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Critical better management practices and critical bio-security measures for prevention of entry and spread of white spot virus and pathogenic *Vibrio* in grow-out farms of cultured *Penaeus monodon* in Sri Lanka

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Abstract Since the first record of white spot disease (WSD) in 1996, PCR tested, white spot virus (WSV) free post larvae of *Penaeus monodon* (Fabricius 1798) are stocked in grow-out farms as the major bio-security measure. However, significantly high mortality in young shrimp and total rejection of harvest have been recorded in North Western Province, Sri Lanka due to WSD and/or vibriosis. Present study was planned to identify critical better management practices (CBMPs) and critical bio-security measures (CBSMs) that should be strictly adopted to prevent the entry and spread of WSV and pathogenic *Vibrio* in grow-out farms of *P. monodon*. A questionnaire survey, carried out at randomly selected 100 grow-out farms located in the North Western Province, revealed that there was a relationship between the occurrence of WSD and /or vibriosis and levels of practicing better management practices (BMPs) and/or bio-security measures (BSMs). Proper pre-stocking pond preparation, adoption of zero water exchange, monitoring and controlling water quality parameters including pathogenic *Vibrio* in culture water and use of a suitable bioaugmenter and a probiotic were identified as the CBMPs while proper disinfection of culture water, stocking of WSV and MBV free post larvae and prevention of WSV contamination through other routes over the rearing period were the identified CBSMs. Entry and spread of WSV and pathogenic *Vibrio* in *P. monodon* grow-out farms in the North Western Province, Sri Lanka could be prevented by strict adoption of CBMPs and CBSMs identified during the present study.

Keywords: *Penaeus monodon,* white spot disease, vibriosis, bioaugmenter/probiotic, Sri Lanka

INTRODUCTION

The shrimp farming generates export earnings with tremendous employment opportunities, and has a great potential for further development in Asia (Subasinghe 2015; Thitamadee et al. 2016). Epizootics of both infectious and non-infectious etiology have continuously plagued both production systems (hatchery system and grow-out system) of the shrimp farming industry (Walker and Mohan 2009). Diseases caused by viruses, bacteria, fungi and parasites are considered to be very important in shrimp culture (Lightner 2003).

Among the recorded pathogenic viruses, white spot virus (WSV), the etiological agent of white spot disease (WSD) is the most serious pathogen in terms of overall production losses because it is lethal for all cultivated shrimp species and as the disease causes rapid and high mortality (Flegel 2006). *Vibrio* species, the most important pathogenic bacteria affects shrimp in both hatchery and grow-out systems and causes chronic or mass mortalities and shell deformities reducing marketability of shrimp (Lavilla-Pitogo et al.1990). Common pathogenic *Vibrio* species both in *P. monodon* hatchery and grow-out systems in the North Western Province, Sri Lanka have been isolated and identified (Kumara 2016; Kumara and Hettiarachchi 2016, 2017).

The most obvious way to prevent a disease is to keep the pathogenic organism out of the farming system (Lightner 2005). In order to evaluate the possibility of such a control strategy, it is necessary to first examine the possible routes by which the pathogen can enter the rearing system of shrimp. Better management practices (BMPs) and bio-security measures (BSMs) are employed to prevent the vertical and horizontal transmission of pathogens, which in turn could prevent the occurrence of diseases reducing the risk of heavy mortalities in cultured shrimp (Lightner 2005).

BMPs have been developed for both agriculture and aquaculture as means for farmers to improve economic gains while reducing negative impacts on the environment (Engle and Valderrama 2004; Béné 2005). BMPs for shrimp culture are based on the principles for sustainable shrimp farming developed by the consortia of international agencies including World Wide Fund for Nature and code of conduct for responsible fisheries prepared by FAO (Béné 2005). Many of the BMPs proposed for cultured shrimp industry are based on published studies on the biology and epizootiology of white spot disease, empirical observations, and provincial, national, and international regulations (Corsin et al. 2001; Engle and Valderrama 2004; Lightner 2005; Walker and Winton 2010). These international principles and codes have been adapted to national levels in each shrimp farming country in order to reduce the risk of diseases, the environmental degradation and to suit the specific demands of intensive and/or extensive production systems (Melba et al. 2005). BMPs are also intended for the development of sustainable shrimp aquaculture industry, to improve food safety of the final product and for social benefits derived from shrimp farming (Walker and Mohan 2009). At the farm level, better management practices (BMPs) are often related to general farm management practices (Flegel et al. 2008). Schuur (2003) had pointed out that, although bio-security measures are widely used in shrimp hatchery systems, under grow-out field conditions such bio-security measures cannot be practiced at absolute levels.

