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A sequential assessment of WSD risk factors of shrimp farming in Bangladesh: Looking for a sustainable farming system

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ABSTRACT

White Spot Disease (WSD) caused by White Spot Syndrome Virus (WSSV), is responsible for widespread mortality and economic losses across almost the entire Asian shrimp farming industry. The distribution of disease prevalence is however uneven, and is likely dependent on a range of management, environmental and socio-ecological factors. In this study, 233 farms were surveyed in southwest Bangladesh, the main shrimp farming zone, to produce a dataset from a range of pond types, culture techniques and farming practices. Four categories of data (site/farm characteristics, environmental variables, disease history, and management variables) with associated risk factors were selected following the development of a conceptual framework and a participatory rural appraisal tool. Factors potentially contributing to WSD prevalence in the current shrimp crop were first screened using univariate analysis and subsequently analyzed using a multivariate logistic regression to highlight significant risk factors. Association of the selected factors with WSD prevalence was examined using multivariate stepwise removal. The multivariate analysis revealed that farms operated by a tenant worker (p: 0.03), mixed use of fertilizer (p: 0.009), poor quality water source (p: 0.001), lack of reservoir for water purification (p: < 0.001), and frequent exchange of water during a single crop culture (p: < 0.001) were significantly associated with WSD prevalence. The results suggest that, where possible, better farm management practices including improving water quality, controlling water exchange and/or maintaining constant salinity, will reduce WSD prevalence.

1. Introduction

The rapid expansion and development of aquaculture and the growth of both domestic and international demand for shrimp produce, has generated the conditions for the intensification of shrimp farming, with increased throughput and crop densities. Animal stress as well as geographical concentration of production have increased the prevalence and risk of White Spot Disease (WSD). WSD accounts for the lion's share of the USD 20 billion economic loss suffered by the Asian shrimp farming industry (Davies, 2016). Ninety percent of global aquaculture production is located in Asia, with China, India, Indonesia, Vietnam and Bangladesh the most significant producers (FAO, 2018). Bangladesh aquaculture production is currently 90% of finfish, 6% of shellfish (shrimp and prawn), and 4% of other aquatic animal production, yet in terms of economic value and foreign exchange, shrimp is highly significant. Almost all finfish are domestically consumed while the vast majority of the shellfish are exported to Europe, the USA and Japan. For Bangladesh's economy, they are the second most important exportable item after readymade garments (DoF, 2017).

Shrimp aquaculture in Bangladesh was originally developed as traditional extensive system with the engagement of over 8.5 million small-scale farmers, middle men, local vendors, and international exporters and importers (DoF, 2013). In many cases in Bangladesh, shrimp live in cultured ponds at relatively low densities. In 1996 WSSV impacted approximately 90% of extensive shrimp farms and led to many farmers going out of business (Debnath et al., 2016). After unplanned and haphazard expansion of coastal shrimp farming and production increases in the following years, there was another disease outbreak in 2001 with roughly 25% of production affected (DoF, 2002; Hossain and Lin, 2001). Partly in response, semi-intensive farming was introduced by Bangladesh Frozen Food Exporters Association (BFFEA) in the southeast region in 2002, and later in the southwest region by CP group. Commercial feed as well as technical assistance to train the farmers in good aquaculture practice were

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provided (Seafood Trade Intelligence Portal, 2019). The introduction of semi-intensive shrimp farming was designed to make Bangladeshi aquaculture internationally competitive and aimed to reduce the risks of WSD through improved biosecurity, feed and seed regimes. Nevertheless, outbreaks continued to rise in prevalence since 2007 and WSD remains the leading cause of production loss in Bangladesh (DoF, 2013). This is possibly related to higher stocking densities maintained in the semi-intensive farms, with the highly transmissible virus moving from traditional and extensive ponds where the disease is considered to be endemic. The attempt to reduce the horizontal transmission of WSSV through intensification and biosecurity effectively failed. Clearly the development, prevalence and transmission of the disease relate to numerous factors involving host, pathogen and their environment (St-Hilaire et al., 1998). For example, water quality and supply, insufficient waste removal, and excessive fluctuation in abiotic factors like oxygen, salinity, and temperature, can lead to the overloading of metabolites and environmental degradation – all of which increase animal stress and disease susceptibility (Kautsky et al., 2000). Moreover, WSSV propagation and transmission are facilitated by spatial clustering of farms; inter and intra farm exchange of water; pond management including sludge removal and crop rotation (culture rice in dry season and continue shrimp culture thereafter); indiscriminate use of chemical and biological treatments.

In addition to horizontal transmission between animals, WSSV can also be transmitted vertically from broodstock (Hsu et al., 1999; Lo et al., 1997). Presence of the virus can be confirmed using a range of techniques including microscopic observation (Kasornchandra et al., 1998), histopathology, immunological methods (Bachère, 2000), conventional PCR (Lo et al., 1996; Otta et al., 1999), Multiplex PCR, qPCR (Jeeva et al., 2014; Mendoza-Cano and Sánchez-Paz, 2013), in situ hybridization method (Chang et al., 1998) and recently developed loop-mediated isothermal method (Chang et al., 1998; Kono et al., 2004; Samyukthaa and Rathinasabapathi, 2013). Among these methods PCR has been considered as the most sensitive and widely adopted 2-step nested protocol to monitor and in turn reduce vertical transmission of WSSV from broodstock by removing infected individuals and pursuing one mother one tank biosecurity at hatcheries (Lightner and Redman, 1998; Lo et al., 1996). Addressing the vertical transmission of WSSV, the government, local and international non-government organizations in Bangladesh developed PCR testing facilities in the shrimp hatchery cluster located in Cox's Bazar, to improve the supply of WSSV free broodstock and post larvae (PL) (Debnath et al., 2016). Other measures to reduce WSSV prevalence and transmission have included training the farmers and raising awareness of the benefits of using PCR tested PL. According to the available scientific literature, these measures were not particularly effective at the farmer level (Chowdhury et al., 2015). A similar attempt to reduce vertical transmission involved the introduction specific pathogen free (SPF) shrimp PL through a collaborative approach involving the Bangladesh Shrimp and Fish Foundation and the Hawaii-based Moana Technologies in 2014 (Chowdhury, 2016) however, results have not yet been encouraging in terms of grow out adoption or success.

In part as a result, in recent years, shrimp aquaculture in Bangladesh has suffered from recurring WSD outbreaks with losses of hundreds of million dollars (Lafferty et al., 2015). 85% of shrimp hatcheries (DoF, 2017) depend on wild caught broodstock from the Bay of Bengal (Mazumder et al., 2015) where there is a high prevalence of WSSV (Ayub et al., 2008; Iqbal et al., 2011). Post larvae from these wild, WSSV positive broods are available at a lower price and given the difficulties in producing enough tested or SPF PL to match demand, farmers often have to rely on untested PL to stock their ponds. Vertical transmission from the hatcheries and the numerous routes for horizontal transfer of WSSV results in endemicity of WSSV in the Bangladeshi system. The openness of the farming systems in Bangladesh, the difficulties faced by farmers in managing their ponds and the impracticability of tight biosecurity approaches arguably make the shrimp sector more prone to WSSV transfer (Bhowmick and Crumlish, 2016; Islam et al., 2014). In addition, WSSV can be transmitted via other routes with around 90 species of arthropods, and some species of other phyla, acting as potential host reservoirs (Sánchez-Paz, 2010). Globally, scientific literature reveals that the transmission of WSSV is attributed to farm site characteristics, culture period, farm preparation, water management, culture method, feed and medicinal inputs, and biosecurity measures (Tendencia et al., 2011). Different biological factors including the invasive and cannibalism behavior of the host (Soto et al., 2001; Wu et al., 2001), age of host (Lightner et al., 1998; Venegas et al., 2000), diversified and high stocking density of shrimp (Lightner et al., 1998; Rajendran et al., 1999; Wu et al., 2001), and the virulence difference of WSSV strains (Marks et al., 2005; Wang et al., 1999; Zwart et al., 2010) were associated with the proliferation of WSD. The complexity of the disease causes, etiology and the range of social and economic factors relating to farmer practice and the implementation of disease management suggest that an integrated approach to WSD is required in Bangladesh. Therefore, shrimp aquaculture requires a multivariate analysis for recommending a comprehensive or joined up strategy to address WSD through developing new guidelines for the farmers to sustain seafood industry.

