Research Article

White Spot Virus (WSV) Resistant Broodstocks of *Penaeus monodon* (Fabricius, 1798) Identified in Sri Lankan Coastal Sea with Microsatellite Markers

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Abstract

White spot disease (WSD) caused by white spot virus (WSV) has been having the greatest negative impact on Sri Lankan shrimp culture industry since its first record in Sri Lanka in 1996. The present study was carried out from 2013 to 2016 to investigate the presence of WSV resistance in broodstocks of Penaeus monodon (Fabricius 1798) in the coastal sea of Sri Lanka using a microsatellite DNA marker. Broodstock samples of P. monodon (n ~10 and 260 samples) were obtained from eight major broodstock collecting sites in Sri Lankan coastal sea. Resistance and susceptibility to WSV were identified using new forward and reverse primers that were designed according to 71 bp microsatellite DNA sequence (following the database of National Centre for Biotechnology Information for P. monodon). Broodstocks resistant to WSV were found in the coastal sea which could be used to produce specific pathogen resistant (SPR) and specific pathogen free (SPF) post larvae for WSV, which would be a breakthrough in cultured P. monodon industry of Sri Lanka. Accession number KX 156833.1 was received for the genomic sequence of 317 bp microsatellite DNA marker (with the absence of 71 bp fingerprint) for screening WSV resistance in P. monodon populations in the Sri Lankan coastal sea.

Keywords*: Black tiger shrimp, genomic resistance, susceptibility to WSV, wild brood stocks*

1. Introduction

White spot virus (WSV) has been identified as the most serious viral pathogen of cultured shrimp, *Penaeus monodon* (Fabricius, 1798). Since the emergence of white spot disease (WSD) in the early 1990s, it has been a major obstacle to the sustainability and profitability of cultured shrimp production in Asia (Walker and Winton, 2010). The Asian WSD epizootics occurred from 1993 to 1996 in China, Taiwan, Japan, Bangladesh, Indonesia, India, Malaysia, Philippines, Thailand, Vietnam and Sri Lanka (Bondad-Reantaso *et al.*, 2005). The cumulative production loss of cultured shrimp in all Asian countries between 1993 and 1996 probably

amounted to several hundred thousand tons. By 2001, cumulative global loss due to WSD was of the order of one million metric tons or more (Flegel *et al.,* 2004).

The first record of WSD in Sri Lanka was in 1996 and it has been having the greatest negative impact on the Sri Lankan cultured shrimp industry for more than twenty years in both hatcheries and grow-out farms (Hettiarachchi, 2017). The virus can be transmitted horizontally and/or vertically; vertical transmission of the virus from infected ovaries of female brood shrimp to eggs and larvae has been demonstrated (Lo *et al.,* 1997; Flegel, 2012; Lightner *et al.,* 2012; Thitamadee *et al.,* 2016). The presence of WSV in wild brood stocks also has been reported (Lo *et al.,* 1996; Otta *et al.,* 1999). According to Withyachumnarnkul *et al.* (2003), vertical transmission of WSV from broodstocks to post larvae significantly contributes to the failure of production of healthy post larvae in tropical shrimp hatcheries. If affected post larvae are stocked, farmers experience very high mortality of juvenile shrimp in grow-out ponds resulting in production failures. Sri Lankan shrimp hatcheries have been depending on the wild brood stocks of *P. monodon* obtained from eight major collecting sites along the coastal sea for the production of post larvae and probably it could have contributed greatly to the recurrence of WSD in grow-out ponds.

Disease resistance has been studied in several species of shrimps and the use of pathogen free (SPF) and pathogen resistant (SPR) post larvae for the stocking of grow-out ponds is the best way to control viral diseases. A microsatellite marker was found to be linked with resistance/susceptibility in *Litopenaeus vannamei*, the cultured American shrimp to Taura syndrome virus disease (TSVD; Xu *et al.* 2003). Xu *et al.* (1999) submitted 42 different specific microsatellite loci from *Penaeus* spp. Mukherjee and Munadal (2009) and Dutta *et al.* (2013) developed a 71 bp microsatellite DNA marker to investigate WSV resistance/susceptibility in brood stocks of *P. monodon*.