Since 1996, environmental degradation due to rapid uncontrolled expansion of the industry brought about diseases, most notably WSD and vibriosis that have severely affected shrimp in grow-out farms of the North Western Province which is the main shrimp farming area of Sri Lanka (Corea et al. 1998; Bergquist 2007). Present study was carried out to identify critical better management practices (CBMPs) and critical bio-security measures (CBSMs) that should be adopted strictly to prevent the entry and spread of white spot virus (WSV) and pathogenic *Vibrio* in grow-out farms of *P. monodon* located in the North Western Province, Sri Lanka.

MATERIALS AND METHODS

Data collection

Out of 643 shrimp farms, which functioned in July 2012 in the North Western Province, Sri Lanka, 100 grow-out farms were selected for the survey by proportional stratified sampling method as described by Khan et al. (2003). The questionnaire was prepared based on manuals of standard better management practices for grow-out farms of black tiger shrimp (FAO/NACA/UNEP/WB/WWF 2006; Sahu et al. 2013; Valderrama et al. 2014) and each selected farm was visited from August to September 2012. Farm managers/farmers were interviewed to find out occurrence of diseases over the previous two consecutive production cycles (from May 2011 to July 2012) in relation to practicing of BMPs and BSMs. Also, available facilities of each farm were observed and recorded.

Adoption quotient for grow-out farms

Descriptive score sheets for each of the recommended practices was constructed by adopting the procedure of Das et al. (1988). A total of 8 recommended BMPs and 4 BSMs, which were most suitable for local conditions were selected for the calculation of adoption quotient using the formula given by Sengupta (1967). Scores were allocated according to available farm facilities and to the responses of farm mangers/farmers regarding adoption of each BMP and BSM. Adoption quotient for each grow-out farm was then computed from these adoption scores. On the basis of adoption quotient, the grow-out farms were categorized into four groups using the scale presented by Sengupta (1967). Accordingly, three levels of adoption quotients, viz. low level (practicing a particular BMP or BSM at 00.01% to 33.33%), medium level (practicing the BMP or BSM at 33.34% to 66.66 %) and high level (practicing the particular BMP or BSM at 66.67% to 100%) were identified. If a particular BMP or BSM had been not practiced the adoption quotient was taken as zero and the farm was categorized as non-practiced.

Data analysis

In the 100 grow-out shrimp farms selected, adoption quotients of BMPs and BSMs (high. Medium, low and zero), were normalized by square-root transformation. Principal component analysis (PCA) was then performed on the normalized data for the 100 grow-out shrimp farms. As BSM 2 (stocking WSV negative post larvae) was adopted in all the farms, it was not used in the analysis. To investigate the influence of level of adoption of BMPs and BSMs (adoption quotients), principal component score of the first component (PC1) were related to mean percentage occurrence of white spot disease (MPWSD) and mean percentage occurrence of different form of vibriosis (MPV) over the two consecutive production cycles. Pearson's product-moment correlation coefficient (r) and coefficient of determination (r^2) were employed to investigate whether they were significantly correlated at 0.05 probability level ($p < 0.05$). MINITAB (version 17) statistical software package was used for the analysis of data.