1.1. Multivariate logistic regression to screen WSD risk factors

Statistical regression models are the most applied techniques for predicting dichotomous outcomes (risk factors) of non-diagnosable disease (Tu, 1996). The statistical methods used for the derivation of empirical relationships between aquaculture system, environment and disease primarily involve two steps; firstly univariate or bivariate analysis is used to reduce the set of possible factors; and secondly a multivariate logistic regression (MLR) model is employed to unpack the key predictive factors (Prein et al., 1993). In this study, an MLR model was used to identify the factors associated with WSSV prevalence. There are a growing number of epidemiological studies that have identified risk factors for WSD (Corsin et al., 2001; Leung et al., 2000; Piamsomboon et al., 2015; Tendencia et al., 2011). Most of these studies used generalized linear modeling of multivariate techniques, such as logistic regression, but due to difficulties in generating accurate measurements or categories, many potentially important variables and subsequent factors were excluded from the models. Moreover, the studies were restricted to binary data and avoided inter-categorization of the variables for easy interpretation, limiting the extent to which inter-relationships of the closely related variables could be identified. Furthermore, the studies were valid only for specific and tightly defined environments (normally those associated with relatively closed production systems). In Bangladesh, the small number of existing studies include Islam et al. (2014) and Karim et al. (2012), and the current studies builds on and extends these studies.

There is a clear need to move beyond relatively abstract or even unattainable notions of biosecurity and develop studies and related guidelines concerning available and easily adopted culture strategies for marginal farmers. However, there were limited studies (Bhowmick and Crumlish, 2016; Debnath et al., 2016; Islam et al., 2014; Rahman et al., 2019) carried out to unpack risk factors related of WSD in shrimp farming in the Bangladeshi context, and minimal information to help farmers understand the source of risk factors. This study first used univariate analysis to reduce the number of variables and then the remaining variables were employed in multivariate logistic regression analysis as a noninvasive diagnosis tool to describe WSD risk factors associated with shrimp farming practices and provide further recommendations for marginal level farmers prioritizing farming system and practices in Bangladesh. The study was modelled to address the gap between the description of existing practices and the identification of potential WSD risk reduction practices.

2. Materials and methods

2.1. Research procedure

The study site was divided into low saline (Khulna), intermediate saline (Bagerhat), and high saline (Satkhira) area (Fig. 1). These areas have been collectively termed as '*shrimp zone'* by Datta et al. (2010) to demarcate an area of shrimp aquaculture development that has risen as a result of the favorable biophysical and agro-climatic conditions, as well as salinity of the near coastal/estuarine waters (Ahmed, 2013). Within the *'shrimp zone'* comprehensive list of WSD experienced shrimp farmers across the three eco-types was developed after discussions with the local Upazila Fisheries Officer of the Department of Fisheries, Bangladesh. The list of the farmers was cross checked at the participatory rural appraisal (PRA) stage of the research. The PRA process involved focus group discussion (FGD), with three FGDs in each site. From each of the farming sites populated with WSSV experienced shrimp farmers (Khulna – 500, Bagerhat – 90 and Satkhira – 190), 30% of farmers (NAO, 2001) from each region {Khulna (150), Bagerhat (26) and Satkhira (57)} were sampled using a simple random sampling technique that was considered to make a robust dataset for multivariate analysis. The questionnaire survey was undertaken with sampled shrimp farmers (N = 233) who reported having been affected by WSD between 2010 and 2017, and also within the survey timeframe of this study.

The stratification of the study site was structured according to salinity gradient as salinity was considered a key determinant in terms of ecology. In terms of culture systems, both extensive and semi-intensive culture was considered for this study. Intensification may lead to adverse changes in water quality and increase the risks of disease due to high stocking density, animal stress and associated feeding rates (Nasrin, 2016).

The selection of potential risk factors was a two-step process. The first step was to build a conceptual framework (Fig. 2) based on a comprehensive literature review to frame the major domains of the explanatory variables (WSSV carrier, farm operation and stressors) with WSD prevalence as the outcome. WSD prevalence was recorded based on farmer reports, developed over several years of farming experiences, and were linked to a number of common disease signs.

The second step involved applying PRA tools (key informant interview and focus group discussion) with shrimp farmers and other value-chain stakeholders to highlight any other potential risk factors. Having gained a suitable set of variables considered to be associated with WSD, a field survey questionnaire was developed. To increase respondent understanding during the interviews, the factors in the questionnaire were categorized into four groups: 1) Site/farm characteristics, 2) Management, 3) Environmental variables (EV), and 4) Disease history (DH).

The questionnaire was designed to include the identified risk factors and provide a clear logic that was accessible to farmers. The questionnaire was first developed in English, and then translated into Bengali. It was piloted and validated with selected farmers and stakeholders from the study area. Questions were then refined based on feedback from the pilot study. The final questionnaire was constructed in a Google form online version (https://goo.gl/forms/ckG1AIf9xMTxtPpf1) for data collection and management. Respondents involved in farm management: farm owners, managers, and technicians were recruited to participate in the survey. The data was collected with the assistance of enumerators from the NGO, ARBAN. Combinations of participatory, qualitative and quantitative methods were used for data collection through Rapid Rural Appraisal (RRA).

2.2. Data management and analysis

The analysis was performed into two sequential steps. A univariate logistic regression was first carried out for all of the independent variables. For the environmental variables (temperature, pH, and salinity) data was only available from 166 respondents, who were provided support to measure these variables by promoting organizations. The univariate analysis for the environmental variables was carried out by excluding the farms with missing values. The independent variables that had a p value of < 0.2 were then analyzed in the second, multivariate step.

For the multivariate analysis, a maximal model was first developed to include all variables selected during the univariate process. Variables were sequentially dropped from the model in a stepwise fashion using log-likelihood ratio tests. The variable was considered to have no significant effect on the model deviance if the p value from the log-likelihood ratio test > 0.05. Finally, the odds ratio for all significant variables was calculated. All analyses were carried out using the lmer package in RStudio. Multicollinearity was detected with the variables water source (direct natural), water source (indirect natural), and water coming via other farms and these could not be included together in the maximal model. As a work around, only the variable with the lowest p value (water source (direct natural) was included. The water source (indirect natural) and water coming via a number of farms variables were subsequently re-inserted into the final model to double check significance. The variables bird scares net, crab fence, and foot bath also suffered from issues of multicollinearity and additionally, had an insufficient number of replicates to be incorporated in a large maximal model. They were also excluded from the maximal model but were again re-inserted into the final model to confirm lack of significance.

3. Results

3.1. Variables of site/gher[1] characteristics, environmental variables and disease history

A total of 233 farms were surveyed and their related characteristics, here termed as variables, are shown in the Appendix A. The majority of farms (85.41%) were initially constructed via conversion of paddy, or other crop fields. Roughly 15% of farms were previously wetlands and/or other types of land. These topographical categories of farms were reported to be affected by WSD to different degrees. Shrimp farms that were previously used for rice or other crops, were found to be highly susceptible to WSD, and the farms developed in wetlands or other type of land areas were moderately susceptible to WSD. Results suggested that smaller farms were less likely to be affected by WSD (Table 1).

The majority of farms had either loamy (61%) or sandy (25%) soil with both being moderately prone to WSD. Although only a small number of farms had clay soil, results suggested these farms maybe less prone to the disease (Table 1).

