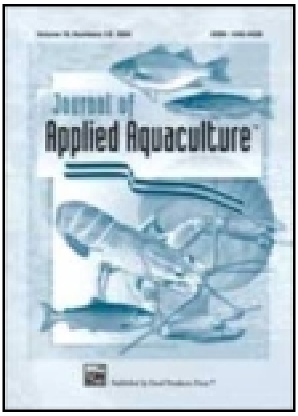

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Epidemiological Investigation of Brackish Water Culture Systems in West Bengal, India

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An epidemiological investigation was done in brackish water culture systems in three coastal districts of West Bengal. A total of 198 farms were randomly surveyed with a structured questionnaire. The data showed that there was a significant difference in outbreak of white spot disease (WSD) ($p < 0.01$), shell-associated problems ($p < 0.01$), and gill-associated problems ($p < 0.05$) among the culture systems. Among all systems, stunted and uneven growth and white fecal disease (only in shrimp monoculture) were the dominant emerging disorders. WSD remained the most prevalent disease. Some farms tested (polymerase chain reaction [PCR]) positive for WSD, but the animals were apparently healthy. Chlorination, use of PCR screening, application of immunostimulants, and strict bio-security measures play major roles in containing disease outbreaks.

KEYWORDS *Shrimp diseases, fish diseases, brackish water culture systems, West Bengal*

INTRODUCTION

West Bengal lies between 21°25′24″–27°13′15′ north latitude and 85°48′20″E–89°53′04″W longitude, stretching from the Himalayas in the north to Bay of Bengal in the south. Covering an area of 88,752 sq. km, the state has a short coastline of 158 km (2.1% of India's coastline) and a

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continental shelf of 17,049 sq. km. The brackish water area in the deltaic region encompasses a territory of 0.2 million ha with very large inland water bodies for capture and culture of shrimp and fish, out of which 85,000 ha has been found suitable for aquaculture. The total area so far developed is 50,438 ha with the total production of 14,483 Mt during 2006–2007 (Marine Products Export Development Authority 2007).

The aquaculture sector has been recognized as a powerful income and employment generator with a number of subsidiary industries, and is a source of cheap and nutritious food besides being a foreign exchange earner. Most importantly, it is the source of livelihoods for a large section of the coastal population. Disease outbreaks have been one of the major challenges faced by aquaculturists, so an epidemiological investigation was done in three coastal districts with specific reference to the traditional Bhery culture system (an extensive fish-shrimp polyculture), shrimp monoculture/zero-water exchange systems, and integrated paddy-cum-fish/shrimp culture.

MATERIALS AND METHODS

A total of 198 shrimp and fish farms were randomly surveyed in North and South 24 Parganas and East Medinipur districts during 2007–2009 using a structured questionnaire that include 115 variables like education, age, experience, ownership, farm area, pond size, source of seed, stocking density, bio-security measures, disease outbreaks, and their relation with other parameters.

From each pond surveyed, at least 50 shrimps were collected for gross examination. Gross lesions were recorded and the representative samples stored in 10% neutral buffered formalin and 95% ethanol for the further investigation.

Shrimp diseases were diagnosed as per Lightner (1977, 1993) and Sudha et al. (1998). Polymerase chain reaction (PCR) for white spot disease (WSD) was performed using four DNA oligonucleotide primers as reported by Kimura et al. (1996).

Survey data and their relationship with disease prevalence were analyzed through one-way ANOVA using SPSS for Windows v.17.0 (SPSS Inc., Chicago, IL, USA) based on the grouping of the three farming systems as follows: Bheries, shrimp monoculture, and paddy-cum-fish/shrimp culture. Location of the farms surveyed is depicted in the Figure 1.