RESULTS

The adoption levels of BMPs and BSMs in shrimp grow-out farms

The recommended BMPs and BSMs for grow-out farms selected in the present analysis and the percentage of farms that fell into high, medium, low and non-practiced categories in relation to each BMP and BSM are given in Table 1. Stocking of high quality post larvae (BMP-4), feed management over the rearing period (BMP-5) and aeration of culture water (BMP-6) were adopted (83%, 76% and 65% respectively) at high category by grow-out farms. There was no farm in the high category in relation to adopting better water exchange system (zero water exchange, BMP-3) and no farm was adopting the use of beneficial bacteria as a probiotic (BMP-8). Most of the grow-out farms (69%) were in the category of low in relation to regular monitoring and maintenance of water quality including monitoring and controlling of pathogenic *Vibrio* populations (BMP-2) and 79% of farms were in low category under proper pre-stocking pond preparation (BMP-1).

All the grow-out farms had adopted the stocking of WSV negative (by nested PCR) post larvae (BSM-2), which is mandated by the National Aquaculture Development Authority, Sri Lanka. However, in adopting the management of incoming and discharged water, 38% grow-out farms fell into the category of low while 26% farms were in non-practiced category (i.e., the farms which did not have reservoir /stock tanks or sedimentation tanks and culture water was not disinfected before pumping into grow-out ponds; BSM-1). Testing post larvae for MBV (before stocking) had been adopted at the high category only by 8% of farms; adoption of this BSM at a medium level had been performed by 92% of farms.

Principal component analysis

The first two principal components (PC1 and PC2) had eigenvalues > 1, explaining 50.9 % of the cumulative variance; PC 1 (eigenvalue = 4.103) accounted for 37.3 % of the variance and PC 2 (eigenvalue = 1.496) accounted for 13.6 % of the variance (Table 2; Fig.1).

The first principal component was positively correlated with the degree of practicing BMPs and BSMs that were adopted in shrimp grow-out farms, i.e., use of disinfection of culture water and having reservoir/stock tanks, etc. (BSM-1; Table 1), proper feed management (BMP-5), proper pre-stocking pond preparation (BMP-1) and frequent checking and maintenance of water quality parameters including monitoring and controlling *Vibrio* (BMP-2). Principal component analysis revealed that occurrence of WSD and vibriosis in shrimp grow-out farms were related to inappropriate pre-stocking pond preparation (BMP-1) and to the usage of non-disinfected water (BSM-1) for rearing shrimp (first principal component was negatively correlated to BMP-1 and BSM-1). Most of the grow-out farms were under the high risk of occurrence of WSD and vibriosis (mainly white feces syndrome, WFS) due to the use of contaminated water from common water sources (not employing BSM-1) for rearing shrimp; some shrimp grow-out farms release their discharged water directly to the same common water source (against BSM-1) and therefore possibility of contamination with WSV and pathogenic *Vibrio* could be high. The score loading in PC1 was low from BSM-4 (i.e., having suitable bio-security measures to prevent WSV contamination over the rearing period through other routes) and BMP-8 (i.e., the use of a bioaugmenter and a probiotic as a better management practice). PC 2 (eigenvalue = 1.496) explained 13.6 % of the total variance and was influenced negatively by BMP-2 (monitoring and maintaining water quality including monitoring and controlling *Vibrio* populations in culture water).

Table 1 Description of recommended better management practices (BMPs) and bio-security measures (BSMs) and percentage of grow-out farms that follow each recommended BMP/BSM