3.2. Variables of management (site/gher management)

Nearly 85% of farms were operated by the farm owners, with the rest being operated by an employee. Irrespective of whether the farm operator was the owner themselves or an employee, WSD prevalence tended to be common across the shrimp sector in Bangladesh (Table 2). Around 50% of the respondents applied inorganic fertilizer to their shrimp farms, with 15% using a mixture of inorganic and organic fertilizer and 5% using organic fertilizer. WSD prevalence was relatively low in the shrimp farms that used either inorganic or organic fertilizers separately but appeared to be high when no fertilizers or a mixture of inorganic and organic fertilizers were applied (Table 2).

All surveyed farmers applied drugs and chemicals of some variety. Chemicals were used without any approval of responsible authorities

[1] *Gher* is a Bangla word meaning 'perimeter' which refers to pond for brackish water shrimp culture in Bangladesh. The term '*gher*' is interchangeably used for 'pond' in the entire manuscript for smooth reading.

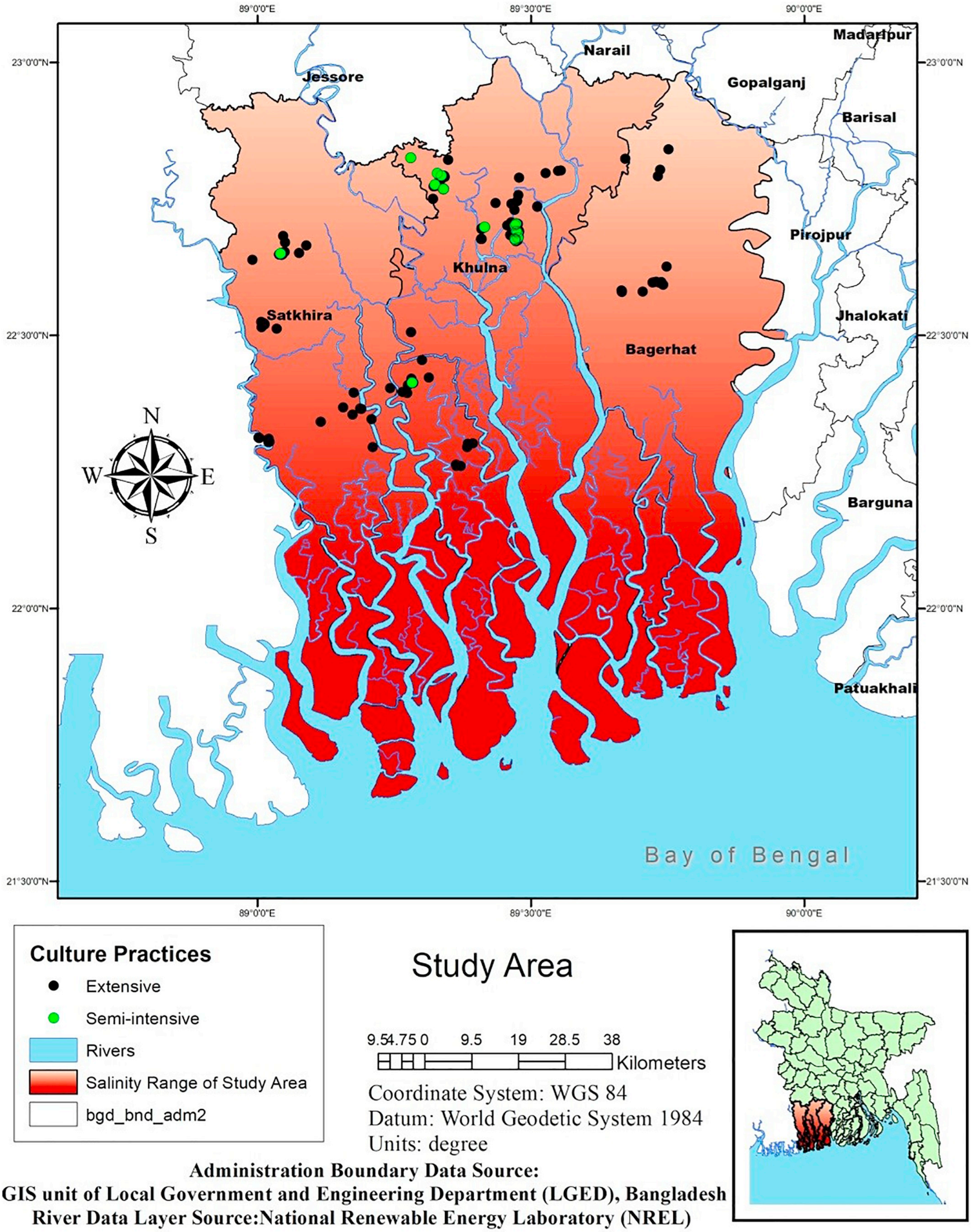

Fig. 1. Inset GIS map of Bangladesh highlighting the study area.

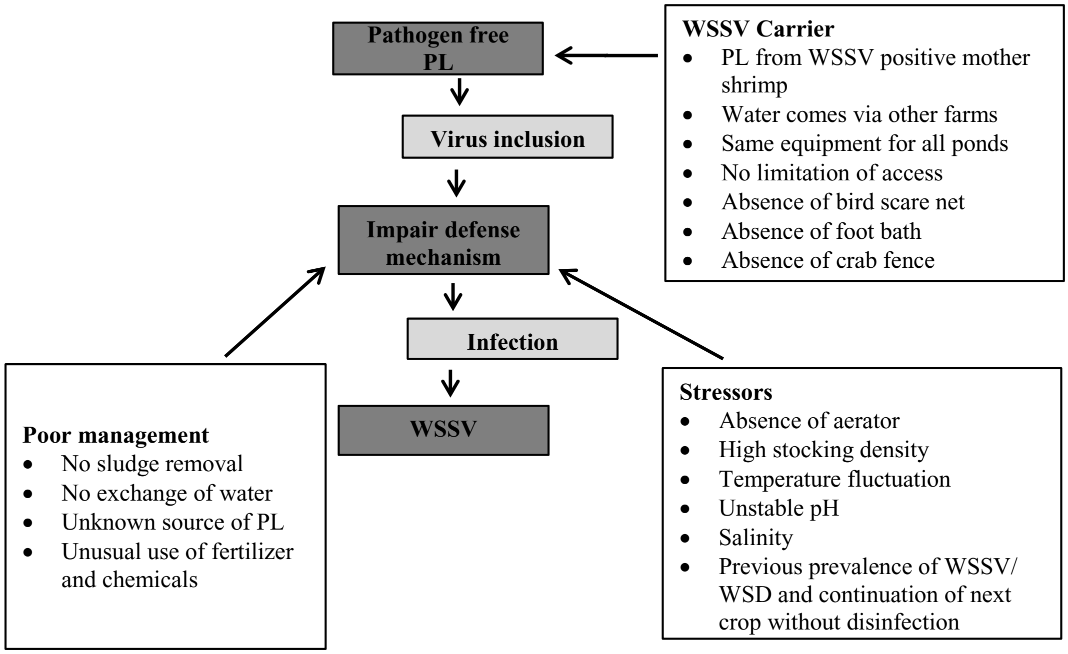

Fig. 2. Conceptual framework for risk factors selection, taking into account the inter-relationship of the proposed risk factors for WSSV prevalence.

Table 1
Descriptive statistics of different type of shrimp farms surveyed ($N = 233$) with their WSD prevalence (d) according to site/gher characteristics, environmental variables and disease history.

