds A, B, C and D respectively. A drastic mortality was observed at the first week of stoking in the ponds. Variation in mean daily growth of *P. monodon* was observed among the ponds. The average daily growth rate were 0.286 g, 0.244 g, 0.192 g and 0.254 g in terms of weight and 1.285 mm, 1.175 mm, 1.18 mm and 1.389 mm in terms of length for the ponds A, B, C and D respectively. Final mean body weight was found highest in pond A followed by B, D and C respectively. The detail stocking, survival, growth and production of shrimp is in detailed in table 2.

Length weight relationship. The value of log C and “n” in length weight relationship (Figure 3) of *P. monodon* for experimental pond A, B, C and D were -12.9533 & 3.264786, -13.184 & 3.349182, -12.4339 & 3.157024 and -12.9814 & 3.281019 respectively. The correlation coefficient “r” was varied from 0.91 to 0.93, which indicates a positive relationship of the body weight on total length in all the experimental ponds.

Outbreak of disease. The WSSV was detected in the pond D after 100 days of culture when the average weight of shrimp was 26.69 g. The outbreak compelled the culture process to be suspended and harvest the growing stock fully.

Table 1

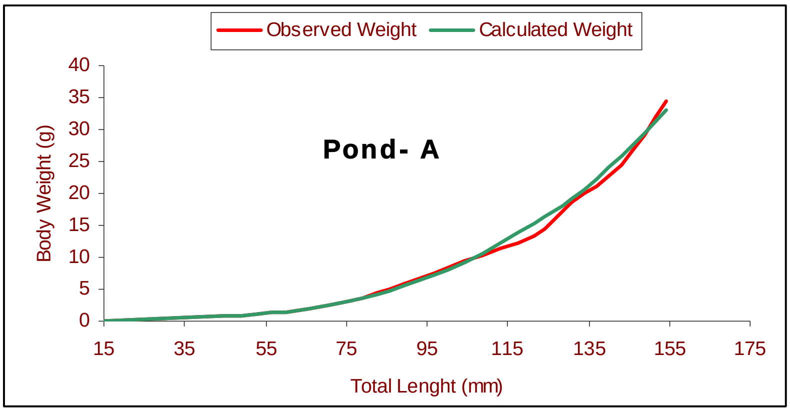
Physico-chemical parameters of the four experimental ponds

Culture period	*Water temp. (°C)*	*Salinity (‰)*	*Water pH*	*DO, ml L^{-1}*	*Alkalinity, mg L^{-1}*	*NH3, mg L^{-1}*	*Secchi depth (cm)*
				Pond A			
Stocking	31	31	8.3	6.2	122	0.1	28
15 days	30.5	29	8	5.6	110	0.2	36
30 days	30	30	7.9	5.8	98	0.2	33
45 days	29.5	30	8.2	6	115	0.3	32
60 days	30.5	29	8	5.8	105	0.4	28
75 days	31.5	27	7.8	6.1	87	0.3	30
90 days	32.5	26	8.1	5.8	118	0.5	25
105 days	31.5	22	7.9	5.4	97	0.4	35
				Pond B			
Stocking	30	10	8.3	6	120	0.1	26
15 days	30	8	8	5.2	108	0.2	34
30 days	30	7	7.8	5.6	98	0.2	32
45 days	29	5	8.1	6.2	110	0.3	25
60 days	30.5	8	7.9	5.8	102	0.4	28
75 days	31	5	7.7	4.9	92	0.4	36
90 days	31.5	4	8.2	5.4	109	0.5	30
105 days	31	3	7.8	5	96	0.4	33
				Pond C			
Stocking	30	0	8.1	5.8	90	0.1	28
15 days	30	0	7.9	4.9	84	0.2	32
30 days	29.5	0	7.6	6.3	78	0.2	23
45 days	29	0	8	5.2	85	0.3	30
60 days	30	0	7.8	6	75	0.4	25
75 days	31	0	7.6	5.5	72	0.6	33
90 days	31	0	7.9	5.4	80	0.7	35
105 days	30	0	7.7	4.8	78	0.8	38
				Pond D			
Stocking	31	29	8	5.4	110	0.1	30
15 days	30	29	7.9	5	90	0.2	35
30 days	29.5	30	7.6	4.6	87	0.4	38
45 days	30.5	28	7.8	4.8	89	0.5	40
60 days	30	27	7.5	5.4	72	0.8	42
75 days	31.5	22	7.4	4.6	68	0.9	40
90 days	30	18	7.5	3.8	70	1.2	45
105 days	32	10	7.1	4.2	60	1.5	50