BMP/ BSM Number	Description of recommended BMPs and BSMs	Level of adoption of each recommended BMP or BSM			
		High	Medium	Low	None
BMP-1	Proper pre-stocking pond preparation (having shut down period, cleaning, ploughing and allowing for oxidation of pond bottom, testing of pond soil parameters and treating soil to have optimum conditions)	5	16	79	0
BMP-2	Regular monitoring and maintenance of water quality parameters (pH, salinity, un-ionized ammonia, alkalinity, transparency, DO; monitoring and controlling pathogenic *Vibrio*)	10	21	69	0
BMP-3	Adoption of zero water exchange system or well planned, limited water exchange system instead of frequent water exchange system	0	21	60	19
BMP-4	Stocking of good quality post larvae (after quality test)	83	17	0	0
BMP-5	Feed management over the rearing period (adjustment of feeding according to survival rate, growth rate, age of juveniles, climatic condition, molting condition, use of suitable sized feed trays in correct number, addition of correct percentage of feed to trays, correct time of observing trays and usage of feed additives and supplementary feed as required)	76	23	01	0
BMP-6	Aeration of ponds (period of starting the aeration, operational hours per day, number and position of paddle wheels installed in ponds)	65	31	04	0
BMP-7	Regular assessing of health status and average body weight by sampling, PCR testing of moribund shrimp once a month for WSV	17	83	0	0
BMP- 8	Use of beneficial bacteria as a bioaugmenter and	0	44	37	19
	Use of beneficial bacteria as a probiotic	0	0	0	100
BSM-1	Management of incoming and discharged water (disinfection of culture water before filling grow-out ponds and use of reservoir/stock tanks to store disinfected water to be used over the production cycle, sedimentation tanks to store discharged water from culture ponds)	4	32	38	26
BSM-2	Stocking WSV negative post larvae (by nested PCR)	100	0	0	0
BSM-3	Testing of post larvae for MBV before stocking	8	92	0	0
BSM-4	Use of other bio-security measures such as bird lines, crab fence, foot bath, hand wash, etc. (to prevent contamination of WSV through other routes)	0	7	93	0

BMPs: better management practices; BSMs: bio-security measures

High: adoption quotient between 66.67 to 100%, Medium: adoption quotient 33.34 to 66.66%, Low: adoption quotient 00.01 to 33.33%, None-practiced: adoption quotient is zero

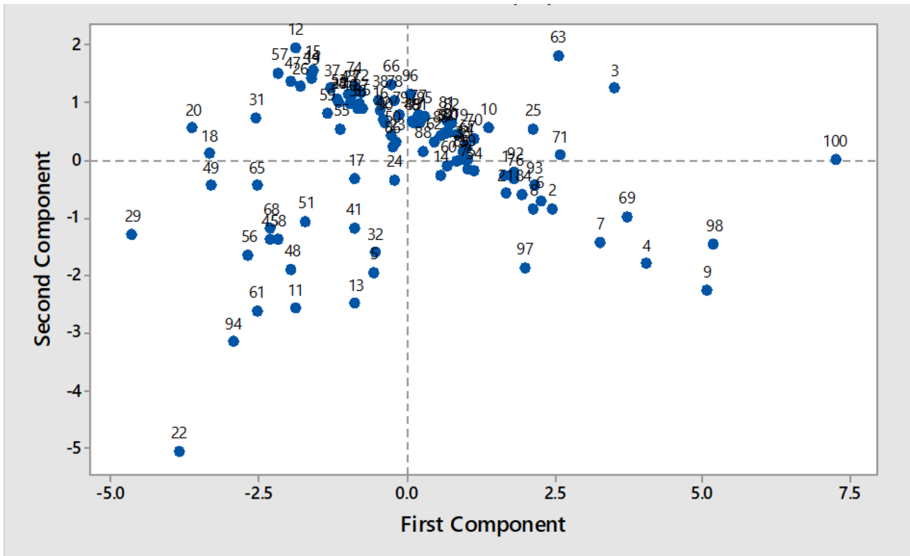

Fig. 1 Two principal component axes; PC 1 and PC 2 based on adoption levels of eight recommended BMPs and four BSMs in randomly selected 100 shrimp grow-out farms in the North Western Province, Sri Lanka (1 to 100 are numbers given to individual shrimp farms)

Adoption of BMPs and BSMs and occurrence of white spot disease

Proper pre-stocking pond preparation, stocking WSV and MBV negative post larvae, use of disinfected culture water (with correct concentration of the disinfectant) and implementation of correct bio-security measures to prevent contamination with WSV through other routes over the rearing period are the critical better management practices (CBMPs) and critical bio-security measures (CBSMs) that should be adopted strictly to prevent the entry and spread of WSV in shrimp grow-out farms in the North Western Province, Sri Lanka (Tables 1 and 2; Fig. 2).