	Variables	Categories	n of N	% of n	*d* of n	% of *d*
Site/gher characteristics	Prior land use	Rice and other crops farming	199	85.41	126	63.31
		Wetland or others	34	14.59	18	52.94
	Area of production gher	Continuous variable				
	Dominant soil type	Sandy soil	58	24.89	38	65.51
		Loam soil	143	61.37	102	71.33
		Clay soil	32	13.74	4	12.5
	Average canal depth	Continuous variable				
	Average gher depth	Continuous variable				
EV[a]	Temperature	Continuous variable				
	pH	Continuous variable				
	Salinity	Continuous variable				
DH[b]	Previous prevalence	Continuous variable				
	Virus detected (current culture)	No	Response variable			
		Yes				

[a] Environmental variable.
[b] Disease history.

for both gher preparation and water treatment during the culture period. Listed chemicals from the survey data were categorized into chemical treatments and therapeutic treatments according to their mode of action, side effect and chemical composition. A large proportion of farmers was found to apply chemical treatments (such as lime, salt, etc.) in their farm operation, and WSD prevalence was lower on these farms compared to those that used therapeutic treatments (Table 2). Use of aerators was relatively rare with only 10% of farmers (predominanlty semi-intensive) employing them during production. Incidence of WSD was lower on farms that used aerators (Table 2).

In between the final harvest and the start of a new crop, almost all respondents completely dried their gher. Five out of the six farms that did not dry the gher between crops reported WSD presence. Sludge removal and suitable dike repair practices (i.e not with the soil from other farms) were also common for the majority of farmers (85% and 96%, respectively). Even so, farmers who regularly removed sludge from the gher and competently repaired dikes were still affected by WSD (ranging around 46 to 77% of farmers). Over 90% of farmers used sludge from the gher floor for their dike repairment, a source of possible WSSV recycling as rain may wash material back into the pond (Table 2).

3.3. Variables of management (water and culture management)

Farms with higher salinity were less susceptible to WSD. There was a large variety in the way water was sourced by farms; from sharing water with neighbouring farms to farms with their own water source that is treated in a separate reservoir within the farm. The farms that sourced water via other farms or were dependent on rainwater, were generally affected by WSD (85% and 80%, respectively). In comparison farms that sourced water direct from the canal and in conjunction with water treatment practices were less likely to be affected (42% incidence of WSD). Those farms that shared water with other farms tended to suffer from WSD incidence (80% of farms). Farms that used a separate water treatment reservoir and separate inlet/outlets had a lower incidence of WSD (41–49%). Interestingly, farms that frequently exchanged water (up to once every 42 days) were affected more with WSD (74–82%) than the farms that never or rarely (less than once every 42 days) exchanged water (26–40%) (Table 3).

Table 2
Descriptive statistics of different type of shrimp farms surveyed (N = 233) with their WSSV prevalence (*d*) according to management variables (site/gher management).

	Variables	Categories	n of N	% of n	*d* of n	% of *d*
Management variables	Site/gher management					
	Farm operated by owner	No	36	15.45	18	50
		Yes	197	84.55	126	63.95
	Use of fertilizer	No	76	32.62	48	63.15
		Inorganic	112	48.07	64	57.14
		Organic	11	4.72	5	45.45
		Mixed – inorganic and organic	34	14.59	27	79.41
	Use of chemicals (pond preparation)	Chemical treatments	20	8.58	19	95
		Therapeutic treatments	213	91.42	125	58.68
	Use of chemicals (water treatment)	Chemical treatments	76	32.62	58	76.31
		Therapeutic treatments	157	67.38	86	54.77
	Use of aerator	No	211	90.56	139	65.87
		Yes	22	9.44	5	22.72
	Gher dry after harvesting the stock	No	6	2.58	5	83.33
		Yes	227	97.42	139	61.23
	Sludge removal methods	No sludge removal	34	14.59	26	76.47
		Flushing, deposit sludge on farm	94	40.34	59	62.76
		Flushing, deposit sludge on and off farm	73	31.33	44	60.27
		Flushing, deposit sludge off farm	32	13.73	15	46.87
	Sludge removal interval	No sludge removal	34	14.59	26	76.47
		≥ 2 years	40	17.17	26	65
		Every year	159	68.24	92	57.86
	Maintain and repair dikes	No repair dikes or repair with the pond bottom soil of other farms	9	3.86	7	77.78
		Repair dikes with the pond bottom soil of farm itself	213	91.42	130	61.03
		Repair dikes with the soil from fallow land	11	4.72	7	63.63
	Period of fallow	Continuous variable				

Table 3
Descriptive statistics of different type of shrimp farms surveyed (N = 233) with their WSSV prevalence (*d*) according to management variables (water-culture management).

	Variables	Categories	n of N	% of n	*d* of n	% of *d*
Management variables	*Water management*					
	Water source (direct natural)	Indirect natural	134	57.51	74	55.22
		Rain water	25	10.73	20	80
		Boring water	13	5.58	9	69.23
		Direct from sea/river	61	26.18	41	67.21
	Water source (indirect natural)	Direct natural	99	42.49	70	70.71
		Water coming via other shrimp farms	20	8.58	17	85
		Canal from sea/river	49	21.03	30	61.22
		Treated water	65	27.9	27	41.54
	Reservoir	No	194	83.26	128	65.97
		Yes	39	16.74	16	41.02
	Water coming via no of farms	No	189	81.12	109	57.67
		Yes	44	18.88	35	79.55
	Frequency of water exchange	No exchange	38	16.31	10	26.31
		> 42 days	50	21.46	20	40
		29–42 days	58	24.89	43	74.13
		< 29 days	87	37.34	71	81.61
	Same passes for inlet/outlet	No	80	34.33	39	48.75
		Yes	153	65.67	105	68.62
	Culture management					
	Culture method	Monoculture	25	10.73	20	80
		Polyculture (shrimp with prawn)	50	21.46	35	70
		Polyculture (shrimp with fish)	158	67.81	89	56.32
	Source of fingerling	Mixed source or non-registered private hatchery	27	11.59	20	70.07
		Registered private hatchery	164	70.39	107	64.24
		Wild	42	18.03	17	40.47
	Stocking density	Continuous variable				
	Stocking age	Continuous variable				
	Quality of PL before stocking	Low	11	4.72	7	63.64
		Medium	194	83.26	123	63.40
		High	28	12.02	14	50
	Crop rotation	No	119	51.07	71	60.16
		Yes	114	48.93	73	63.47

Polyculture of shrimp with other salt tolerant freshwater fish was the main culture practice (68%) and farmers in this category reported lower incidence of WSD (56%). The prevalence of WSD was high (70–80%) on both monoculture (only shrimp) and bi-species culture (shrimp with prawn) farms. Farms that reported that their main source of PL was registered private hatcheries (70%) had moderate prevalence of WSD (64%). WSD prevalence was slightly higher (70%) on farms that stocked PL from mixed sources or non-registered private hatcheries.

Table 4
Descriptive statistics of different type of shrimp farms surveyed (N = 233) with their WSD prevalence (*d*) according to management variables (feed-biosecurity management).

	Variables	Categories	n of N	% of n	*d* of n	% of *d*
Management variables	*Feed management*					
	Types of feed use	Live food	33	14.16	28	84.84
		Formulated homemade pellet feed	43	18.45	33	76.74
		Mixed use of homemade and commercial pellet feed	59	25.32	43	72.88
		Formulated commercial pellet feed	56	24.03	24	42.85
		No supplement of feed	42	18.03	16	38.09
	Use of feed additives	No	143	61.37	97	67.83
		Yes	90	38.62	47	52.22
	Biosecurity management					
	Bird scare	No	221	94.85	141	63.80
		Yes	12	5.15	3	25
	Crab fence	No	206	88.41	135	65.53
		Yes	27	11.59	9	33.33
	Foot bath	No	211	90.56	139	65.87
		Yes	22	9.44	5	22.72
	Limited access	No	205	87.98	135	65.85
		Yes	28	12.02	9	32.14
	Same equipment for whole culture area	No	36	15.45	16	44.44
		Yes	197	84.55	128	64.97

Wild sources of PL may be less susceptible (40%) to WSD although this is an illegal practice. In spite of this, the quality of PL did not seem to be associated with WSD prevalence. Furthermore, some farmers rotate crops in their gher for cultivating other agricultural crops in the off-season to maintain their households. However, this practice appeared to have no significant impact on WSD occurrence (Table 3).

