Vol. 124: 193–199, 2017
https://doi.org/10.3354/dao03128

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published May 11

# Risk factors associated with white spot syndrome virus outbreaks in marine shrimp farms in Rayong Province, Thailand

**Sompit Yaemkasem[1,2], Visanu Boonyawiwat[1], Jiraporn Kasornchandra[3], Chaithep Poolkhet[1,*]**

[1]Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom 73140, Thailand
[2]Rayong Coastal Fisheries Research and Development Center, Rayong 21000, Thailand
[3]Department of Fisheries, Chatuchak, Bangkok 10900, Thailand

ABSTRACT: The purpose of this study was to determine risk factors for white spot disease (WSD) in Rayong, Thailand. A study was conducted from October 2014 to March 2015 to identify potential farm-level risk factors using a validated questionnaire. We completed 1 questionnaire for each farm; a total of 38 questionnaires from the case farms and 127 questionnaires from the control farms were collected. The results showed that the presence of WSD in previous crops and the use of seawater were risk factors ($p < 0.01$), indicating that the environment plays an important role in WSD outbreaks in Rayong. Good management practices for pond preparation and other mitigation steps should be part of a control measure program for WSD in this region.

KEY WORDS: Shrimp · Epidemiology · Risk factors · White spot syndrome virus



## INTRODUCTION

White spot disease (WSD) is a highly fatal and contagious disease in marine shrimp that is caused by the white spot syndrome virus (WSSV). The disease is globally distributed. The first official reports of WSD were confirmed in Taiwan and China in 1992 (Chou et al. 1995, Flegel 1997, Zhan et al. 1998). The virus was then reported from other countries in Asia and the Americas (Flegel & Alday-Sanz 1998, APHIS 1999, Walker & Mohan 2009). In Asia, it was estimated that the economic impact of WSSV was $US 4 to 6 billion between 1992 and 2002 (Lightner 2003). The application of disease control measures would have significantly lessened the economic impacts of these disease incursions (Cock et al. 2009). Therefore, studies of risk factors associated with WSD are needed to better understand the disease pattern in different geographical locations, and to enable relevant authorities to apply the most appropriate control measures for their region.

WSSV is hosted in crustaceans such as shrimp, crabs, crayfish, and lobsters (Maeda et al. 2000, Mello Junior et al. 2011, OIE 2014). The virus has the potential to infect its host through vertical (Lo & Kou 1998) or horizontal routes (Tuyen et al. 2014). However, the most frequent route of transmission is either through an infected carrier or contaminated water (Rajendran et al. 1999, Flegel 2006, Waikhom et al. 2006). The virus is more likely to proliferate in a host that is exposed to stressful conditions (Jiravanichpaisal et al. 2004, Du et al. 2006, OIE 2014). Reddy et al. (2013) explained that contamination of farm water sources by wastewater from plants processing infected shrimp can cause dissemination of WSSV. Tendencia et al. (2010) showed that fluctuations in

*Corresponding author: fvetctp@ku.ac.th

temperature and pH affected the shrimp and influenced WSD infection. Corsin et al. (2003) indicated that, in Karnataka, India, potential risk factors for WSSV included improper salinity in transportation bags, improper post-larvae (PL) length, and late stocking date. However, very little information is available about risk factors at the pond or farm level. Corsin et al. (2001) reported that the location of a pond close to the sea was a risk factor in Vietnam. In the Philippines, an important risk factor was feeding live mollusks to the shrimp (Tendencia et al. 2011). Recently, in Thailand, Piamsomboon et al. (2015) indicated that risk factors included the sourcing of water from communal canals, the use of year-round cultures, and an owner operating multiple farm sites.

The purpose of the present study was to identify farm-level risk factors for WSD in Rayong, Thailand. Results of this study will be used to improve or establish measures that can be used to prevent or control WSD in this area. This will reduce the economic losses of farmers in Rayong. In addition, conclusions from this study may also instruct disease evaluation and control in other geographical areas, both inside and outside Thailand.