Preliminary investigations showed that some brood stocks of *P. monodon* collected from the wild, along the coastal sea of Sri Lanka, were positive for WSV (by nested PCR) but they did not succumb to death (indicating the occurrence of some resistance) while some brood shrimp died after the spawning with white spot disease (indicating WSV susceptibility). Therefore, the present study was planned to investigate whether WSV resistance and WSV susceptibility are present in the genome of wild *P. monodon* broodstocks that inhabit the coastal sea of Sri Lanka using selected microsatellite-based DNA fingerprints.

2. Material and Methods

2.1. Collection of broodstock samples of *P. monodon*

Random samples of live *P. monodon* broodstocks were obtained from the broodstock collectors of the eight major collecting sites, *viz*. Beruwala, Hendala, Negombo, Marawila, Chilaw, Muallatittivu, Pottuvill and Valachenai (sampling period varied following the period of collection from deep sea depending on the effect of monsoon seasons to the area). Each shrimp was kept separated in labeled polythene bags containing oxygenated, UV sterilized, sea water. From each individual of each sample, DNA materials were extracted separately for identification of WSV resistance and WSV susceptibility using 71 bp microsatellite DNA marker and for the investigation on the severity of infection (by nested PCR) in WSV susceptible brood shrimp. Figure1 and Table 1 show the sampling sites (with GPS locations), the number of samples collected from each site with the total number of individuals used to extract DNA from each site.

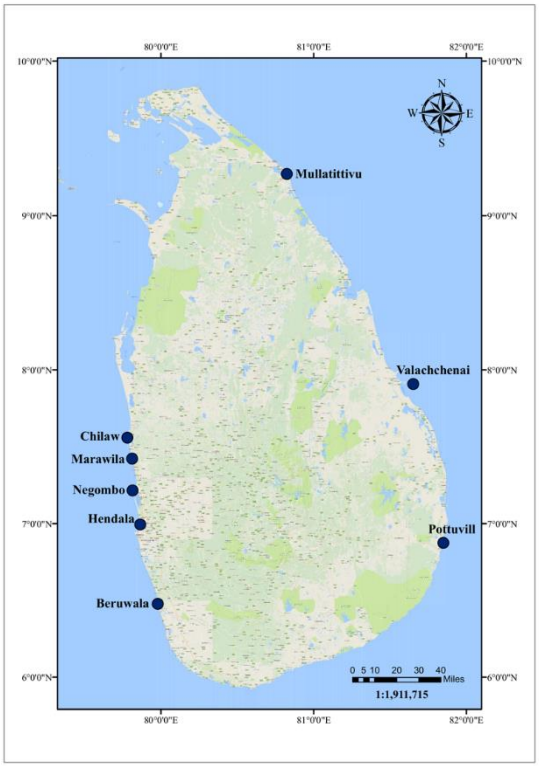

Figure 1: Global positioning system (GPS) locations of the collecting sites of wild *monodon* broodstocks along the coastal sea of Sri Lanka

Table1: Broodstock collecting sites with Global positioning system (GPS) locations and number of broodstock samples collected from each site for DNA extraction of *P. monodon*

Broodstock collecting site	GPS location	No. of random samples collected from each site (over one year)	No. of individuals collected (over one year)	Total no. of individuals used for DNA extraction
Beruwala	6°28'39.28"N 79°58'45.33"E	24	850	96 (11.3 %)
Hendala	6°59'40.00"N 79°51'52.31"E	67	13500	475 (3.51 %)
Negombo	7°12'56.38"N 79°48'52.49"E	49	7168	389 (5.42 %)
Marawila	7°25'22.94"N 79°48'41.41"E	17	350	85 (24.2 %)
Chilaw	7°33'32.18"N 79°46'48.08"E	26	1200	149 (12.4 %)
Mullatittivu	9°16'11.11"N 80°49'25.94"E	29	1340	183 (13.7 %)
Valachchenai	7°54'26.82"N 81°39'6.67"E	30	1200	196 (16.4 %)
Pottuvill	6°52'28.79"N 81°50'56.32"E	18	650	97 (14.9 %)

2.2. Extraction of DNA from tissues of *P. monodon* brood shrimp

A small piece weighing about 100 mg was dissected out from a pleopod of each brood shrimp and DNA was extracted using phenol chloroform method as described by Sambrook and Russel (2006). A sample from extracted DNA (from each brood shrimp) was electrophoresed in 0.8% (w/v) agarose gel to determine the quality; only the extracts that gave a clear band on agarose gel indicating that the DNA had been extracted properly were chosen for subsequent PCR.