Table 2

Stocking, survival, growth and production in the ponds

Pond	*Stocking density (indiv m^{-2})*	*Final survival rate (%)*	*Final length (mm)*	*Final weight (g)*	*Production (kg ha^{-1})*
A	3	70.16	154.23	34.35	723
B	3	55.73	140.95	29.3	490
C	3	63.23	141.6	23.4	437
D	3	49.3	145.8	26.69	395

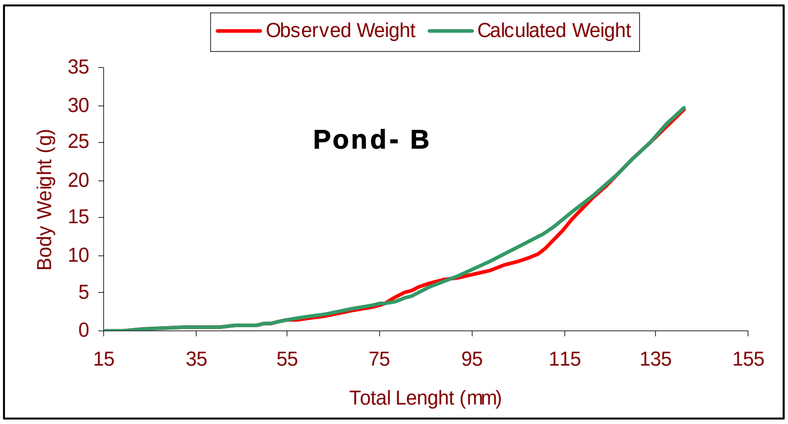


Log C = -12.9533

n = 3.264786

Log W = -4.26811

r = 0.91

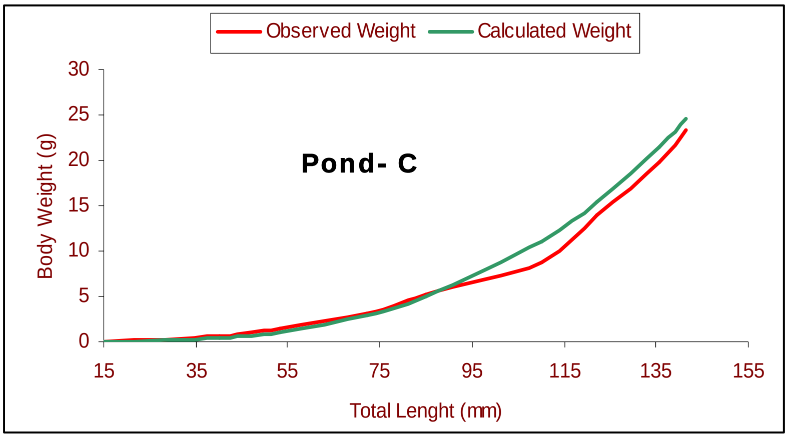


Log C = -13.184

n = 3.349182

Log W = -4.06771

r = 0.91

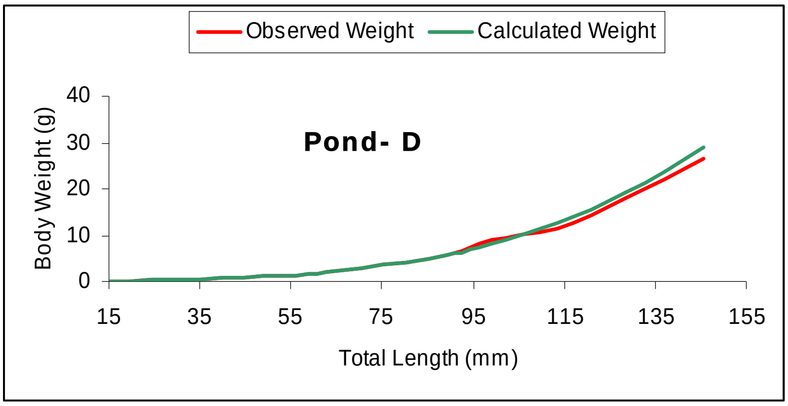


Log C = -12.4339

n = 3.157024

Log W = -3.86351

r = 0.93



Log C = -12.9814

n = 3.281019

Log W = -4.25303

r = 0.91

Figure 4. Relation between length & weight of *Penaeus monodon* in culture ponds.