## MATERIALS AND METHODS

### Target population and study framework

All active marine shrimp farms in Rayong were examined in this study. We selected this area because marine shrimp culture is predominant. Most of the farms in this area are located in 2 districts: the Mueang Rayoung District and the Klaeng District (Fig. 1). A study was conducted from October 2014 to March 2015 to identify potential among-farm (farm-level) risk factors using a validated questionnaire. At the initiation stage of this study, the laboratory results from passive surveillance by the Department of Fisheries (DoF) of Thailand were used to categorize shrimp farms as either cases or controls. To qualify as a suspected farm for WSD, a farmer had to have called in a report to the local DoF and had to have brought at least 15 suspected shrimp to the laboratory. The following clinical signs were used to define the suspected cases and identify suspected shrimp: moribund shrimp or living shrimp with white spots on the exoskeleton with a reddish discoloration of the body, the presence of floating shrimp around the edges of the pond's surface, a decrease in food consumption, or a surge in the mortality rate (OIE 2014). The suspected shrimp were confirmed using WSSV strip test kits (EnBiotech Laboratories; sensitivity = 34.7 %, specificity = 100 %) and/or the polymerase chain reaction (PCR) method (OIE 2014). Farms with at least 1 infected shrimp were assigned to the case group. The

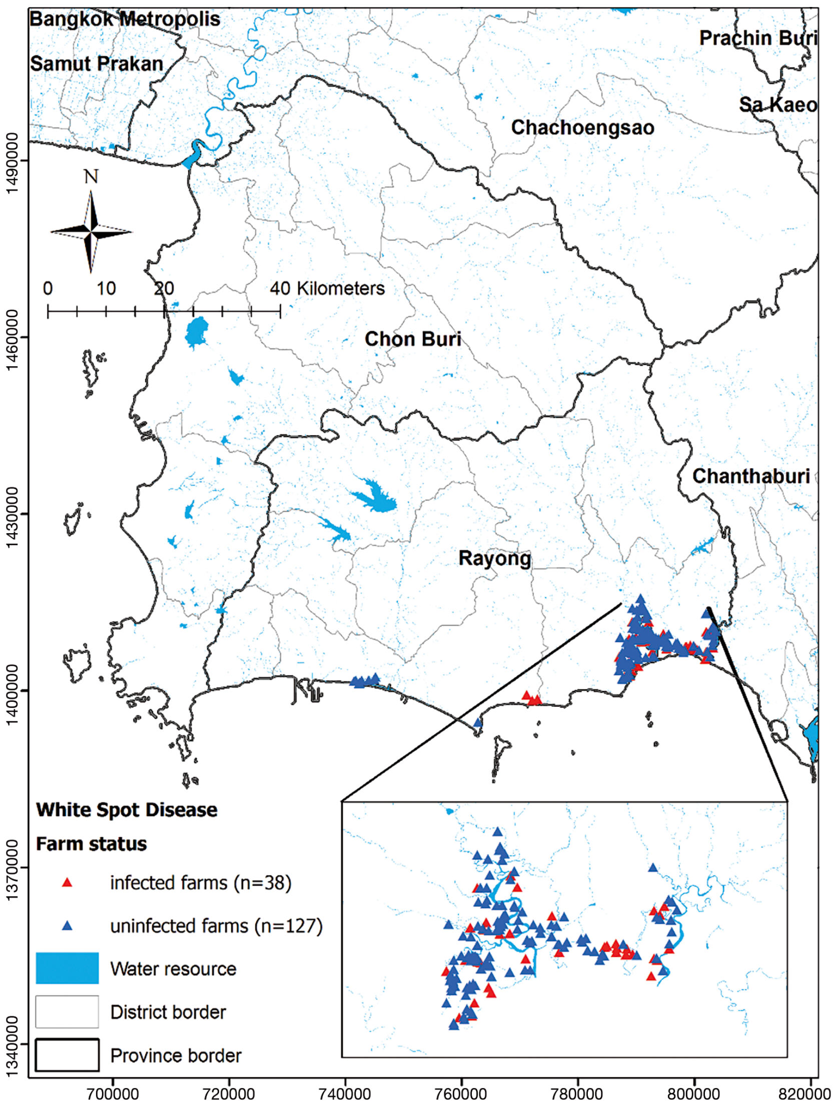


Fig. 1. Geographic distribution of the farms sampled in Rayong, Thailand (coordinates: UTM system). Case (red triangle) and control (blue triangle) farms are represented by different colored symbols

control farms were all the remaining farms in which clinical signs of WSD were absent over the study period. At the end of the production cycle, the production index, mortality rate, and feed conversion ratio of all control farms were also checked for verification their status. A questionnaire was administered to 38 case farms and 127 control farms.

### Questionnaire design and factors associated with WSD

The questionnaire contained both open and closed questions that were asked of the respondents in face-to-face interviews. Aquaculture (n = 2) and epidemiology (n = 1) experts proofread the questionnaire and provided their feedback to clarify any unclear information in the questions and to strengthen the items in the questionnaire. Before starting data collection, the questionnaire was tested using 20 farmers. Then, the questionnaire was discussed with local fisheries officers (n = 2) who performed the data collection.