2.3. Designing new primers and identification of WSV resistance and susceptibility in wild broodstocks of *P. monodon*

The sequence of microsatellite DNA marker was obtained from the National Centre for Biotechnology Information (NCBI) database of *P. monodon* (GenBank Accession No. AF077565, Nucleotide Division of National Centre for Biotechnology Information, Bethesda, Maryland, USA). The forward and reverse

primer sequences for PCR amplification were designed using Primer blast software (NCBI) from the flanking regions of their respective microsatellite loci (F: 5' - TCG GCT GTT TTC TCT CGT TCA - 3' and R: 5' - TGG ACT GGC ATG TGA ATG ACT -3') to identify the WSSV resistant and susceptible *P. monodon* in Sri Lankan coastal sea.

Extracted DNA from each brood shrimp of *P. monodon* (collected from the Sri Lankan coastal sea) was used for PCR. The PCR amplification was carried out using newly designed primers in 25 µL reaction mixture containing 400 ng of shrimp DNA, 15 pmol of each forward and reverse primer, 0.2 mM deoxyribonucleotide triphosphate (Sigma Aldrich Inc, Germany), 1 x buffer with 10 mM Tris-HCl (pH, 9.0), 50 mM KCl, 2.0 mM $MgCl_2$, 0.01% gelatin (Themo Fisher Scientific Inc, USA) and 0.7 U Taq polymerase (Sigma Aldrich Inc, Germany) and then the reaction mixture was placed in a thermo cycler. The thermal profile for PCR was 94℃ for 3 min followed by 30 cycles of 94℃ for 1 min, annealing temperature of 35℃ for 30 s and extension at 72℃ for 5 min. Amplified PCR of DNA fragments were electrophoresed in 2% agarose gel at 90 V for 1 h, stained with ethidium bromide (l μgmL^{-1}) and subsequently visualized. Amplification of WSV resistant and WSV susceptible microsatellite DNA markers resulting from the PCR with newly designed primers was confirmed by the presence of 317 bp and 71 bp DNA bands (in gel photographs) from the extracted DNA of *P. monodon.* Sequence analysis of the isolated microsatellite DNA marker (71 bp) was performed at Macrogen Company, Korea. Finally, the sequence was verified to be similar (99.9% to 100%) to the existing gene sequence of *P. monodon* clone TUZX4-6:6 microsatellite sequences in the NCBI database. Genomic sequence of microsatellite-based DNA marker (317 bp) was deposited in NCBI database and a specific accession number (KX 156833.1) was obtained; this DNA maker (317 bp) together with the absence of 71 bp DNA finger print could be used to identify WSV resistance (SPR for WSV) and WSV susceptibility (by the presence of 71 bp DNA finger print) of broodstocks of *P. monodon* populations inhabiting the Sri Lankan coastal sea.

2.4. Prevalence of WSV resistance and susceptibility in broodstocks of *P. monodon* obtained from major collecting sites

Polymerase chain reaction was performed separately (using newly designed primers) with extracted DNA from tissues of each brood shrimp of each random sample collected from the eight major collecting sites along the coastal sea of Sri Lanka to identify WSV resistance (SPR for WSV) or WSV susceptibility in each individual

(using 317 bp and 71 bp microsatellite DNA markers employing the same procedure described earlier; refer 2.3). The percentage prevalence of WSV resistance and WSV susceptibility in brood shrimp of each random sample collected from each collecting site was recorded separately. Data recorded for all the broodstock samples collected from one particular site were used to obtain the mean percentage prevalence of WSV resistance (MWSVR) and the mean percentage prevalence of WSV susceptibility (MWSVS) in broodstocks of that site.

2.5. Severity of WSV infection in broodstocks of *P. monodon* in major collecting sites

Each brood shrimp in each broodstock sample was examined separately for the severity of WSV infection by nested PCR using primers from IQ2000 TM WSV detection kit (GeneReach Biotechnology Corp, Taiwan); PCR products were electrophoresed in 2% (w/v) agarose gel and stained with ethidium bromide. The gel photographs were then visualized and documented in a gel documentation system and the severity of infection was categorized into three levels according to the method presented by Lightner (1996). Percentage prevalence of WSV in different severity in each sample of broodstock (by the presence of 1 to 3 DNA bands of the viral genome) obtained from each collecting site was then calculated separately.