A significant negative linear relationship was evident between PC1 scores (accounted for 37.3% of variance) and mean percentage occurrence of white spot disease (MPWSD) in grow-out farms ($r^2 = 0.358$; $p < 0.05$; Figure 2). Farms with negative PC1 scores that had not correctly adopted BMP-1 (proper pre-stocking pond preparation) and BSM-1 (use of disinfected water for rearing shrimp while having stored disinfected water to be used for water exchange whenever necessary) had high MPWSD. Conversely, shrimp grow-out farms with low MPWSD and positive loading of PC 1 scores were characterized by having a frequent checking and maintenance of water quality parameters, viz. pH, salinity, unionized ammonia, alkalinity, transparency, DO while monitoring and controlling *Vibrio* populations (BMP-2) in culture water (by exchanging water with disinfected water). Inadequacy of adopting BMPs and BSMs were closely related to the occurrence of outbreaks of white spot disease in grow-out farms of *P. monodon*.

Adoption of BMPs and BSMs and occurrence of vibriosis

The first principal component had a significant negative relationship ($r^2 = 0.206$; $p < 0.05$) with mean percentage occurrence of vibriosis. In other words, shrimp grow-out farms with negative PC 1 scores had relatively high occurrence of vibriosis due to the use of non-disinfected culture water (against BSM-1) with frequent water exchange directly from main water source (against BMP-3), none-use of a probiotic and inadequacy of using a bioaugmenter (against BMP-8) and none-maintenance of water quality including none-monitoring and none-controlling pathogenic *Vibrio* populations in culture water (against BMP-2). Low occurrence of vibriosis in shrimp grow-out farms (with positive PC 1 scores on MPV) were associated with use of disinfected culture water (BSM-1), stocking with good quality post larvae (BMP-4) that are free from WSV and MBV (BSM-2 and BSM-3), maintenance of water quality within optimum ranges for *P. monodon* (BMP-2) and regular application of a suitable bioaugmenter to culture water (BMP-8); those BMPs and BSMs are the critical better management practices (CBMPs) and critical bio-security measures (CBSMs) that should be adopted strictly to prevent the occurrence of different form of vibriosis in shrimp grow-out farms in the North Western Province, Sri Lanka (Tables 1 and 2; Fig. 2).

Table 2. Eigenvalues, percentage variance explained and coefficients of the principal component analysis for adoption behavior of recommended better management practices (BMPs) and bio-security measures (BSMs) at randomly selected 100 grow-out shrimp farms in the North Western Province, Sri Lanka

	PC 1	PC 2	PC 3
Eigenvalue	4.103	1.496	1.067
Proportion	0.373	0.136	0.097
Cumulative variance explained	0.373	0.509	0.606
Variable			
BMP -1	0. 355	-0.155	-0.138
BMP - 2	0.352	-0.253	-0.280
BMP - 3	0.325	-0.211	-0.099
BMP - 4	0.323	0.601	0.032
BMP- 5	0.372	0.578	0.018
BMP - 6	0.291	-0.064	0.427
BMP - 7	0.326	-0.071	0.261
BMP - 8	0.034	0.214	-0.654
BSM - 1	0.391	-0.181	0.124
BSM - 3	0.299	-0.239	-0.265
BSM - 4	0.034	-0.027	0.358