The identification of risk factors was based on a literature review (Flegel et al. 1997, Corsin et al. 2001, 2005, MPEDA/NACA 2003, Esparza-Leal et al. 2009, Gao et al. 2011, Tendencia et al. 2011, Pradeep et al. 2012, Kumar et al. 2013, Reddy et al. 2013, OIE 2014) and the field experiences of the present study's authors. Questions focused on 8 main issues that covered the disease status of the farm, potential risk factors, respondent's information, farm characteristics, farm environment, farm management, presence of the vector, and geographic information (Table 1).

### Data analysis

Descriptive, univariate, and binary logistic regression analyses were conducted using NCSS 11 statistical software (NCSS). The WSD status of a farm was used as the dependent variable. In univariate analysis, all independent variables (Table 1) were analyzed using a chi-squared test with $\alpha = 0.05$. Continuous independent variables were converted to binary outcomes using their medians as cut-off points (Corsin et al. 2001). To compensate for the small values, the Zero Count Adjustment value was applied to all count data by adding one. Then, significant factors from univariate analysis were included for variable selection using all possible regressions. In this method, the factors with high R-squared values and the smallest of the square root of the mean square error were selected. The selected set of independent variables was used in binary logistic regression. Moreover, during the analysis all independent variables were checked for multicollinearity using Pearson's correlation. For mapping display, ArcGIS version 10.3.1 (ESRI) was used for data visualization.

## RESULTS

### General information

A total of 165 questionnaires were collected from all active shrimp farms during the study period. By this study's definition of cases, the farm-level pre-

Table 1. Factors possibly associated with white spot disease (WSD). WSSV: white spot syndrome virus; PL: post-larvae

| Type of factor | Factors |
|---|---|
| **Dependent variable** | |
| Disease status | Clinical signs confirmed by laboratory results |
| **Independent variables** | |
| Biosecurity measures | Bird and crab protection; Use of fencing; Control of people and cars before entry to the farm; Control of pet animals; Lining type; Vector control; Disease protection; Waste management; WSSV screening test |
| Farm characteristics | Farm location; Pond characteristics; Disease history; Source and characteristics of the PL; Source of water and usage method; Stocking density |
| Feeding management | Source and type of feed; Use of antibiotics; Use of natural feed; Use of probiotics and supplements |
| General information | People at the farms; The farm's experience with the culture; The culture method used |
| Geographical factors | Distance to roads; Distance to the sea; Distance to neighboring farms |
| Pond preparation | Chemical usage; Liming; Water preparation; Tea seed cake application; Sludge removal; Plowing; Drying and flushing before stocking |
| Water management | Sedimentation; Water added; Water coloring; Water filter; Water reuse; Water treatment; Water retention |