RESULTS AND DISCUSSION

The total farm area surveyed was 797 ha, which included North 24 Parganas (626.64 ha), South 24 Parganas (38.48 ha), and East Medinipur (131.88 ha),

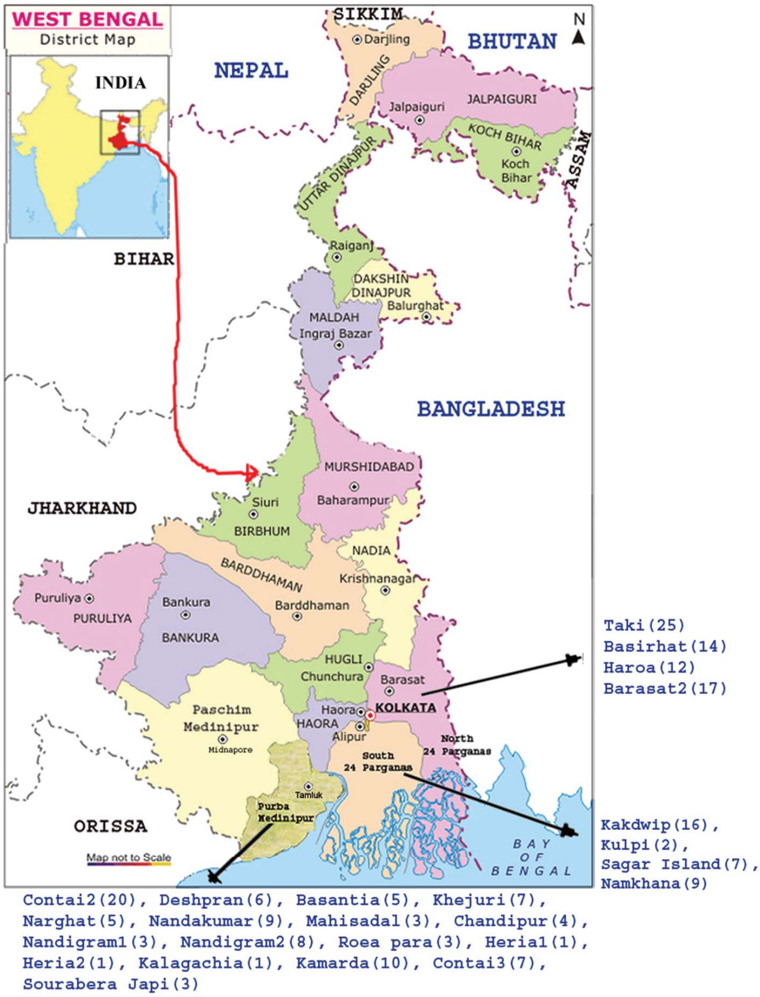

FIGURE 1 District map of West Bengal showing the area surveyed in the three coastal districts. The value in the parenthesis indicates the number of the farmers surveyed in the block. (Color figure available online.)

with the area under different types of culture systems such as the Bhery system, shrimp monoculture, and paddy-cum-fish culture being 143.6, 636.45, and 16.95 ha, respectively. Bhery culture was found in North 24 Parganas and shrimp monoculture in East Medinipur. Both these systems of culture were also observed in South 24 Parganas. Typical paddy-cum-fish culture was observed along the Ichchamatir River bank of North 24 Parganas.

In shrimp monoculture in East Medinipur, the growing season starts in April/May (pre-monsoon) and ends by August/September (monsoon). In South 24 Parganas it starts in June (monsoon) and ends in October/November (post-monsoon). WSD outbreaks are known to occur more frequently during the monsoon season (Withyachumnarnkul et al.

2003). Invariably all the farmers doing shrimp monoculture grow one crop per year. In Bhery and paddy-cum-fish culture/shrimp systems, stocking of *Penaeus monodon* starts in February and is completed by September, with harvesting from May to December followed by a one-month fallow period. Growout ranges from 120 to 165 days, with an average of 140. Maximum survival rate reported was 91%, with 65%–80% being typical. Other characteristics of the culture systems are shown in Table 1.

The educational status of the farmers doing different types of aquaculture varied from illiterate to graduate. Most of the farmers practicing Bhery and paddy-cum-fish/shrimp culture were at the literacy level of primary (42.6% and 54.2%, respectively) as reported by Alam and Philips (2004). Among the graduate farmers (18.9% of the total), the incidence of various diseases was less than 15%, indicating that literacy level and awareness played a role in disease control. The age of the farmers varied between 20 and 69 years. It was observed that the farmers practicing Bhery and paddy-cum-fish/shrimp culture tended to be somewhat older (36 to 55), while those doing shrimp monoculture were younger (26 to 45). Farmers with a wide range of experience are being drawn into aquaculture by perceived profits, but many lack the technical skills required to succeed at shrimp farming.