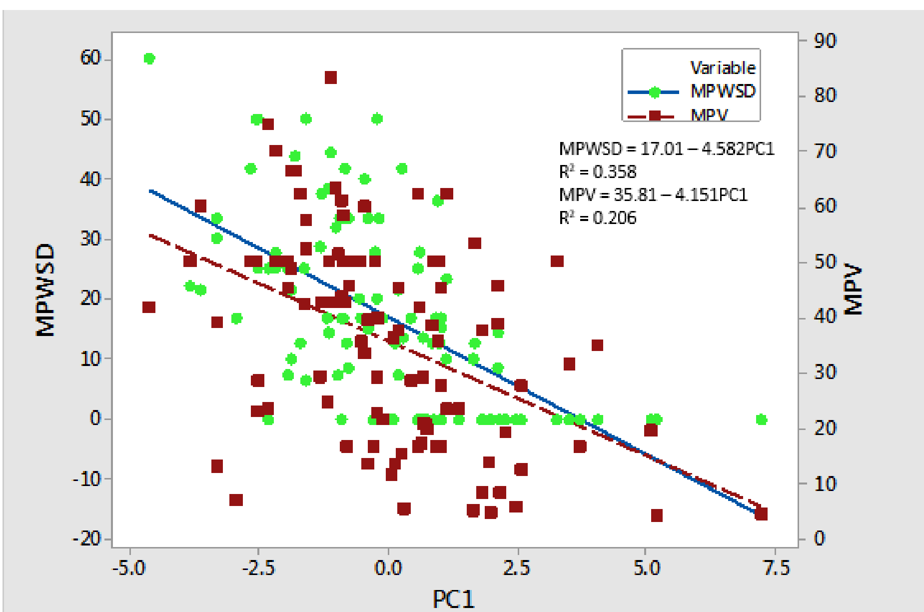

Fig. 2 Relationships between the scores of first principal component (based on level of adoption of the recommended BMPs and BSMs) and mean percentage occurrence of white spot disease (MPWSD) and mean percentage occurrence of vibriosis (MPV) in randomly selected 100 grow-out farms in the North Western Province.

Occurrence of white spot disease and vibriosis

Negative significant linear relationships ($p < 0.05$) were evident having an inverse correlations of mean percentage occurrence of WSD (MPWSD) and mean percentage occurrence of vibrosis (MPV) with PC 1 scores, depending on the degree of practicing recommended BMPs and BSMs in grow-out farms. Shrimp grow-out farms that had high levels of adopting BMPs and BSMs, specially the use of disinfected culture water (BSM-1) with limited water exchange system (BMP-3), regular monitoring and maintenance of water quality parameters at optimum ranges for shrimp and controlling *Vibrio* populations (BMP-2) seemed to prevent the entry and spread of WSV and pathogenic *Vibrio* species in shrimp grow-out farms.

Shrimp grow-out farms of the category of low (due to low adoption quotient of recommended BMPs and BSMs; Table 1) had recorded significantly higher mean percentage occurrence of vibriosis (mainly white feces syndrome, WFS) due to none-practice of BMP-2 compared to the shrimp grow-out farms in the category of medium (farms with medium adoption quotient of BMP-2). The results of questionnaire survey revealed that shrimp grow-out farms of the category of low adoption quotients of recommended BMPs and BSMs had experienced frequent occurrence of white spot disease and vibriosis as a dual infection.

The water pumped into grow-out ponds directly from main water sources would have facilitated horizontal transmission of white spot virus and pathogenic *Vibrio* species; those grow-out farms had contributed greatly to have a significant negative relationship between PC1 scores and mean percentage occurrence of WSD ($r^2 = 0.358$; $p < 0.05$) as well as between PC1 scores and mean percentage occurrence of vibriosis ($r^2 = 0.206$; $p < 0.05$) over the two consecutive production cycles (from May 2011 to July 2012) in the present questionnaire survey. Significant negative relationships between PC 1 scores and occurrence of WSD as well as between PC 1 scores and the occurrence of vibriosis (r^2 = 0. 358 and 0.206 respectively; $p < 0.05$) indicated that shrimp grow-out farms under low category (having low adoption quotient of recommended BMPs and BSMs) were more susceptible to dual infection (infected with both WSV and pathogenic *Vibrio*; Tables 1 and 2; Fig. 2).

Some shrimp grow-out farms with medium adoption quotient (of recommended BMPs and BSMs) also had experienced occurrence of both vibriosis and WSD as a dual infection during the two consecutive production cycles (from May 2011 to July 2012). This could be related to inappropriate use of bioaugmenters to control pathogenic *Vibrio* populations in culture water and none-use of a probiotic to prevent pathogenic *Vibrio* establishing within gastrointestinal tract of shrimp. Those grow-out farms also had contributed for the negative significant relationship between PC 1 scores and occurrence of vibriosis ($r^2 = 0.206$; $p < 0.05$).