valence of WSD in Rayong was 23.03% (38/165). The results indicated that most of the farms raise shrimp by monoculture with *Penaeus vannamei*. During the study period, only 2 farms (1.21%) raised a monoculture of *P. monodon*. According to the number of active ponds in each farm, 75 farms (45.45%) contained 1 to 5 ponds, 78 (47.27%) contained 6 to 10 ponds, and 12 (7.27%) contained more than 11 ponds. PL were sourced from hatcheries located in Trad, Chachoengsao, Chonburi, Chumphon, and Songkla. Before the PL were sent to farmers, either the DoF or a private laboratory sampled all the batches and tested for WSD using PCR. All batches tested negative for WSD. When the PL batches were moved to the ponds, all of the hatcheries packed them into plastic tanks. The estimated number of PL in each tank was 35000 to 50000 per 100 l of water. During the study, the average (range) price for PL was 0.11 (0.06–0.14) Baht larvae$^{-1}$ (~$US 0.0034 [0.0018–0.0043] larvae$^{-1}$). The farmers began raising the shrimp at PL 12 (range: 10 to 18). For farm-level biosecurity, 124 farms (75.15%) utilized a fence. Only 19 farms (11.52%) applied visitor biosecurity measures, for example, by using vehicle tire disinfection and visitor disinfection protocols (showers and changing clothes), before entering the farms.

### Univariate analysis and binary logistic regression

Univariate analysis using the chi-squared test identified 9 significant risk factors for WSD (Table 2). The DoF staff visiting the farm during the culture process, the truck and driver of the feed company visiting the farm during the culture process, and the use of probiotics during the culture process were significant protective factors ($p < 0.05$). A positive WSD designation in a previous crop, a stocking density of more than 100000 PL per 0.16 ha, use of water from the sea, and use of added water during culture without disinfection were significant risk factors ($p < 0.05$). These factors were analyzed by all possible regression methods, and it was found that the truck and driver of the feed company visiting the farm during culture could be removed from the model. The final model of binary logistic regression is shown in Table 3.

Table 2. Farm-level univariate analysis of risk factors for white spot disease (WSD) in marine shrimp farms in Rayong, Thailand. To compensate for the small values in WSD-positive and WSD-negative columns, the Zero Count Adjustment value was applied to all counts by adding one. OR: odds ratio; PL: post-larvae; DoF: Thailand Dept. of Fisheries

| Factors | WSD-positive | WSD-negative | OR (95% CI) | p |
|---|---|---|---|---|
| Farm with WSD in a previous crop | | | | |
| No | 23 (57.50) | 17 (13.18) | 8.91 (4.00, 19.88) | <0.01 |
| Yes | 17 (42.50) | 112 (86.82) | | |
| DoF staff visited during the culture | | | | |
| No | 23 (57.50) | 106 (82.17) | 0.29 (0.14, 0.63) | <0.01 |
| Yes | 17 (42.50) | 23 (17.83) | | |
| Stocking density >100 000 PL per 0.16 ha | | | | |
| No | 25 (62.50) | 54 (41.86) | 2.31 (1.12, 4.76) | 0.02 |
| Yes | 15 (37.50) | 75 (58.14) | | |
| Source of water from the sea | | | | |
| No | 15 (37.50) | 23 (17.83) | 2.77 (1.28, 6.01) | 0.01 |
| Yes | 25 (62.50) | 106 (82.17) | | |
| Truck and driver of feed company visited during the culture | | | | |
| No | 22 (55.00) | 95 (73.64) | 0.44 (0.21, 0.91) | 0.03 |
| Yes | 18 (45.00) | 34 (26.36) | | |
| Use of probiotics during the cultivation period | | | | |
| No | 24 (60.00) | 106 (82.17) | 0.33 (0.15, 0.70) | <0.01 |
| Yes | 16 (40.00) | 23 (17.83) | | |
| Water added without treatment during the cultivation period | | | | |
| No | 5 (12.50) | 3 (2.33) | 6.00 (1.50, 23.86) | <0.01 |
| Yes | 35 (87.50) | 126 (97.67) | | |
| Water held in a reservoir for >7 d before applying it to the culture | | | | |
| No | 29 (72.50) | 38 (29.46) | 6.31 (2.89, 13.76) | <0.01 |
| Yes | 11 (37.50) | 91 (70.54) | | |