3. Results

3.1 Presence of WSV resistance and susceptibility in broodstocks of *P. monodon* in the Sri Lankan coastal sea

Amplification of WSV resistant and WSV susceptible microsatellite DNA markers resulting from the PCR, with the primers designed during the present study, confirmed the presence of WSV resistant and WSV susceptible broodstocks of *P. monodon* at major broodstock collecting sites in the Sri Lankan coastal sea. The identified micro satellite DNA marker was verified for the shrimp genome with the NCBI blast program and the accession number: KX 156833.1 was received for the nucleotide sequence of microsatellite DNA marker (317 bp).

3.2. Prevalence of WSV resistance and susceptibility in brood shrimp of *P. monodon* at major broodstock collecting sites

Table 2 shows the mean percentage prevalence of WSV resistance (MWSVR) in brood shrimp of *P. monodon* which were identified by being negative for 71 bp and

positive for 317 bp microsatellite DNA markers and WSV susceptibility (MWSVS) in brood shrimp which were identified by being positive for both 71 bp and 317 bp DNA markers in random samples collected from eight major collecting sites along the coastal sea of Sri Lanka. The mean percentage prevalence of WSV resistance in brood shrimp at Beruwala sea was 87.49 ± 2.42% which was the highest recorded value; significantly higher MWSVR were also recorded for brood shrimp collected from the coastal sea of Pottuvill (85.38 ± 2.79%) and Valahchenai (78.53 ± 0.98%; $P < 0.05$). The lowest MWSVR was recorded for the broodstocks obtained from the Chilaw coastal sea (21.02 ± 3.17%). The mean percentage prevalence of WSV susceptibility (being positive for 71 bp as well as for 317 bp DNA markers) in broodstock samples collected from the sea of Chilaw, Hendala and Negombo were significantly higher (78.04 ± 2.17%, 65.07 ± 5.81% and 62.06 ± 3.64% respectively; $P < 0.05$). The lowest susceptibility to white spot virus was recorded for the broodstock samples obtained from Beruwala coastal sea (MWSVS was 12.51 ± 1.23%; Table 2). According to 317 bp and 71 bp microsatellite DNA markers, the prevalence of WSV resistance in broodstock samples collected from the eight major collecting sites when ranked in the descending order was Beruwala, Pottuvill, Valahchenai, Mullattivu, Marawila, Negombo, Handala and Chilaw (Table 1). Figure 2 shows only 317 bp DNA fingerprint confirming the resistance to WSV in brood shrimp and the presence of both DNA fingerprints, viz. 71 bp DNA and 317 bp DNA display WSV susceptibility of brood shrimp of *P. monodon.*

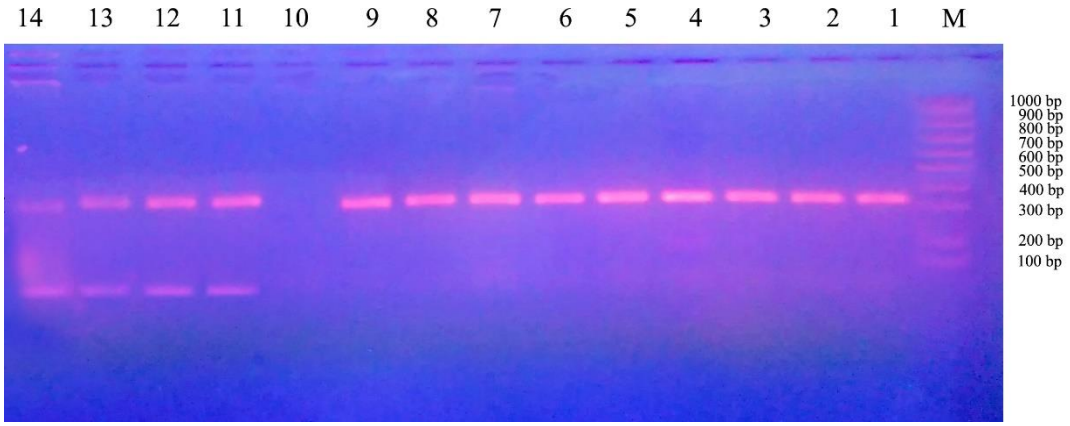

Figure 2: Microsatellite DNA marker displaying WSV resistance and WSV susceptibility of *P. monodon* brood shrimp collected from Sri Lankan coastal sea.
M: molecular weight marker; Lanes 1 to 9 absence of 71 bp DNA fingerprint from *P. monodon* with the WSV resistance in the genome (with presence of only 317 bp DNA

fingerprint), Lanes 11 to 14 presence of both 71 bp DNA and 317 bp DNA fingerprints from shrimp with the WSV susceptibility in the genome

Table 2: Mean percentage prevalence of WSV resistance (MWSVR) and WSV susceptibility (MWSSV) in *P. monodon* brood shrimp at major collecting sites - identified by 71 bp microsatellite DNA marker (data are provided as mean ± SE).