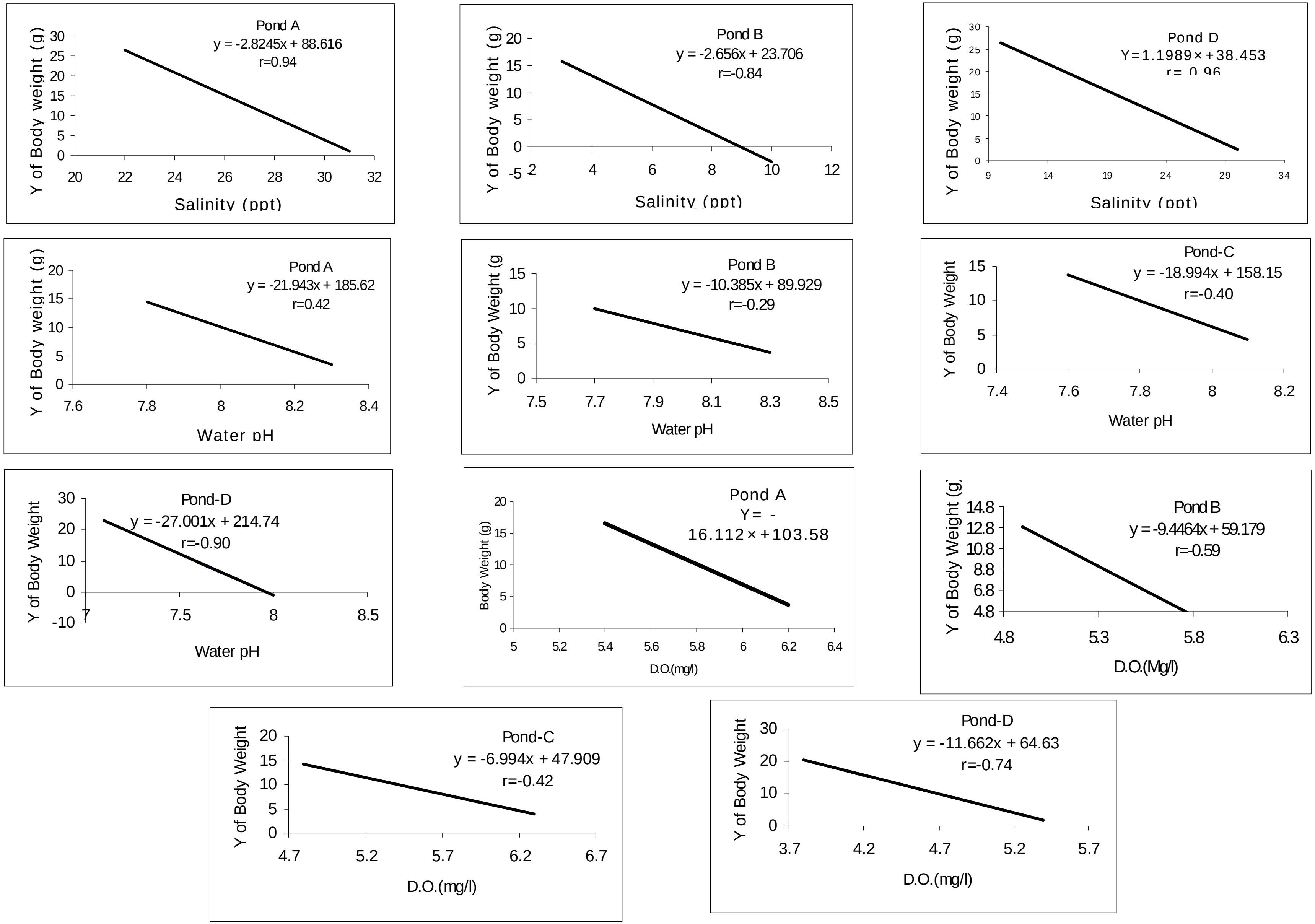


Figure 5a. Correlation and regression (Y on X) of body weight on some parameters of *Penaeus monodon.*