Table 3. Binary logistic regression of the risk factors associated with white spot disease (WSD) in marine shrimp farms in Rayong, Thailand. OR: odds ratio; PL: post-larvae; DoF: Thailand Dept. of Fisheries. Final log likelihood = −59.21; model $R^2$ = 0.49

| Factor | β | SE | OR (95% CI) | Wald *Z*-test | p |
|---|---|---|---|---|---|
| Intercept | 0.04 | 0.61 | 1.05 (0.32, 3.43) | 0.07 | 0.94 |
| Farm with WSD in a previous crop | 2.61 | 0.52 | 13.53 (4.87, 37.58) | 5.00 | <0.01 |
| Source of water from the sea | 1.42 | 0.55 | 4.15 (1.42, 12.14) | 2.60 | <0.01 |
| DoF staff visited during the culture | −1.70 | 0.55 | 0.18 (0.06, 0.54) | −3.10 | <0.01 |
| Stocking density >100 000 PL per 0.16 ha | 0.88 | 0.50 | 2.42 (0.91, 6.41) | 1.78 | 0.08 |
| Use of probiotics during the cultivation period | −0.84 | 0.56 | 0.43 (0.15, 1.29) | −1.51 | 0.13 |
| Water added without treatment during the cultivation period | 1.88 | 1.05 | 6.57 (0.84, 51.19) | 1.80 | 0.07 |

Multicollinearity was checked before analyzing the binary logistic regression. No evidence of severe collinearity was found. The final model showed 3 significant variables ($p < 0.05$). The DoF staff visiting during the culture was a protective factor. Two remaining factors were found to be risks: WSD in a previous crop and use of water from the sea (Table 3).

## DISCUSSION

The purpose of this case-control study was to identify potential farm-level risk factors associated with WSD in Rayong, Thailand. We found that the presence of WSD in previous crops and the use of improperly treated seawater were risk factors. In this study, the farm-level prevalence of WSD in Rayong was 23.03%. The prevalence was high because the study period was the most opportune time for WSD proliferation in Rayong. In our experience, farm-level prevalence of WSD in Rayong tends to be high in the winter season in Thailand. This correlates with Boonyawiwat (2009), who reported that the prevalence of WSD dramatically increases in shrimp from June to December. While factors related to this seasonal increase are unclear, a study utilizing a temporal analysis could help clarify this relationship.

The case definition impacts the apparent prevalence rate of WSD. In this study, the case definition was highly sensitive, resulting in a high number of farms identified as positive for WSD. Even if only 1 infected shrimp was detected at a farm, the farm was categorized into the case group. An advantage of a sensitive case definition is that it includes most or all of the infected farms. If the authorities design the control measures based on the results of this study, the measure will cover all possible scales of outbreak, whether small or large. This situation can be optimized to control WSD and the impact the disease has on the economic value of shrimp industries in Thailand.

A monoculture of *Penaeus vannamei* was the most common type of shrimp culture in Rayong during the study period. Most of the farms (n = 153; 92.73%) were small (1 to 10 ponds farm$^{-1}$). However, we observed that each farm still contained inactive ponds. This is because the farmers need to reduce the risk of monetary loss through diseases such as WSD or early mortality syndrome throughout the year. Per our conversations with the farmers, they choose to minimize the number of active ponds. This helps them obtain the best benefit for their culture because they are able to spend more time on farm biosecurity.