3.4. Variables of farm management (feed and biosecurity management)

The descriptive data suggests that producing shrimp without supplementing feed may result in a lower prevalence of WSD (this is the case for the most extensive and low input farms). However, most shrimp farmers use supplementary feeds or various kinds (homemade and commercial). The data suggests that disease is less likely with the use of commercial feed. Those farms applying commercial feed were less prone to WSD (43%) in comparison to farms that used homemade feed (77%). Combining homemade and commercial feeds had a similar incidence of WSD (73%). More than 80% of farmers that used live feed experienced WSD outbreaks on their farms (Table 4).

About one third of the respondents reported using different antibiotics, chemicals and probiotics as feed additives. The respondents that used antibiotics or different chemicals as additives were moderately affected by WSD (52%). The respondents who used probiotic additives were surprisingly more prone (68%) to WSD. Most farms (85–95%) did not implement additional biosecurity measures and of these, approximately 66% suffered from WSD outbreaks (Table 4).

3.5. Farming intensity and WSD prevalance

The survey consisted of 21 semi-intensive (9%) and 212 extensive (91%) farms. WSD was present (*d*) in 144 farms (62%) comprising of 5 (24%) semi-intensive and 139 (66%) extensive ones. The odds ratio (OR) 6.09 (95% CI: 2.14–17.29) suggested WSD presence is six times more likely on extensive compared to semi-intensive farms in Bangladesh (Table 5).

3.6. Univariate analysis

Univariate logistic regression reduced and prevented collinearity effects of variables included in the multivariate analysis (Table 6). Variables with a p-value > 0.2 were not included in multivariate analysis. The qualifying variables were then fitted into a maximal model.

Table 5
Farming intensity and the prevalence of WSD.

Data	Total	WSD emerged	OR	95% CI
No of farms	233			
No of farms manifested WSD (%)[a]	144 (61.8)			
Semi-intensive	21 (9.01)	5 (23.81)		
Extensive	212 (90.99)	139 (65.57)	6.09	2.14–17.29

[a] Figures in parentheses are percentage.

3.7. Multivariate analysis

Univariate analysis unpacked 24 potentially significant variables that were included in the multivariate analysis. Five variables were identified as potential risk factors for WSD presence. The risk of WSD prevalence in the post-stocking period was around three times greater in a mismanaged gher than in a well-managed gher. Whether the farm operator, the type of fertilizer used, water source (direct natural), presence or absence of a reservoir, and frequency of water exchange were all retained in the final model:

$$\text{WSD} \sim \text{farm operator} + \text{use of fertilizer} + \text{water source (direct natural)} + \text{reservoir} + \text{frequency of water exchange.}$$

The farms that were not operated by the farmer were 2.7 (95% CI: 1.1–6.5) times more likely to report WSD ($p = 0.03$, dev = 4.8) (Table 7). The type of fertilizer was also associated with WSD occurrence. Results suggest the use of a combination of both organic and inorganic fertilizer is associated with an increased risk of WSD ($p = 0.009$, dev = 11.7). Organic, inorganic, and the use of no fertilizer were associated with a 0.08 (0.01–0.4), 0.2 (0.07–0.6), and 0.3 (0.09–0.9), respectively, reduction in odds of WSD presence compared to farms that used both fertilizers (Table 7).

The risk of WSD occurrence was associated with water source, and particularly the use of indirect sources (water coming via other shrimp farms, canal from sea/river, and treated water). The odds of WSD presence on the farms is multiplied by 1.4 (0.7–3) for direct from river, sea or tidal, by 0.2 (0.05–0.4) for ground/bore hole water, and by 0.5 (0.08–3.2) for rain water ($p < 0.001$, dev = 16.2).

The use of a reservoir on farms appeared to provide a level of protection against WSD with odds of WSD presence being 0.05 (0.003–0.3) less likely compared to farms that have no reservoir

Table 6
Univariable – Adjusted p value for WSD according to site/farm characteristics, environmental variables, disease history and management variables.

	Variables	p Value		Variables	p Value
SC	Prior land use	0.7	Management variables	*Water management*	
	Area of production pond	0.11		Water source (direct natural)	0.007
				Water source (indirect natural)	0.002
	Dominant soil type	0.01		Water coming via no of farms	0.09
	Average canal depth	0.42		Reservoir	< 0.001
	Average farm depth	0.003		Frequency of water exchange	< 0.001
EV	Temperature[a]	0.26		Same passes for inlet/outlet	0.001
	pH	0.65		*Culture management*	
	Salinity	0.01		Culture method	< 0.001
DH	Previous prevalence of WSD	< 0.001		Source of PL	0.14
	Virus detected	Response variable		Stocking density	0.99
				Stocking age	0.99
Management variables	*Site/farm management*			Quality of PL	0.5
	Farm operated by owner	0.12		Crop rotation	0.68
	Use of fertilizer	0.15		*Feed management*	
	Chemicals use (pond preparation)	0.59		Types of feed use	0.73
	Chemicals use (water treatment)	0.11		Use of feed additives	0.2
	Use of aerator	< 0.001		*Biosecurity management*	
	Gher drying after harvest	0.59		Bird scare net	0.006
	Sludge removal method	0.1		Crab fence	< 0.001
	Sludge removal interval	0.14		Foot bath	< 0.001
	Maintain and repair dikes	0.57		Limited access	< 0.001
	Period of fallow	0.23		Same equipment for whole farm	0.005

[a] Before analysis the missing values from dataset have been removed. This was also done for other environmental variables like for pH and salinity.

Table 7
Logistic regression analysis of risk factors associated with WSD prevalence.

Variable	Deviance	p-Value	Treatment	OR (95% CI)
Farm operated by owner	4.8 (1 d. f)	0.03	No	Ref.
			Yes	2.7 (1.1–6.5)
Use of fertilizer	11.7 (3 d. f)	0.009	Mixed – inorganic and organic	Ref.
			Organic	0.08 (0.01–0.4)
			Inorganic	0.2 (0.07–0.6)
			None	0.3 (0.09–0.9)
Water source (direct natural)	16.2 (3 d. f)	0.001	Indirect natural	Ref.
			Direct from river, sea or tidal	1.4 (0.7–3)
			Boring water	0.2 (0.05–0.4)
			Rain water	0.5 (0.08–3.2)
Reservoir	13.8 (1 d. f)	< 0.001	No	Ref.
			Yes	0.05 (0.003–0.3)
Frequency of water exchange	20.8 (3 d. f)	< 0.001	Never	Ref.
			> 42 days	0.29 (0.09–0.93)
			29–42 days	0.3 (0.1–0.8)
			< 29 days	1.8 (0.8–4.3)

($p < 0.001$, dev = 13.8) (Table 7).

The frequency at which farms exchanged water within single crop cultivation was associated with WSD prevalence ($p < 0.001$, 20.8). The odds of WSD prevalence increased in farms that frequently exchanged water { < 29 days (OR = 1.8 (0.8–4.3)} in comparison to a farm that did not exchange water. On the contrary, there appears to be a protective effect in the farms that exchanged water a moderate amount [29–42 days {OR = 0.3 (0.1–0.8)} or > 42 days {OR = 0.29 (0.09–0.93)}]. The goodness of fit for the final model was tested using McFadden's (Pseudo) R^2 with a value of 0.22 suggesting that a high portion of the variance in the data remained unexplained by the variables.