Broodstock collecting site	MWSVR (negative for 71 bp and positive for 317 bp)	MWSVS (positive for both 71 bp and 317 bp)
Beruwala	87.49 ± 2.42 [a]	12.51 ± 1.23 [d]
Hendala	30.20 ± 3.24 [cd]	65.07 ± 5.81 [ab]
Negombo	35.54 ± 4.07 [bcd]	62.06 ± 3.64 [b]
Marawila	41.04 ± 3.99 [bc]	54.10 ± 2.65 [c]
Chilaw	21.02 ± 3.17 [d]	78.13 ± 2.23 [a]
Mullatittivu	47.02 ± 6.12 [b]	52.11 ± 1.75 [c]
Valahchenai	78.53 ± 0.98 [a]	13.72 ± 2.47 [d]
Pottuvill	85.38 ± 2.79 [a]	14.98 ± 1.91 [d]

(Means with different superscripts in a column are significantly different from each other ($P < 0.05$, ANOVA and Tukey's Pairwise Test)

3.3. Severity of WSV infection in broodstocks of *P. monodon* in major collecting sites

The mean percentage prevalence of white spot virus infection with varying degree of severity in *P. monodon* brood shrimp collected from the eight major broodstock collecting sites along the coastal sea of Sri Lanka is given in the Table 3. Nested PCR detection of white spot virus (performed with IQ 200 TM WSV detection kit) with different severity of infection, *viz*. low, moderate and severe (having one, two and three DNA bands respectively) is displayed in Figure 3. According to Lightner (1996) very severe WSV infection with 10^5 viral particles or above produces all three DNA bands (at 720 bp, 310 bp and 210 bp), moderate infection having 10^3 to $< 10^5$ viral particles produce two DNA bands (at 310 bp and 210 bp) and low infection with 10 - 200 viral particles produce only the 210 bp DNA band.

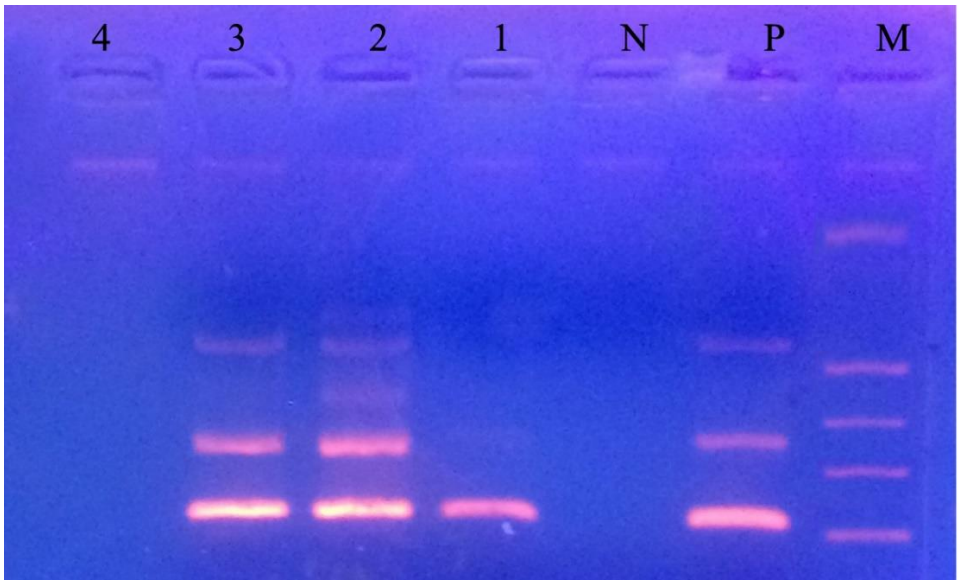

Figure 3: DNA bands displaying differences in degree of severity of WSV infection among brood shrimp of *P. monodon* (from major collecting sites)
Lane M: molecular weight marker, P: WSV positive control, N: WSV negative control, Lane 1: light infection of WSV (only 210 bp DNA band); Lane 2 and 3: severe infection of WSV (having 210 bp, 310 bp and 720 bp DNA bands)