Some 46.5% of farmers own their farms, while 52.0% lease and 1.4% have both personal and leased ponds. Only 36.8% and 2.9% of the farmers practicing shrimp monoculture and integrated systems, respectively, have approval from the Coastal Aquaculture Authority. As reported by Kumaran et al. (2003), no organized farmers groups were observed in the survey. Farmers rely heavily on credits provided by sometimes-unscrupulous fish traders and a non-transparent bargaining system that fixes prices and remunerations.

Shrimp monoculture ponds tend to be newer (1 to 15 years), while traditional and paddy-cum-fish culture ponds were older, 4 to 75 years and 9 to 29 years, respectively. Age of the pond had no specific relationship with disease incidence if proper care is taken for pond preparation. Total organic carbon (TOC) of the sediments in ponds accumulates over time and has a positive linear relation with the pond age (Biao & Kaijin 2007) and disease outbreaks.

Location of farms relative to the sea and other water bodies, villages, and agricultural land is highly variable. The distance of the pond from the sea was inversely proportional to the disease incidence. This might be due to the entry of wild species to the pond or be related to microbial diversity. It was also noticed that the distance between creek and pond, and inlet and outlet of the neighboring farm should not be less than 100 meters to minimize disease occurrence. Most farms (84.3%) used the same inlet and outlet for water input and drainage. These farms experienced 35.9%, 49.7%, 67.7%, and 27.8% of disease outbreaks over 2006–2009, respectively, less than the

TABLE 1 Comparison of different farming system in West Bengal

Parameters	Shrimp monoculture	Traditional Bheries	Paddy-cum-fish culture
Dry fallow period	7 months	1 month	1 month
Chemical Treatments	Extensive	Occasional	Rice pesticides
Species (stocking density per m^2)	*P. monodon* (14–46)	*P.* monodon (3.0), *M. rosenbergii* (0.4), *L. calcarifer* (accidental), *O. mossambicus* (0.04), *L. parsia* (0.7), Indian major carps (0.4), *M. cephalus* (0.1), *M. gulio* (0.3)	*P. monodon* (5.0), *M. rosenbergii* (0.2), *L. calcarifer* (0.1), *O. mossambicus* (0.5), *L. parsia* (0.3)
FCR	1.12–2.25	<0.6	<0.3
Pond size (ha)	0.05 to 12	0.07 to 100	0.05 to 3.0
Pond depth (feet)	4.5 to 9	2 to 3	2 to 3
Salinity (ppt)	3–15	0–18	0–18
Reservoir pond size (ha)	0.01 to 0.80	Nil	Nil
Water intake	Tidal + pumping	Tidal	Tidal
Disease prevalence (2008)	**Infectious diseases:** WSD (9.4%), WSD + black gill (1.9%), vibriosis (1.9%), vibriosis + rough shell (2.8%), vibriosis + thumb impression inside carapace of cephalothorax (0.9%), vibriosis + black gill (0.9%), vibriosis + microsporidiasis (1.9%), vibriosis + antenna cut (0.9%), vibriosis + swollen tail (0.9%), loose shell (4.7%) **Non-infectious diseases:** Black gill (5.7%), double shell (3.8%), rough shell (6.6%), rough shell + algal bloom (0.9%), thumb impression inside the carapace of cephalothorax (0.9%), antenna cut + thumb impression (0.9%), stunted growth with epicommensal infection (0.9%), white fecal disease (2.8%), slow growth (5.7%)	**Infectious diseases:** WSD (58.8%), vibriosis + algal bloom (1.5%), vibriosis + red ulcers in finfishes (1.5%) **Non-infectious diseases:** Black gill (2.9%), gill choke + algal bloom (1.5%), rough Shell (1.5%), rough shell + algal bloom (1.5%), uneven growth + jellyfish (1.5%), stunted and uneven growth (1.5%), uneven growth + red ulcers in seabass (1.5%), algal bloom + rough shell + red ulcers in Indian major carps (1.5%)	**Infectious diseases:** Vibriosis (4.2%), vibriosis + black gill (12.5%), vibriosis + microsporidiasis (4.2%), vibriosis + algal bloom (4.2%), vibriosis + black gill + algal bloom (4.2%), vibriosis + black gill + weeds (4.2%), vibriosis + weeds + rough shell (4.2%), vibriosis + loose shell + weeds (4.2%), vibriosis + weeds (4.2%) **Non-infectious diseases:** Black gill (4.2%), gill choke + algal bloom (4.2), double shell (4.2%), rough shell (4.2%), rough shell + algal bloom (8.3%), rough shell + jellyfish (4.2%), uneven growth + jellyfish (4.2%), stunted growth with epicommensal infection (8.3%), weeds + red ulcers in finfishes (4.2%).
Production (t/ha)	3.0 to 7.0	1.0 to 1.5	2.0 to 3.0
Production Cost (Rs/kg)	180–200	75	100–120
Average Net Income (Rs/ha)	210,000–490,000	140,000–160,000	160,000–180,000