3.8. *Temperature-salinity interaction*

None of the environment variables were significant in the univariate analyses with the exception of salinity ($p = 0.01$). Despite this we plotted a response surface for temperature and salinity against mortality rate (%) to highlight the relationship (Fig. 3). This part of the analysis utilized in order to provide a description of the ideal environmental ranges for shrimp farming in Bangladesh.

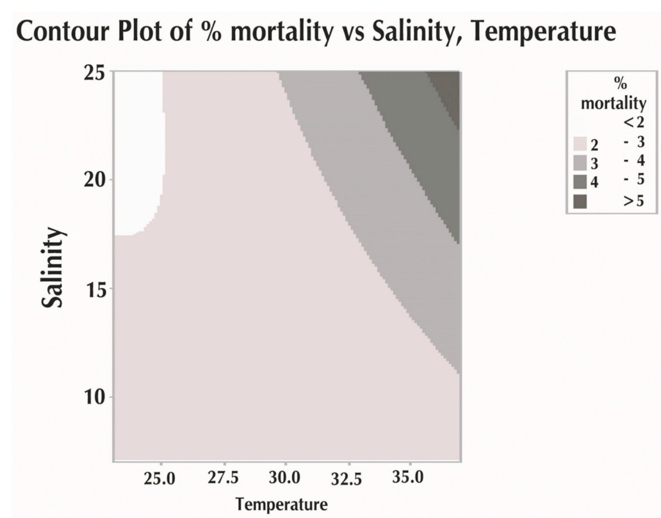

Fig. 3. Response surface showing percent mortality of shrimp at different gradient of temperature and salinity.

The complex co-relations between the effects of salinity and temperature on mortality rate are clear from the response surface. The response surface confirms high tolerance of salinity and a wide range of temperature tolerance of shrimp. Disease susceptibility was considerable both in high as well as low temperature and within the range of medium to high salinity exposure. The optimum temperature and salinity were determined at 25 °C and 17 ppt, respectively.

4. Discussion

This study analyzed the risk factors associated with WSD using a series of methodological steps (Fig. 4). These steps unpacked a number of variables associated with the deadly disease that accounts for

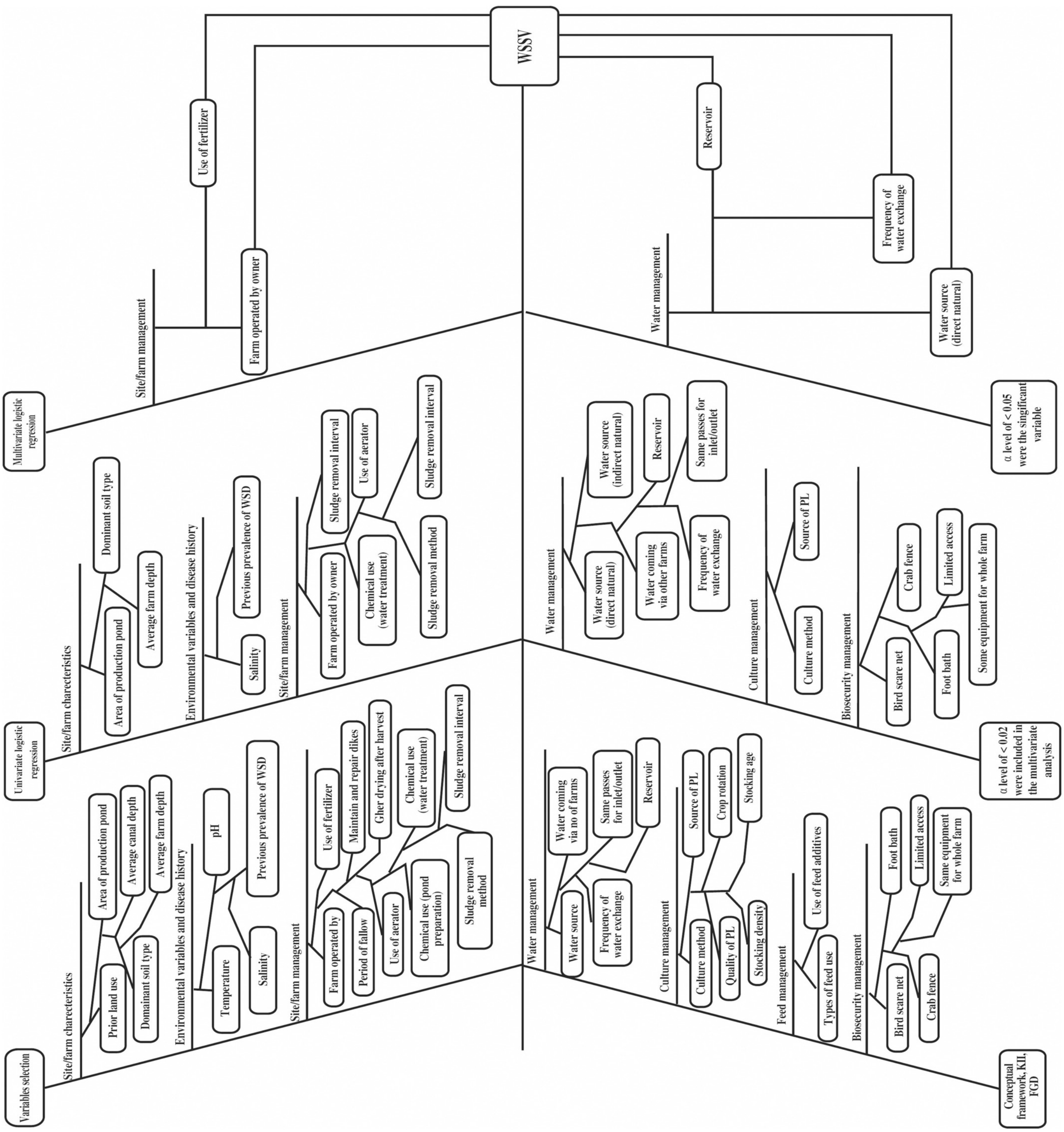

Fig. 4. Fish bone diagram showing whole process of the study ranging from methodology to results.

economic losses of between US$ 832 and 3928 ha^{-1} in Bangladesh (Jahan et al., 2015). After selecting the main producing region of southwest Bangladesh, a questionnaire survey of 233 farmers, with a set of 38 variables, data was conducted. Risk factors associated with WSD were highlighted through a combination of descriptive, univariate and multivariate approaches.

In univariate analysis, some of the variables under site/pond characteristics were found to be significant. However, they did not remain in the final multivariate model. This is perhaps due to the amalgamation of numerous interconnected shrimp farms in a vast area with similar ecological conditions of high salinity that reduced inter-farms variabilities. Therefore, WSD in Bangladesh is beyond these factors as the major shrimp farming area (Khulna, Satkhira, and Bagerhat) is located adjacent to mangrove area, a high saline zone in the south. However, several studies reported that farms in low saline zones (Begum and Alam, 2002), far from mangrove areas (Belak et al., 1999) and large farms with shallow water (Boyd and Tucker, 1998; Fast et al., 1988; Tendencia et al., 2011) are susceptible to WSD in the Philippines, China, Thailand, and Indonesia.

In terms of environment, salinity was the most important conditioner of WSD susceptibility suggested by univariate analysis. Abrupt fluctuation of salinity leads to considerable stress in the shrimp and can trigger WSSV infection. Below or above the optimum salinity range (10-25 ppt), the virus replicates and infection rates can increase (Du et al., 2006; Granja et al., 2006; Moser et al., 2012; Rahman et al., 2007). Lower water salinity was also detected as an incremental risk factor as it increases the osmotic gradient between the hemolymph and passively passed external water through the water pores, which increases access for WSSV via the digestive system and the gills (Capen, 1972; Escobedo-Bonilla et al., 2007; Freire et al., 2008; Henry et al., 2012; Huong et al., 2010; Parado-Estepa et al., 1987). The effect of anthropogenic activities and climate change are gradually altering the coastal environment which is likely to change the salinity and therefore impact the prevalence of shrimp diseases (Haque et al., 2017). However, in the form of clustered community-based shrimp farming, farmers can develop water inlet and outlet channels that in turn allow them to control water salinity and other water quality parameters. Pamini River Shrimp Farmers Association in South India is a community-based shrimp farmers association that encourages better management practices like scheduled farm operations, quality seed procurement, simultaneous stocking, water exchange and harvesting regimes, in order to contribute to the substantial reduction in vertical and intra cluster transmission of disease (Kumaran, 2009).