The lowest mean WSV prevalence in *P. monodon* broodstocks was recorded for Beruwala coastal sea where only 9.80 ± 0.45% were found to be infected (Table 3); among those infected shrimp, 2.94 ± 0.13% had a low infection, 4.90 ± 0.21% had moderate infection and only 1.96 ± 0.04% had a severe infection. From the brood shrimp samples tested from the Chilaw coastal sea, 62.2 ± 3.51% were positive for WSV and among those infected shrimp 53.66 ± 2.84% were severely infected with WSV (had all three DNA bands). Only 20.75 ± 1.37% of brood shrimp collected from the Pottuvill coastal sea were WSV positive and out of that 15.09 ± 1.81% had a low infection and 3.77 ± 0.27 % had a severe infection (Table 3).

Table 3: Mean percentage prevalence of WSV infection with different severity in brood shrimp of *P. monodon* (from major collecting sites; data are provided as mean ± SE).

Broodstock collecting site	Mean percentage prevalence of white spot virus infection with different severity			
	Low infection	Moderate infection	Severe Infection	Total prevalence of infection
Beruwala	2.94 ± 0.13^{e}	4.90 ± 0.21^{d}	1.96 ± 0.04^{d}	9.80 ± 0.45^{e}
Hendala	6.35 ± 0.26^{d}	4.76 ± 0.17^{d}	47.62 ± 1.62^{b}	58.73 ± 2.02^{a}
Negombo	12.07 ± 1.34^{c}	6.96 ± 0.43^{c}	41.38 ± 2.04^{c}	60.41 ± 3.12^{a}
Marawila	2.7 ± 0.4^{e}	5.41 ± 0.31^{d}	51.35 ± 3.12^{a}	59.46 ± 4.03^{a}
Chilaw	0^{f}	8.54 ± 1.71^{b}	53.66 ± 2.84^{a}	62.20 ± 3.51^{a}
Mullatittivu	32.39 ± 1.81^{b}	9.86 ± 0.63^{a}	1.41 ± 0.07^{d}	43.66 ± 2.71^{c}
Valahchenai	37.25 ± 3.14^{a}	9.80 ± 1.37^{a}	1.96 ± 0.23^{d}	49.01 ± 3.23^{b}
Pottuvill	15.09 ± 1.81^{c}	1.89 ± 0.08^{e}	3.77 ± 0.27^{d}	20.75 ± 1.37^{d}

(Means with different superscripts in a column are significantly different from each other ($P < 0.05$, ANOVA and Tukey's Pairwise Test)

3.4. Relationship between mean percentage prevalence of WSV susceptibility and mean percentage prevalence of WSV infection in *P. monodon* brood shrimp

Figure 4 shows the relationship between the mean percentage prevalence of WSV susceptibility (by 71 bp microsatellite DNA marker) and the mean percentage prevalence of WSV infection (by nested PCR) in broodstocks collected from the eight major collecting sites of the Sri Lankan coastal sea. Broodstock samples collected from Beruwala and Pottuvill coastal sea showed lower mean percentage prevalence of both WSV susceptibility (Table 2) and WSV infection (Table 3) compared with other collecting sites. Significantly higher ($P < 0.05$) mean percentage prevalence of WSV susceptibility was recorded for broodstocks of Chilaw (78.13 ± 2.33%), Hendala (65.07 ± 5.81%) and Negombo (62.06 ± 3.64%); mean percentage prevalence of WSV infection observed for these three sites also was high (62.2 ± 3.51%, 58.73 ± 2.02% and 60.41 ± 3.12%, respectively). Though the broodstocks from Valahchanai had 49.01 ± 3.23% prevalence of WSV infection, higher percentage of them had a low infection; MWSVR recorded for those broodstocks was significantly high (78.53 ± 0.98%; $P < 0.05$) which must have kept the infection at a low level.