levels observed by Nagesh, Abraham, and Ghosh (2009). The importance of separate drains and fills is widely accepted in aquaculture, but may not be as important in our study area, possibly related to distance between inlets among farms. The distance between neighboring farm inlets in West Bengal varied from 5 meters to 2 km, with an average of 169 meters.

Out of 198 farmers, 85.9% were using some kind of filtration for water inlets. Mesh hole sizes vary: 1.6 mm (8 × 8 holes per square inch), 1.04 (12 × 12), 0.6 (20 × 20), 0.4 (30 × 30), 0.3 (40 × 40), 0.21 (60 × 60), 0.16 (80 × 80), 0.13 (100 × 100), and 0.1 (120 × 120). Among these, the smaller mesh sizes of 1.04 mm (12 x 12 holes/sq. in.) used in Bhery and paddy-cum-fish/shrimp culture, and 0.1 mm (120 x 120 holes/sq. in.) in shrimp monoculture were the most effective in controlling infectious diseases. Predation by carnivorous fish that managed to elude the filtration system was reported by 6.6% of shrimp monoculture farmers and 4.2% of paddy-cum-fish/shrimp farmers.

Penaeus monodon seed is supplied for shrimp monoculture by input dealers-cum-farmers in Tamil Nadu, Puduchcheri, Andhra Pradesh, and Orissa at 40–50 paise (∼1 US cent) per piece and transported by air in plastic bags containing 2,000–2,300 post-larvae (PL 20–50) in 3 to 4 liters of water with an equal volume of oxygen. Farmers practicing Bhery and paddy-cum-fish/shrimp culture use wild-caught seed available locally at 30 paise per piece. The use of wild seed was related to a significant level of WSD in these systems. Wild fish seed of *Lates calcarifer* at Rs. 6 per piece, *Liza parsia* Rs. 200/kg (0.2–0.3 gm size), *Mugil caphalus* at Rs. 10 per piece (50 gm size), and *Mystus gulio* at Rs. 100/kg (2–3 gm size) were also available locally. Hatchery seed was available for the Indian major carps: *Catla catla*, *Labeo rohita,* and *Cirrhinus mrigala* at Rs. 80/kg (10 gm size), *Oreochromis mossambicus* at Rs. 40/kg (50 gm size), and juvenile *Macrobrachium rosenbergii* at Rs. 5 each (post-larvae of *M. rosenbergii* sell for Rs. 1.50 each).

The age of the PLs stocked varied between 12 and 30 days. Commercially available pelleted feeds were used in shrimp monoculture, while Bhery and paddy-cum-fish/shrimp farmers used ground nut cakes, mustard cake, rice bran, poultry waste, and hide-scrapings from tanneries as feed. Feeds should be stored for a maximum of 15 days to avoid aflatoxicosis.