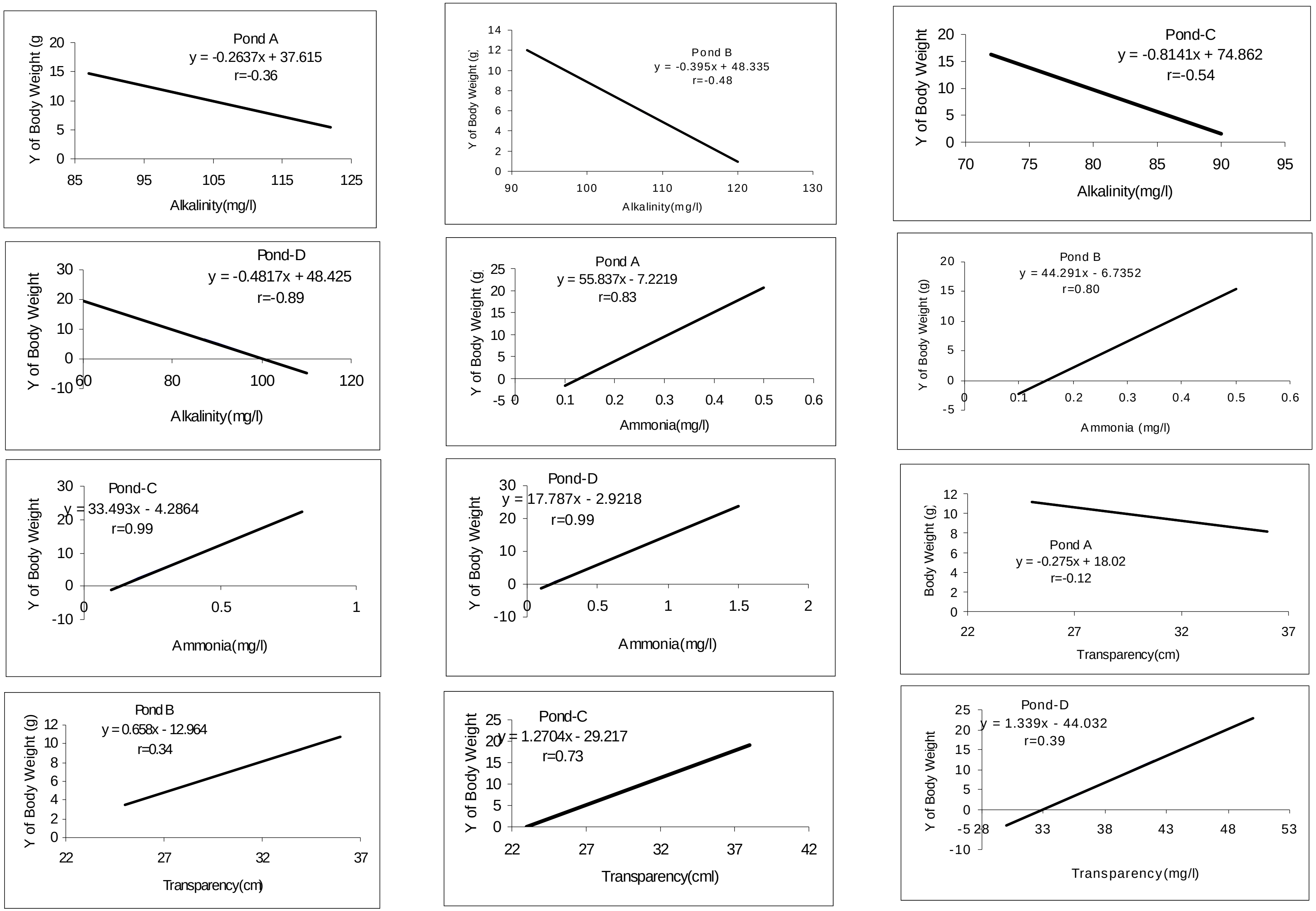


Figure 5b. Correlation and regression (Y on X) of body weight on some parameters of *Penaeus monodon*

Water parameters during disease outbreak. Water temperature Varied from 31 to 34.5ºC, salinity varied from 7 to 18 ‰ and the variation of pH, transparency, D.O. alkalinity, ammonia ranged from 6.7 to 7.1, 48 cm to 55 cm, 3.8 to 4.3 mL L^{-1}, 55 to 65 mg L^{-1} and 1.4 to 1.7 mg L^{-1} respectively in pond D. The temperature was too high (34.5ºC) and salinity was rapidly declined (18 to 7 ‰) due to heavy rainfall.

WSSV confirmation by PCR method. After the primary detection of WSSV from the shrimp, the effected pond D was further sampled and samples were confirmed WSSV positive by PCR test at the Center for Health and Population Research of International Center for Diarrhoeal Disease Research, Bangladesh (ICCDR'B).

Symptoms of disease. WSSV infections typically cause lethargic behavior in affected shrimps, cessation of feeding, followed within a few days by the appearance of moribund shrimp swimming near the surface, at the edge of ponds. These affected animals display a pink to reddish-brown coloration due to the expansion of their cuticular chromatophores, and also show white inclusions embedded in the cuticle. These inclusions range from minute spots to discs, several millimeters in diameter and they may converge into larger spots. These spots are most easily observed by removing the cuticle over the shrimp head, scraping away any attached tissue with the thumbnail, and then holding the cuticle against a light.