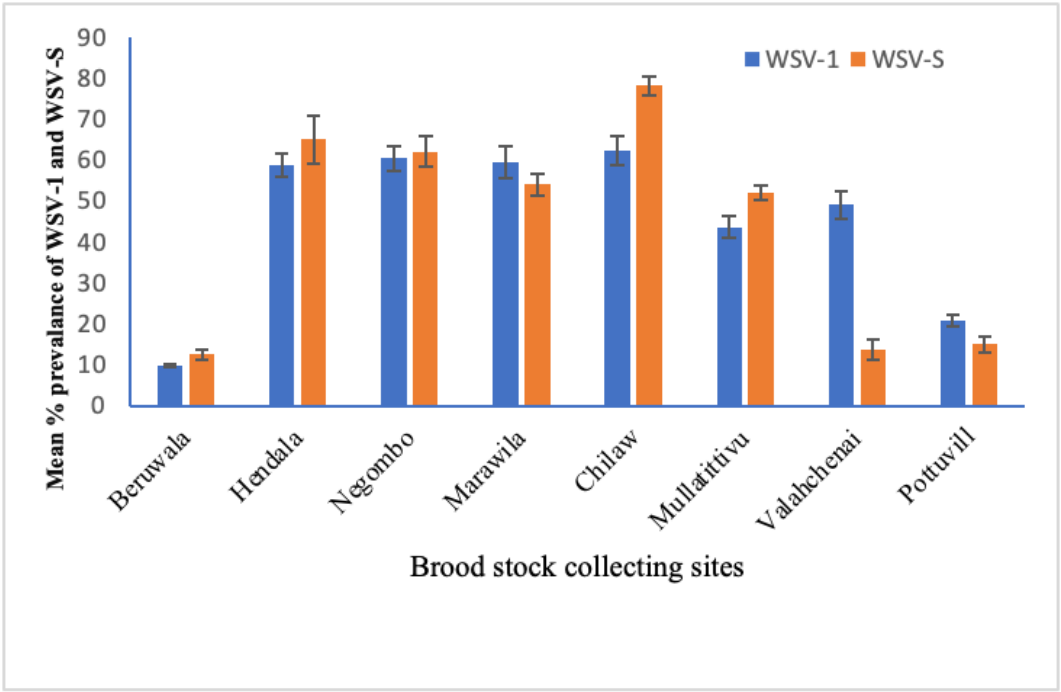

Figure 4: Mean percentage prevalence of WSV infection (WSV-I by nested PCR) and mean percentage prevalence of WSV susceptibility (WSV-S by 71 bp microsatellite DNA marker) in *P. monodon* brood shrimp at eight major collecting sites.

4. Discussion

Amplification of WSV resistant and WSV susceptible microsatellite DNA markers resulting from the PCR, with the primers designed during the present study, confirmed the presence of WSV resistant and WSV susceptible broodstocks of *P. monodon* at major broodstock collecting sites in the Sri Lankan coastal sea. Since the identified micro satellite DNA marker was verified for the shrimp genome with the NCBI blast program, the accession number: KX 156833.1 was received for the nucleotide sequence of microsatellite DNA marker (317 bp). Xu *et al.* (2001) reported that shrimp DNA that are negative for 71 bp microsatellite marker and positive for 317 bp microsatellite DNA marker was WSV resistant while shrimp DNA that are positive for both microsatellite markers (71 bp and 317 bp) were WSV susceptible.

Mukherjee and Mandal (2009) and Dutta *et al.* (2013) developed 457 bp RAPD SCAR marker and 71 bp microsatellite DNA marker for the identification of WSV resistant and susceptible populations of *P. monodon*; those authors also reported that

71 bp microsatellite DNA marker was present in WSV susceptible shrimp. During the present study also 71 bp microsatellite DNA marker was found in the genome of *P. monodon* that were susceptible to WSV and therefore 71 bp microsatellite DNA marker was used to identify WSV susceptible individuals in random samples of broodstocks obtained from the major broodstock collecting sites along the coastal sea of Sri Lanka. Accordingly, brood shrimp that were negative for 71 bp and having only one DNA band of 317 bp were with WSV resistance in their genome while shrimp that were positive for 71 bp fingerprint and therefore having two DNA bands (at 71 bp and at 317 bp) were with genomic characteristics that could make them to be WSV susceptible. The prevalence of genomic resistance to WSV (being negative for 71 bp microsatellite DNA marker) in broodstock samples collected from eight major broodstock collecting sites, when ranked in descending order was Beruwala, Pottuvill, Valahchenai, Mullattivu, Marawila, Negombo, Handala and Chilaw. Dutta *et al.* (2013) observed in a WSV challenged experiment having 1.21×10^3 and 2.25×10^3 fold higher WSV propagation identified by the presence of 71 bp microsatellite DNA marker with the absence of 457 bp RAPD SCAR DNA marker in WSV susceptible shrimp; these findings have established a significant link between those DNA markers and WSV susceptibility or WSV resistance. Dutta *et al.* (2014) also have recorded the presence of 457 bp RAPD SCAR marker in WSV resistant shrimp.