Disease prevalence differed significantly among the different culture systems in terms of outbreak of WSD ($p < 0.01$), shell-associated problems ($p < 0.01$), and gill-associated problems ($p < 0.05$). Newly emerging disease conditions such as stunted and uneven growth was observed among all culture systems, while white fecal disease was observed only in shrimp monoculture (Table 2). Ponds with stunted and uneven growth showed more than 60% of the shrimp with less than 10 g size after 100 days of culture at a stocking density of 20–29 per m^2. The expected size of *P. monodon* after four months of culture at 30–50 per m^2 is 24 to 40 g (Chayaburakul et al. 2004). Between 70 and

TABLE 2 Culture system level analysis of disease prevalence (%) in West Bengal (2006–2009)

Diseases	Shrimp monoculture	Bhery	Paddy-cum-fish/shrimp
No disease ($p < 0.01$)	66.9 ± 7.64^b	37.7 ± 8.15^a	25.0 ± 9.64^a
White spot disease ($p < 0.05$)	9.2 ± 1.56^a	55.9 ± 4.48^b	12.5 ± 7.22^a
Vibriosis	5.3 ± 1.75	0.5	22.4 ± 12.41
Shell problems ($p < 0.05$)	7.1 ± 3.13^a	2.0 ± 1.0^a	20.9 ± 0.03^b
Gill problems ($p < 0.01$)	4.7 ± 1.49	1.5 ± 1.47^a	8.4 ± 2.4^b
Stunted & uneven growth	4.0 ± 1.77	1.0	4.2 ± 4.17
White fecal disease	2.1 ± 1.34	0	0
Gas bubble disease	0	0	1.4
Yellow discoloration	0.7	0	1.4
Finfish diseases	0	1.5	4.2 ± 2.42

Values bearing different superscripts in a row differ significantly.
*Only observed in one year.

80 days into the culture cycle, shrimps from the affected ponds exhibited clinical signs of white fecal disease—long strings of white feces floating on the water surface as reported by Chuchird et al. (2008).

Among the different culture systems, regular pond drying, scraping, and liming were followed by 80.2% of shrimp monoculture farmers, 41.2% of the Bhery farmers (drying and liming only), and 83.3% paddy-cum-fish/shrimp farmers (drying, ploughing, and liming). Scrapings were dumped outside of the peripheral dyke for strengthening.

Some 95% of shrimp monoculturists sterilize incoming water with bleaching powder (8–60 ppm; 63% use 15–30 ppm). Among those who did not use bleaching powder, 1.9% applied 1 ppm of benzalkonium chloride (banned in India) for disinfection. Only 35.3% of Bhery farmers applied 15 ppm of chlorination, and none of the paddy-cum-fish/shrimp farmers did so. The WSD incidence was estimated to be 3.0%, 6.6%, and 5.1% with chlorination during 2006, 2007, and 2008, respectively, compared to 19.2%, 22.7%, and 20.2% in ponds without chlorination during the same period. Oseko et al. (2006) reported effective inactivation of WSSV even at a low free chlorine concentration of 0.5 ppm. The free chlorine concentration used by surveyed farmers was between 3.1 and 23.4 ppm. As the WSS virus is hosted by a range of decapod crustaceans (Bonilla et al. 2008), further study is needed to estimate the minimum free chlorine concentration required for pathogen inactivation from carriers.

All the shrimp monoculture farmers used hatchery seed, 99% of which were PCR screened for WSSV. Paddy-cum-fish/shrimp farmers used only unscreened wild seed while Bhery farmers used both unscreened wild (64.7%) and screened hatchery (35.3%) seed. In 2007 and 2008 (data not available for 2009), WSD was much less prevalent among PCR-screened seed than among non-screened seed (Figure 2). WSD outbreaks were observed from days 15 to 110 in shrimp monoculture, days 50 to 70 in Bhery culture, and days 48 to 60 in paddy-cum-fish/shrimp culture. As previously described by Wongteerasupaya et al. (1995), Tan et al. (2001), Wu et al. (2001), Abraham and Sasmal (2008), and Stalinraj et al. (2008), shrimp infected with WSD showed a range of clinical signs such as white spots, red discoloration, and white spot plus red discoloration (Figure 3). About 6% of screened PLs appeared healthy but PCR tested positive (Figure 3). Magbanua et al. (2000) observed gross manifestations of WSD ranging from lack of appetite to reddish discoloration, external fouling, and mortality without white spots; 62% of apparently healthy shrimp were PCR positive for WSSV. Emergency harvest was done when disease outbreak occurred after 60 days of culture permitting farmers to recoup part of their expenditures, but not earning enough to make a profit at the local market price of Rs. 80–90/kg. Following an outbreak, only 9.1% of farmers chlorinated their pond water at the end of the culture cycle. An average of 59% of farmers disposed of moribund shrimp while the rest left the dead in the pond either to decompose or be consumed by co-stocked finfish. In only 2.5% of cases did disease in one pond spread to neighboring ponds of the same farm, indicating that farmers were taking remedial action when a disease was identified.