The incidence of WSD tended to be higher in extensive farms in comparison to semi-intensive farms. This suggests that, where practicable, the adoption of semi-intensive farming practices can reduce the risks of WSD. These changing practices include; operation of the farm by the owners themselves, sole use of approved fertilizers following the appropriate application rules, ensuring a safe water source for the farm, construction of a separate water reservoir, and limiting water exchange during crop culture. Improvements in biosecurity practices for the majority of the 200,000 or so farms would also improve matters.

A farm operated by the owner him/herself was the first risk factor derived from the multivariate analysis associated with WSD prevalence. WSD was more prevalent in the surveyed farms operated by an employed worker or technician compared to the farms operated by the owner. This is possibly due to the fact that owners derive the financial benefits from a disease-free production by employing sustainable practices, whereas employees see little of these financial rewards. According to Haque et al. (2014), the successful operation of rice-fish farms was due to multiple socio-economic and biophysical reasons; with a key factor being the owners involvement in the farming.

Use of fertilizer for pond preparation and other activities of farm operation was identified as another potential risk factor of WSD prevalence within the multivariate analysis. Fertilizers used in shrimp farming are either organic or inorganic with the purpose of stimulating healthy blooms of zooplankton as well as phytoplankton and to make natural food organisms available to the newly stocked larvae. Lower disease prevalence was reported in ponds infected with WSSV when the farms were fertilized with inorganic fertilizers like phosphorus (Corsin et al., 2001). Among the organic fertilizers, manure or cow dung produced the best results against WSD outbreaks in the farms (Tendencia et al., 2011). Cow dung was also found to be an effective alternative to inorganic fertilizer, helping to enhance productivity, improve water filtration, and minimize the chances of viral, bacterial and fungal pathogenic disease. The prophylactic properties of cow dung can regulate the pond microbiome, which also acts as an Ayurveda purifier for different wastes in the waterbody (Dhama et al., 2005; Randhawa and Kullar, 2011). Another case study conducted by Begum and Alam (2002) in the Khulna region of Bangladesh, revealed that the application of both organic and inorganic fertilizers resulted in no disease incidence for the first four crops, though WSD tended to affect any subsequent crops.

In the univariate analysis, the unplanned use of chemicals for water treatment was a significant conditioner, while in the multivariate analysis it was excluded. Some studies (Devi and Prasad, 2006; Gräslund et al., 2003; Reyes et al., 1999) based on multivariate analysis reported the significant associations of disease occurrences with the application of chemicals for water treatment. This suggests that the unregulated growth of drugs and chemicals marketing in Bangladesh stimulated farmers into using them to eliminate diseases. Farmers are experiencing financial losses of about USD 46.3/ha/yr (Phillips, 2000) for drugs and chemicals purchase without having proper treatments. This cost is limited to extensive farms, while in semi-intensive farms this varies from natural to synthetic compounds (Shamsuzzaman and Biswas, 2012). There are only six approved chemicals for use in aquaculture as designated by the US Food and Drug Administration (FDA) and US Environmental Protection Agency (EPA) (Benbrook, 2002). Farmers often apply a group of chemicals either based on their previous experience or from the suggestions from chemical vendors, without truly understanding the mode of action of the chemical. There were lots of discussions among concerned organizations to appoint professional personnel under the Department of Fisheries (the governments drugs and chemical regularity authority) to prescribe the appropriate drugs and chemicals to be used by aquaculture farmers. In Bangladesh, the aquaculture drugs and chemical industry is dominated by manufacturers and traders without strong control by the regulatory authority. All of these stakeholders need to be united in a common platform to ensure the responsible use of drugs and chemicals by manufacturing species and system-specific chemicals, increasing on-farm management of chemicals use, and developing awareness of the health consequences of chemical misuse.

Univariate analysis and descriptive statistics showed that the farmers who did not use an aerator were more prone to WSD. In shrimp farms, oxygen is an important physiochemical limiting factor as crustaceans cannot survive in limited oxygen levels unlike many finfish species (Dean and Richardson, 1999). Shrimp ponds in Bangladesh tend to be shallow, with many aquatic plants grown on the bottom and decomposition tends to limit available oxygen. Oxygen-stressed stock are more susceptible to the onset of viral disease with viral loads rapidly amplified. Aerators, used correctly, can maintain dissolved oxygen at an optimum level and balances other physicochemical parameters. According to Boyd (1989) and Le Moullac et al. (1998), the absence of an aerator indirectly provokes disease by the impairment of the host defense mechanism. Many other studies indicate that the installation of aerators improved the water quality of shrimp ponds, enhanced digestion and metabolism of the animals, and kept them disease-free resulting in higher yield (Jiang et al., 2005; McGraw et al., 2001; Neill and Bryan, 1991). The installation of aerators in aquaculture ponds is growing in Bangladesh because of the increasing availability of aerators on the markets and improvement and distribution of electricity across the country. Many local and international companies have started marketing paddle wheel and ring blower aerators in Bangladesh. The efficiency of ring blower aerators is very high compared to others and 0.6–0.8 HP aerators can aerate about 1 ha of shrimp farm depending on the semi-intensive to intensive culture. The electricity cost associated with this practice is no more than USD 200/ha. Installation of aerators would increase the cost of USD 0.1/Kg but increase the yield of shrimp by 50% (Banrie, 2013; NRCS-USDA, 2011; Tucker, 2005), therefore it is likely to be cost-effective to the farmers in Bangladesh, depending upon the economic margins and profitability of the farms.

The prevalence of WSD was shown to be particularly high in the farms where the farm owners did not remove the sludge from the bottom of the pond after every crop. The reluctance of the farmers to de-sludge ponds resulted in sediment deposition in the pond, impairing soil and water quality (Munsiri et al., 1996; Steeby et al., 2004; Thunjai et al., 2004). The sludge removal practices varied from several years to never. Munsiri et al. (1996) reported that, in Asian shrimp aquaculture, sediment is often removed after every crop. Though sludge removal has significant benefits (Boyd, 1995; Karim et al., 2012) it is common only to remove sediment at intervals of several years or when it becomes problematic (Boyd, 1995; Thunjai et al., 2004). According to Karim et al. (2012), sludge removal from shrimp farming has a significant relationship with lower susceptibility of WSD. Shrimp farmers in Bangladesh who removed sludge from the pond regularly were found free from WSD. Farmers can use a cost-effective sludge remover device with the capacity to remove 0.078 ha/h of sludge from an intensive farm, resulting in good production and economic performance through lower disease presence, feed conversion ratio (FCR) and specific growth rate (SGR) improvement (Hossain et al., 2016). This sludge remover was locally developed by the Bangladesh Agricultural Research Institute (BARI) with the capability to remove sludge up to 40–50 mm from the aquaculture pond. Moreover, sludge has a high level of nutrients and organic matter which has the potential to be recycled and used as organic manure for fodder and other crop production (Haque et al., 2016).