Kumara and Hettiarachchi (2015) have reported on low mean percentage prevalence of WSV in brood shrimp collected from Beruwala and Pottuvill sea. Present study has recorded very severe infection in broodstocks of *P. monodon* collected from the sea of Chilaw, Marawila, Hendala and Negombo areas of Sri Lanka. These collecting sites of broodstocks are adjacent to the area where some shrimp processing plants, hatcheries and grow-out farms also are located; infected discards or escaped individuals from infected farms or extra viral load that has been discharged to coastal water via processing plants and fish markets could have contributed for the recorded severity of WSV infection in broodstocks of those collecting sites. Peña *et al.* (2007) and Chakrabarty *et al.* (2014) had reported similar results in relation to certain broodstock collecting sites of coastal sea of Philippine and India respectively.

It is quite expected that the MWSVS in shrimp should be proportional to mean percentage prevalence of WSV infection while MWSVR being inversely proportional to mean percentage prevalence of WSV infection, which was confirmed by the results of present work. Chakrabarty *et al.* (2014) stated that the phenomenon of disease resistance depends upon the genomic content of the organism. According

to Dutta *et al.* (2015) WSV may not be prevalent at a particular site due to climatic condition and the physicochemical parameters of the surrounding water and therefore the shrimp residing there may not face the WSV threat. However, if the genotype of shrimp living in the place is the WSV susceptible type and if there are favorable conditions for WSV to propagate, the shrimp will be infected. Therefore, screening broodstocks that are collected from natural populations living in the coastal sea for disease resistance by the absence of 71 bp microsatellite DNA marker with the primers designed during the present research will be highly beneficial in selecting brood *P. monodon* for the production of post larvae in Sri Lankan shrimp hatcheries. As most of the shrimp farms and hatcheries are located along Northwestern coastal belt of the country, collection of broodstocks for production of post larvae also has been carried out mainly from the same coastal belt due to easy transportation, etc. Results of present study reveals that Beruwala and Pottuvill coastal seas are better sites for the collection of healthy, WSV resistant broodstocks in order to produce WSV resistant post larvae.

Present research has provided a clear picture of broodstocks of *P. monodon* available at major collecting sites along the coastal sea of Sri Lanka that could be greatly supportive in selecting broodstocks with WSV resistant genome (being negative for 71 bp microsatellite DNA marker). Genomic sequence of 317 bp microsatellite DNA marker with accession number KX 156833.1 and the forward and backward primers designed during the present study could also be successfully employed to screen and to stock only the WSV resistant post larvae in grow-out ponds as a critical bio-security measure that must be adhered to which could be a great breakthrough in *P. monodon* culture industry of Sri Lanka. Specific pathogen resistant (SPR) broodstock production through marker assisted selection (MAS) of breeding programs are yet to be developed for *P. monodon*. Therefore, vertical and horizontal transmission of viral pathogen could be prevented for commercial advantage by selecting WSV negative (by nested PCR) as well as WSV resistant brood shrimp (without 71 bp microsatellite DNA marker) for production of "SPF" and "SPR" post larvae of *P. monodon* for WSV. Mukherjee and Mandal (2009) have also reported on possibility of achieving great success in long-term disease control in shrimp culture industry by molecular base identification of disease resistance (such as microsatellite DNA markers) and selective breeding.

5. Conclusions

Genomic sequence of microsatellite DNA marker (317 bp) with the accession number KX 156833.1 which was identified during the present work together with the absence of 71 bp DNA finger print could be used to screen and select WSV resistant wild brood shrimp of *P. monodon.* Beruwala and Pottuvill coastal seas are better sites for the collection of healthy, and WSV resistant broodstocks for the production of WSV resistant post larvae.

6. Acknowledgements

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