In the study's analysis, factors related to biosecurity such as bird-protection using nets, vector-control using chemical agents, and farm fencing were analyzed separately and in combination. Univariate analysis did not find any single measure to be statistically significant. A possible reason for this is that an effective biosecurity program must address several measurements, not just one.

The binary logistic regression results (Table 3) indicated that the history of a WSD outbreak in a previous crop was a significant risk factor. If a farm is infected, it is very important to eliminate the causative agent. Ineffective disinfection methods can lead to WSSV remaining in the environment. Likewise, good pond preparation is an important practice for terminating disease transmission in a farm between crop cultures. In this study's investigation, most of the farms did not employ pond drying, liming, or fertilization. Therefore, this study could not evaluate the effect of these risk mitigation measures. However, we found that some farms implemented vector eradication using a chemical agent. For example, some farmers used trichlorfon to eliminate undesirable species during pond preparation. Nevertheless, they did not remove or dry the bottom soil. Chemical agents may not be enough to eliminate infestations completely. MPEDA/NACA (2003) suggested that inadequate pond preparation has led to the persistence of WSSV. In our study, most of the infected farms used improperly treated water from the sea for their culture, and most cultured the shrimp using one-time stocking and periodic harvesting. Toward that end, this study found that the farmers possibly added untreated water from the sea to the culture pond. This is risky behavior that could introduce the WSSV from a natural source. However, the factor of water being added without treatment during the cultivation period was not significant in the logistic model. It is possible that the number of cases in this study was too small. Waterborne virus has been shown to play a role in other WSD outbreaks. In the Philippines, sharing infected water has been identified as a WSD risk factor (Tendencia et al. 2011). A previous study showed that WSSV can survive and infect shrimp for at least 40 d at 30°C in seawater under laboratory conditions and feasibly for 3 to 4 d in ponds (OIE 2014). In addition, based on our personal experience, some farmers do practice good pond preparation before they culture their shrimp and still encounter WSD outbreaks. This may be because they treated the water in the reservoir but left it too long before using it. It is possible that the water was exposed to the infectious agent during that interval. According to Bondad-Reantasco (2001), treating the water with 30 ppm chloride for 4 d before using is sufficient to kill WSSV.

As a protective factor, a visit by DoF staff (Table 3) appears to be an important activity. This may not be a predictive factor, however, as it is normal for the DoF staff to visit healthy farms. Rather, it seems that the fishery authorities avoid visiting infected farms regularly because they do not want to expose other farms to the disease and risk transmission. However, when DoF staff visit healthy farms, they always inform farmers about any WSD outbreak in their area. These visits help farmers refine their biosecurity to prevent a WSD outbreak at their farms. The present study attempted to analyze this factor by removing and adding the variable in the logistic model, but we did not find any evidence for a confounding effect. We also analyzed a multicollinearity test for these and did not meet any potential bias or confounding in this study.

In conclusion, our analysis indicated that the risk factors for WSD in shrimp farms in Rayong include WSD outbreaks in a previous shrimp crop and the sourcing of influent water from the sea. This correlates with findings reported by Corsin et al. (2005), who explained that water is probably a major pathway for WSSV entry onto a farm. In shrimp farming with poor practices, wastewater is discharged routinely into the adjacent environment without any treatment. It is possible that this waste contains WSSV and that this water is an ongoing source of infection. The authorities should consider control measures to prevent farmers from releasing untreated wastewater into the environment.

*Acknowledgements.* We thank the officers of the Rayong Coastal Fisheries Research and Development Center for their valuable help and support. We also thank all the farmers who participated in this study for their time and their contributions.