The majority of risk factors identified by multivariate analysis were related to water management measures. These were water source (direct natural), presence or absence of a reservoir and the frequency of water exchange. Among these conditions, it could be recommended that farmers avoid the exchange of untreated water within a culture period as the addition of water may have stressed the shrimp and set off an epizootic outbreak and/or potentially introduced vectors and/or viruses into the pond. A good alternative for shrimp farmers could be the adoption of an environmentally friendly zero water discharge technology (Suantika et al., 2015) or green water technology (Bosma and Tendencia, 2014). Such technology could maintain water quality, and prevent pathogen spread as well as wastewater discharge to the environment. Tendencia et al. (2011) also suggested reducing water exchange as a means to reduce WSSV transmission. This study highlighted how sharing a water source increases biosecurity risks. These results are also in agreement with Mohan et al. (2008), who highlighted the risk of water exchange within single crop farming. Where practical, the farmers should develop a sole water source, that has not been introduced via other farms, as the water from other farms tended to allow entry to disease-carrying vectors. The irregular and unapproved development of the clustered farms amalgamates water sources. Individual and dedicated reservoirs can reduce this kind of contamination problem. The dedicated and planned reservoir system can mitigate the contamination of water source by discharge effluents (Corea et al., 1998) and facilitate better circulation and aeration in the farms.

Biosecurity measures are often suggested as a means to reduce pathogen transmission and disease risks (Lotz, 1997; Mohan et al., 2008). In Bangladesh, the most common biosecurity measures practised on shrimp farms are tangle bird scare nets, installing crab fences, limiting human access, using separate equipment between the ponds and foot baths for visitors. It is logical to think that farms with high biosecurity have a lower risk of WSD prevalence. The univariate results of this study indicate that the use of separate equipment to limit the horizontal transmission of WSSV may be effective. Contamination from the feces of predatory birds (Garza et al., 1997), and the crawling and burrowing behavior of the crab can limit the biosecurity efficiency of crab fences. There is scientific evidence that WSSV is transmitted by people, and the results suggest that use of foot baths and limited access are significant. Though biosecurity measures for shrimp farms were criticized by Tendencia et al. (2011), Mohan et al. (2008) recommended biosecurity measures as a disease prevention tool. More advanced biosecurity measures including zero water discharge and water remediation (biofloc, RAS); highly energy-efficient aeration-mixing systems; automation (electro-valves, feeder); organic waste recycling (sludge, animal tissue) and GMP (good management practice) can be adapted to minimize the risks of disease introduction and spread within the farm. However, these can be costly and need to be measured against the practicalities of farms and farming practice in Bangladesh. The overall discussion of the results indicates that the stocking of PCR tested or pathogen free PL is not the only important factor with regards WSD. Instead, an array of labour, the application of fertilizers and poor water management practices in and around the shrimp farms were complexly linked to WSD incidence.

5. Conclusion

According to the available literature, no comprehensive and systematic investigation on risk factors under different domains of shrimp farming practices such as site/gher characteristics, environmental variables, disease history and management (site/gher, water, culture, feed, and biosecurity) variables, has been carried out in Bangladesh. This study considered multiple variables under those domains as risk factors to draw a conclusion on WSD prevalence in shrimp farming and its sustainable operation. All these factors were framed in a structured questionnaire and data was generated from different representative regions (Khulna, Bagerhat, and Satkhira) of shrimp farming in Bangladesh. The data were double screened by univariate and multivariate logistic regression analysis to uncover the responsible composite risk factors for WSD. The key risk factors associated with WSD are farm owner-labour relations (and the absence of owners from the farm); use of inorganic and organic fertilizer separately; unregulated and shared sources of water; the lack of reservoir and frequent exchange of water in the farms. These all indicate that avoiding WSD is not only related to the stocking of pathogen free PL or specific pathogen free PL, but more closely linked to some integrated management practices of farms. According to this study, if the shrimp farmers operate their own farms or appointed operators follow better management practices, use only approved fertilizers in a safe manner, avoid unsafe sources of water, construct a distinct reservoir for water refining before introduction into the culture pond, and control water exchange at a standard level, they can reduce WSD incidence. These better management practices are promoted by different aquaculture farm operation standards in many countries and need to be adopted and taught at the farmer level by giving advanced training in cluster forms so that the awareness among the farmers builds rapidly in the wider geographical area of Bangladesh, populated by > 200,000 shrimp farmers.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author statement

Neaz Al Hasan: Contributed to conceptualization of the research, data collection, data cleaning, analysis, interpretation and drafting the manuscript.

Mohammad Mahfujul Haque: Contributed to conceptualization and supervision of the research, data analysis, interpretation, editing and discussion of the results of the manuscript.

Steve J. Hinchliffe: Contributed to conceptualization of the research, interpretation of the results, discussion of the results and editing of the manuscript.

James Guilder: Contributed to data analysis for statistical modeling using the software RStudio and interpretation of the results to an extent.

Appendix A. Categorization of the variables

	Variables	Variables narration
Site/farm characteristics	Prior land use	Rich or other crops farming: 3, Wetland or others: 1
	Area of production pond	Continuous variable
	Dominant soil type	Sandy soil: 3, Loam soil: 2, Clay soil: 1
	Average canal depth	Continuous variable
	Average farm depth	Continuous variable
	Culture practice	Extensive: 2, Semi-intensive: 1
EV	Temperature	Continuous variable
	pH	Continuous variable
	Salinity	Continuous variable
DH	Previous prevalence of WSD	Continuous variable
	Virus detected (current culture)	No: 0, Yes: 1

	Variables	Variables narration
Management variables	Site/farm management	
	Farm operated by owner	No: 0, Yes: 1
	Use of fertilizer	No: 4, Inorganic: 3, Organic: 2, Mixed – inorganic and organic: 1
	Chemicals use (pond preparation)	Chemical treatments: 3, Therapeutic treatments: 1
	Chemicals use (water treatment)	Chemical treatments: 3, Therapeutic treatments: 1
	Use of aerator	No: 0, Yes: 1
	Gher drying after harvest	No: 0, Yes: 1
	Sludge removal method	No: 5, Flushing, deposit sludge on farm: 3, Flushing, deposit sludge on and off farm: 2, Flushing, deposit sludge off farm: 1
	Sludge removal interval	Never: 1, 1 year: 2, $\geq$2 year: 3
	Maintain and repair dikes	No repair dikes or repair with the pond bottom soil of other farms: 4, Repair dikes with the pond bottom soil of farm itself: 2, Repair dikes with the soil from fallow land: 1
	Period of fallow	Continuous variable
	Water management	
	Water source (direct natural)	Rain water: 3, Boring water: 2, Direct from sea or river/tidal: 1, If not direct natural: 0
	Water source (indirect natural)	Water coming via other shrimp farms: 4, Canal from sea/river: 2, Treated water: 1, If not indirect natural: 0
	Water coming via other farms	No: 0, Yes: 1
	Reservoir	N0: 0, Yes: 1
	Frequency of water exchange	$\leq$ 7–28 days: 4, 29–42 days: 3, $>$ 42 days: 2, No exchange: 1
	Same passes for inlet/outlet	No: 0, Yes: 1
	Culture management	
	Culture method	Monoculture: 4, Polyculture (shrimp with prawn): 3, Polyculture (shrimp with fish): 1
	Source of PL	Mixed source or non-registered private hatchery: 3, Registered private hatchery: 2, Wild: 1
	Stocking density	Continuous variable
	Stocking age	Continuous variable
	Quality of PL	Low: 3, Medium: 2, High: 1
	Crop rotation	No: 0, Yes: 1
	Feed management	
	Types of feed use	Live food: 5, Homemade pellet feed: 4, Mixed use of homemade and commercial pellet feed: 3, Formulated commercial pellet feed: 2, No: 1
	Use of feed additives	No: 0, Yes: 1
	Biosecurity management	
	Bird scare net	No: 0, Yes: 1
	Crab fence	No: 0, Yes: 1
	Foot bath	No: 0, Yes: 1
	Limited access	No: 0, Yes: 1
	Same equipment for whole farm	No: 0, Yes: 1

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