Discussion

Fish survival & growth. Survival rates obtained in the culture ponds were 70.16%, 55.73% and 63.23% in ponds A, B and D respectively. Chakraborti et al (1986), Rubright et al (1981), and Caces-Borja & Rasalan (1958) reported a maximum survival of 60-80% with supplemental feeding and improved management at a density of 1-2 indivs m^{-2}. The percentage recovery in the present study confirms the view of Mahmood (1987) who reported the survival rate as 60-80%, under suitable rearing conditions with the absence of predators, sub-optimal temperature/salinities. The present result is in close agreements with Ali (1981) who reported a survival rate of 50.86% and Alam (1989) who reported a 68-70% survival at a density of 2 m^{-2}. In case of fresh water pond C, the survival rate was 63.23%, which was very close to Ali (1996), 71% at complete fresh water culture ponds.

Shrimp growth obtained from the present study may be compared with the results of 0.129 g day^{-1} (Chen 1976), 0.23 g day^{-1} (Apud et al 1983) 0.163 g/day^{-1} at a density of 10.5 indivs/m^{-2} (Alam 1989) and 0.23 g/day^{-1} in a traditional culture system (Rahman & Bhuiyan 1979). But the result was not satisfactory when compared with the results of 0.328 g/day^{-1} (Bal & Rao 1984), 0.36 g/day^{-1} at a density of 3 indivs/m^{-2} (Apud et al 1983) and 0.39 g/day^{-1} without supplemental feeds (Sundararajan et al 1979) and 0.31-0.39 g/day^{-1} (Precilla & Myrna 1991). Ali (1996) gets 0.233 g/day^{-1} in complete fresh water. From the aforesaid statement, the survival rate and the daily growth rate were satisfactory in the present study except pond D. It may be due to the better management practice which maintained good water quality.

Length-weight relationship. The value of 'n' in fish and shrimp usually lies between 2.5 and 4.0 (Hile 1936). The value of 'n' will be exactly 3.0 when the growth is isometric in length-weight relationship of fish (Ricker 1963). In present study, the values of 'n' in all ponds were always above 3.0, which may be safely concluded that the growth was not isometric.

WSSV vulnerability. WSSV vulnerability of the cultured shrimp was described by Dixon (2000). The recommendation to avoid contamination were use of germ-free seeds, reduce stocking stress, culture in close condition, provide stable environmental condition and use of filtered water. The present study showed significant asymmetry with the recommendation. The two closed ponds (B & C) neither exposed to environmental degradation nor to WSSV contamination. Between the ponds facilitated by water exchange, the filtered intake in pond A, saved the animals from WSSV contamination. Rapid change of salinity, use of uncontrolled water exchange facility, poor DO and ammonia level of pond D made the pond more vulnerable to WSSV.

Conclusions. Probably the biggest single problem faced by shrimp farmers aside from the actions of their associates will be the sudden environmental fluctuations that accompany the rainy season. Sudden changes in salinity and temperature have been implicated in many disease outbreaks. As the disease moves from one area to another the viral load in the environment will increase to the point where the virus will be ever present. Ideally shrimp should be destroyed once, when they are ill to prevent entering high loads of viruses from the environment. Unfortunately this is usually not practical. Harvesting shrimp is and should be encouraged even if shrimp are too small to sell. Cutting losses and minimizing the spread of the virus are to the farmer's advantage. Therefore, it may be expected that the present study will give some information to develop and demonstrate an improved shrimp culture technology for the country and may serve as a source of information for the researcher who will be interested to work on shrimp disease like WSSV in the coastal water of Bangladesh.

We suggest a few recommendations for the field level implementations are as follows:

1. Proper pond preparation includes complete drying, bottom-cracking, removal of black soil (if applicable), liming, tilling etc.
2. Use of good quality WSSV free PL for stocking, preferable without any treatment by drugs, *i.e.*, using healthy and WSSV free brood shrimp in the hatchery.
3. Stocking rate should be adjusted to the carrying capacity of ponds, as well as, of the management efficiency.
4. Stocking season (February/March) and stocking time (early morning/late evening) should be proper.
5. The chance of abrupt change in the environmental factors should be prevented.
6. For water exchange, the use of filter (usually 250 microns mesh) can efficiently reduce the introduction of virus carrier vectors.
7. Regular and accurate monitoring of water parameters, pond bottom condition, growth & survival rates and health of shrimps should be practiced.

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