## LITERATURE CITED

APHIS (Animal and Plant Health Inspection Service) (1999) Outbreak of shrimp viral disease in Central America: situation report. US Department of Agriculture, Animal and Plant Health Inspection Service, Riverdale, MD. www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/downloads/wssv.pdf (accessed 15 Jan 2016)

Bondad-Reantaso MG, McGladdery SE, East I, Subasinghe RP (2001) White spot disease. In: Bondad-Reantaso MG, McGladdery SE, East I, Subasinghe RP (eds) Asia diagnostic guide to aquatic animal diseases. FAO Fisheries Technical Paper No. 402/2. FAO, Rome, p 178–182

Boonyawiwat V (2009) Traditional and molecular epidemiology to determine risk factors for outbreaks of shrimp white spot disease in Thailand. PhD dissertation, Kasetsart University, Bangkok

Chou HY, Huang CY, Wang CH, Chiang HC, Lo CF (1995) Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. Dis Aquat Org 23:165–173

Cock J, Gitterle T, Salazar M, Rye M (2009) Breeding for disease resistance of penaeid shrimps. Aquaculture 286: 1–11

Corsin F, Turnbull JF, Hao NV, Mohan CV and others (2001) Risk factors associated with white spot syndrome virus infection in a Vietnamese rice-shrimp farming system. Dis Aquat Org 47:1–12

Corsin F, Thakur PC, Padiyar PA, Madhusudhan M and others (2003) Relationship between white spot syndrome virus and indicators of quality in *Penaeus monodon* postlarvae in Karnataka, India. Dis Aquat Org 54:97–104

Corsin F, Turnbull JF, Mohan CV, Hao NV, Morgan KL (2005) Pond-level risk factors for white spot disease outbreaks. In: Walker P, Lester R, Bondad-Reantasco MG (eds) Diseases in Asian aquaculture, Vol 5. Fish Health Section, Asian Fisheries Society, Manila, p 75–92

Du HH, Li WF, Xu ZR, Kil ZS (2006) Effect of hyperthermia on the replication of white spot syndrome virus (WSSV) in *Procambarus clarkii.* Dis Aquat Org 71:175–178

Esparza-Leal HM, Escobedo-Bonilla CM, Casillas-Hernández R, Álvarez-Ruíz P and others (2009) Detection of white spot syndrome virus in filtered shrimp-farm water fractions and experimental evaluation of its infectivity in *Penaeus* (*Litopenaeus*) *vannamei.* Aquaculture 292: 16–22

Flegel TW (1997) Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. World J Microbiol Biotechnol 13:433–442

Flegel TW (2006) Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. Aquaculture 258:1–33

Flegel TW, Alday-Sanz V (1998) The crisis in Asian shrimp aquaculture: current status and future needs. J Appl Ichthyology 14:269–273

Flegel TW, Boonyaratpalin S, Withyachumnarnkul B (1997) Progress in research on yellow-head virus and white spot virus in Thailand. In: Flegel TW, Macrae IH (eds) Disease in Asian aquaculture, Vol 3. Asian Fisheries Society, Manila, p 285–296

Gao H, Kong J, Li Z, Xiao G, Meng X (2011) Quantitative analysis of temperature, salinity and pH on WSSV proliferation in Chinese shrimp *Fenneropenaeus chinensis* by real-time PCR. Aquaculture 312:26–31

Jiravanichpaisal P, Söderhäll K, Söderhäll I (2004) Effect of water temperature on the immune response and infectivity pattern of white spot syndrome virus (WSSV) in freshwater crayfish. Fish Shellfish Immunol 17:265–275

Kumar SS, Bharathi RA, Rajan J, Alavandi S, Poornima M, Balasubramanian C, Ponniah A (2013) Viability of white spot syndrome virus (WSSV) in sediment during sun-drying (drainable pond) and under non-drainable pond conditions indicated by infectivity to shrimp. Aquaculture 402-403:119–126