Among shrimp monoculture farmers using commercially available immunostimulants or pond treatments aimed at disease reduction (e.g.,

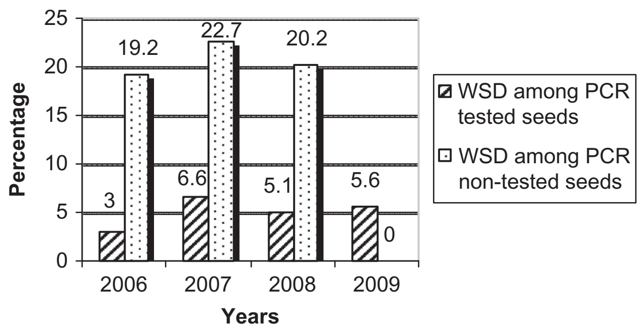

FIGURE 2 Comparison of WSD outbreaks among PCR-tested and non-tested seed.

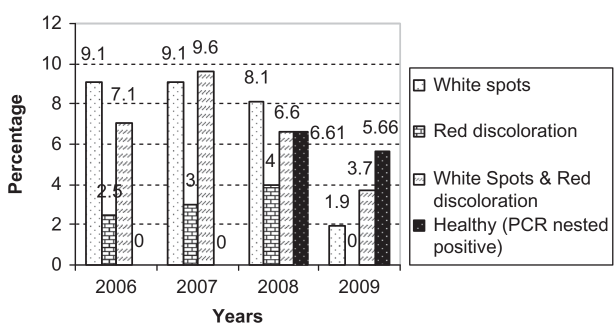

FIGURE 3 Prominent clinical signs of white spot disease in *P. monodon*.

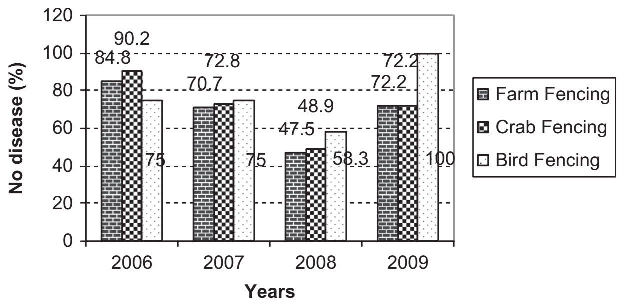

FIGURE 4 Relationship between bio-security and absence of disease outbreaks in shrimp monoculture system.

chemicals such as lime, bleaching powder, dolomite, gypsum, zeolite, and a range of "probiotics"), overall disease incidence was 41.2% in 2008 and 17.6% in 2009. Immunostimulants have been reported to reduce shrimp mortalities associated with vibriosis (Itami 1996) and WSSV (Balasubramanian et al. 2008).

Among shrimp monoculture farms, bio-security farm fencing, crab fencing, and bird fencing were observed in 93.4%, 86.8%, and 11.3% of the shrimp monoculture farms, respectively, while none of the Bhery and paddy-cum-fish/shrimp farms were fenced. Disease outbreak was found to be less on farms with bio-security fencing (Figure 4) as reported by the National Institute of Agricultural Extension Management (2008). It's interesting to note that none of the shrimp farmers were using antibiotics due to export awareness.

Overall, disease outbreaks were found to be more among Bhery system users and among farmers with less educational background (Bhattacharya & Ninan 2009). The use of known bio-security methods had a dramatic impact on disease prevalence. Making information available to illiterate farmers who depend upon lower technology systems would make a strong positive contribution to the sustainability of shrimp farming in West Bengal.

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