DISCUSSION

The questionnaire survey revealed that stocking high quality post larvae (83%), feed management over the rearing period (76%) and aeration of culture water (65%) were adopted in grow-out farms of the North Western Province representing high category (Table 1). Browdy (1998) stated that stocking of high health post larvae of *P. monodon* in grow-out ponds offers economic advantages to farmers while contributing for industry sustainability. However, Fegan and Clifford (2001) pointed out that the success in preventing the introduction of WSV infected post larvae (Pl) into shrimp grow-out farms by PCR testing of Pl before stocking will not prevent the risk of occurrence of WSD. Controlling the possible contamination of the pathogens through other routes over the culture period is also essential (Flegel et al. 2008). All the grow-out farms used for the present study had adopted the stocking of WSV negative post larvae (by nested PCR, BSM-2; as it was mandatory according to conditions imposed by National Aquaculture Development Authority). However, WSD had been recorded in many farms confirming the importance of adopting other recommended BMPs and BSMs in order to protect the shrimp from WSD.

The practice of some BMPs and BSMs such as pre-stocking pond preparation, stocking of seed that are negative for WSV, maintaining water quality within optimum ranges for cultured shrimp together have significantly reduced WSV outbreaks but not prevented the occurrence completely in Vietnam, India and Indonesia (Bondad-Reantaso et al. 2005; Taslihan et al. 2015). Wyban et al. (1993) and Moss et al. (2012) had pointed out that the use of none-disinfected water was responsible for contamination when ponds were stocked with high-health shrimp seed. They also had stated that water use for filling the grow-out ponds initially and the water use to exchange culture water during the entire rearing period need to be disinfected.

During the present study, no farm was found to have zero water exchange system while limited water exchange with disinfection was done at a medium level by a low percentage of farms and at a low level by more than 50% of farms. Chamberlian and Hopkins (1994) had pointed out that zero water exchange culture systems are the most promising systems that could offer reduced water intake, thereby increased bio-security with reduced feed costs too. According to Tacon et al. (2002), zero water exchange systems could increase the possibility of moving the cultured shrimp industry along a path of greater sustainability and environmental compatibility that would be compliance with the food safety systems.

Wang et al. (2008) pointed out that the use of suitable bioaugmenters in aquaculture would be accepted to be compliance with the food safety regulations while satisfying environmental concerns too. Application of useful bacteria to maintain water quality (as bioaugmenters) had been practiced at a medium level by some grow-out farms; however useful bacteria as probiotics had never been used by any of the farms selected for the present study. Probiotics are known to activate immune responses, reduce the establishment of pathogenic bacteria in the alimentary track and increase survival and growth of cultured hosts including fish and shrimp (Irianto and Austin 2002; Balcazar and Rojas-Luna 2007; Verma and Gupta 2015). Hettiarachchi and Hettiarachchi (2005) reported on the development of a bioaugmenter/ probiotic locally and on successful performances of the product in *P. monodon* grow-out farms with zero water exchange systems.

Present study revealed that contributory effect of none-monitoring and none-maintenance of water quality (including monitoring and controlling of pathogenic *Vibrio* populations), improper pre-stocking pond preparation and improper management or none-management of incoming and discharge water (such farms did not have reservoir/stock tanks, sediment tanks and did not disinfect culture water before using) seemed to be the cause of frequent disease outbreaks recorded in the grow-out farms in the North Western Province. Virus or bacteria that get into such grow-out ponds could multiply, increase their populations, invade shrimp bodies and could cause diseases; where essential precautionary measures were not taken, pathogens (WSV and pathogenic *Vibrio*) from those ponds with affected shrimp had spread (even into the grow-out ponds of the farms that had practiced BMPs and BSMs at higher levels) causing outbreaks of WSD and/or vibriosis. Recommended biosecurity measure (BSM-4) to prevent contamination had been employed at a low level by the farms in the present study allowing easy spread of the pathogens. Horowitz and Horowitz (2001) described physical, chemical, and biological precautionary measures that could be taken against potential disease outbreaks; the use of iodine and chlorine to disinfect tools, foot ware, hands, etc. are among suggested precautionary measures by those authors.