Lightner DV (2003) The penaeid shrimp viral pandemics due to IHHNV, WSSV, TSV and YHV: history in the Americas and current status. In: Sakai Y, McVey JP, Jang D, McVey E, Caesar M (eds) Aquaculture and pathobiology of crustacean and other species. Proc 32nd Joint UJNR Aquaculture Panel Symp, 17, 18 and 20 Nov 2003, Davis and Santa Barbara, CA. US Dept of Commerce, Silver Spring, MD, p 17–20

Lo CF, Kou GH (1998) Virus-associated white spot syndrome of shrimp in Taiwan: a review. Fish Pathol 33:365–371

Maeda M, Itami T, Mizuki E, Tanaka R and others (2000) Red swamp crawfish (*Procambarus clarkii*): an alternative experimental host in the study of white spot syndrome virus. Acta Virol 44:371–374

Mello Junior CC, Delsol GYL, Motte E, Escobar VAC and others (2011) Selection of shrimp breeders free of white spot syndrome and infectious hypodermal and hematopoietic necrosis. Pesqui Agropecu Bras 46:530–536

MPEDA/NACA (Marine Products Export Development Authority/Network of Aquaculture Centers in the Asia-Pacific) (2003) Shrimp health management extension manual. www.enaca.org/modules/library/publication.php?publication_id=55 (accessed 4 May 2016)

OIE (World Organization for Animal Health) (2014) Manual of diagnostic tests for aquatic animals. Chapter 2.2.6: white spot disease. www.oie.int/international-standard-setting/aquatic-manual/ (accessed 17 Aug 2014)

Piamsomboon P, Inchaisri C, Wongtavatchai J (2015) White spot disease risk factors associated with shrimp farming practices and geographical location in Chanthaburi province, Thailand. Dis Aquat Org 117:145–153

Pradeep B, Rai P, Mohan SA, Shekhar MS, Karunasagar I (2012) Biology, host range, pathogenesis and diagnosis of white spot syndrome virus. Indian J Virol 23:161–174

Rajendran KV, Vijayan KK, Santiago TC, Krol RM (1999) Experimental host range and histopathology of white spot syndrome virus (WSSV) infection in shrimp, prawns, crabs and lobsters from India. J Fish Dis 22:183–191

Reddy AD, Jeyasekaran G, Shakila RJ (2013) Morphogenesis, pathogenesis, detection and transmission risks of white spot syndrome virus in shrimps. Fish Aquacult J 2013:FAJ-66

Tendencia EA, Bosma RH, Verreth JA (2010) WSSV risk factors related to water physico-chemical properties and microflora in semi-intensive *Penaeus monodon* culture ponds in the Philippines. Aquaculture 302:164–168

Tendencia EA, Bosma RH, Verreth JA (2011) White spot syndrome virus (WSSV) risk factors associated with shrimp farming practices in polyculture and monoculture farms in the Philippines. Aquaculture 311:87–93

Tuyen NX, Verreth J, Vlak JM, de Jong MC (2014) Horizontal transmission dynamics of white spot syndrome virus by cohabitation trials in juvenile *Penaeus monodon* and *P. vannamei.* Prev Vet Med 117:286–294

Waikhom G, John KR, George MR, Jeyaseelan MP (2006) Differential host passaging alters pathogenicity and induces genomic variation in white spot syndrome virus. Aquaculture 261:54–63

Walker PJ, Mohan C (2009) Viral disease emergence in shrimp aquaculture: origins, impact and the effectiveness of health management strategies. Rev Aquacult 1: 125–154

Zhan WB, Wang YH, Fryer JL, Yu KK, Fukuda H, Meng QX (1998) White spot syndrome virus infection of cultured shrimp in China. J Aquat Anim Health 10:405–410

*Editorial responsibility: Lori Gustafson, Fort Collins, Colorado, USA*

*Submitted: July 8, 2016; Accepted: March 23, 2017*
*Proofs received from author(s): May 7, 2017*