Qung et al. (2009) stated that WSV may survive in water for 20 months (possibly in tissues of crustacean carriers). According to Natividad et al. (2008) WSV could persist in sediments of pond bottoms (in fertilized eggs of crustaceans in dormant stages) for more than 10 months. These reports confirm the negative effect that could occur due to inappropriate pre-stocking pond preparation (not practicing BMP-1 correctly), use of non-disinfected water to rear shrimp in grow-out ponds and release of pond effluents directly into water sources from where water may be pumped into other grow-out farms. WSV that could stay in crustacean carriers in sediments and in water for longer time could be recycled among grow-out ponds if complete disinfection of pond bottoms, incoming water and discharged water from ponds is not carried out using suitable disinfectant in correct concentrations with correct exposure time (BSM-1).

The present study did not record any farm in the high category in relation to adoption of the use of beneficial bacteria as bioaugmenters (BMP-8). Use of suitable bioaugmenters to pond bottom and to culture water would be of great importance to keep the ammonia, nitrite and water pH within optimum ranges while controlling pathogenic *Vibrio* also (Kumara 2016). According to Corsin et al. (2001) and Kakoolaki et al. (2015) outbreaks of WSV were preceded or coincided with high pH and unionized ammonia in culture water; although stress seems to be the most likely explanation for those association, it is also possible that these variables exert impacts through other mechanisms (Tendencia et al. 2014). High pH, high unionized ammonia and nitrite level of culture water were identified as stress factors that lead to disrupt the immune ability and metabolic performances of shrimps increasing the susceptibility to microbial infections (Joseph and Philip 2007).

The relationship between inadequacy of adopting BMPs and BSMs and mean percentage occurrence of vibriosis (MPV; Fig 2) highlighted that diseases caused by *Vibrio* species were prevalent in shrimp grow-out ponds where BMP-2 was practiced at a low level. Five different types of vibriosis were prevalent in cultured shrimp, of which tail necrosis, shell diseases, loose shell syndrome (LSS) and red disease were recorded earlier from many regions (Lightner 1998; Sung et al. 2001). White feces syndrome (WFS) is a disease condition that has caused a severe loss to the shrimp culture industry in India from 2000 to 2001 (Jayasree et al. 2006), in 2010 in Thailand (Somboon 2012) and in Sri Lanka from 2010 (Kumara and Hettiarachchi 2017). According to Lee (2003), adequate detection and diagnostic methods for excludable diseases, methods for pathogen eradication and disinfection are key elements of bio-security that can contribute to successful production of cultured shrimp. Kumara and Hettiarachchi (2017) had reported on the detection of white feces disease (WFS) in *P. monodon* in grow-out ponds of North Western Province. The successful control of the causative *Vibrio* species was reported using bioaugmenter/probiotic produced locally by Hettiarachchi and Hettiarachchi (2005). Moriarty (1999) and Salência et al. (2016) also have observed the ability of bioaugmenting beneficial bacteria in keeping the pond environment healthy while reducing the risk of development of pathogenic microbial populations in culture water.

Present questionnaire survey revealed that the grow-out ponds that were managed under intensive culture system with frequent introduction of beneficial bacteria to the culture water as bioaugmenters (BMP-8) had completed the production cycles with less occurrence of disease outbreaks of vibriosis or WSD. The introduced beneficial bacteria must have contributed to achieve a dynamic balance within the pond environment suppressing populations of pathogenic bacteria and reducing the accumulation of organic sediments thereby reducing stress of shrimp leading to enhance innate immunity to fight against WSV.

CONCLUSIONS

Recommended Critical Better Management Practices (CBMPs) were,

- Proper pre-stocking pond preparation,
- Adoption of zero water exchange,
- Monitoring and controlling water quality parameters including pathogenic *Vibrio* in culture water and
- Use of a suitable bioaugmenter and a probiotic.

Recommended Critical Bio-Security Measures (CBSMs) were,

- Proper disinfection of culture water,
- Stocking of WSV and MBV free post larvae and
- Prevention of WSV contamination through other routes over the rearing period.

Entry and spread of WSV and pathogenic *Vibrio* in *P. monodon* grow-out farms in the North Western Province, Sri Lanka could be prevented by strict adoption of those CBMPs and CBSMs.

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