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White spot disease risk factors associated with shrimp farming practices and geographical location in Chanthaburi province, Thailand

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ABSTRACT: Over the past 2 decades, shrimp aquaculture in Thailand has been impacted by white spot disease (WSD) caused by white spot syndrome virus (WSSV). Described here are results of a survey of 157 intensive shrimp farms in Chanthaburi province, Thailand, to identify potential farm management and location risk factors associated with the occurrence of WSD outbreaks. Logistic regression analysis of the survey responses identified WSD risks to be associated with farms sharing inlet water and culturing shrimp year round and with a single owner operating more than 1 farm. The analysis also showed WSD risks to be reduced at farms that used probiotics and applied lime to pond bottoms when fallow to neutralize acidity and kill microorganisms. Regression modeling identified no association of geographical location with WSD. The data should assist shrimp farms in mitigating the effects of WSD in Thailand.

KEY WORDS: Epidemiology · Risk factors · Shrimp farming · Thailand · White spot disease · WSSV · Pacific white shrimp · *Litopenaeus vannamei*

INTRODUCTION

White spot disease (WSD) is caused by white spot syndrome virus (WSSV), which has been responsible for substantial economic losses to shrimp farming industries in most parts of the world. WSSV has a wide host range, including most freshwater or marine decapod crustaceans and all commercially important cultured shrimp species (OIE 2014). WSD can result in up to 100% farm pond mortality within 3 to 10 d (Nunan et al. 2001) and was first reported in Taiwan and China in 1992 before spreading to become pandemic within a decade (Dieu et al. 2010, Vijayan & Sanil 2012). In Thailand, WSD outbreaks were first reported in 1995 (Flegel 1997), and the virus rapidly became endemic throughout shrimp farming areas.

As no resistant shrimp or effective commercial treatment options for WSD are currently available, disease control and prevention have relied mainly on management strategies, including virus exclusion and reducing shrimp stress (OIE 2014). Control measures for reducing the incidence of WSD currently rely on stocking ponds with WSSV-free postlarvae, using closed zero-water-exchange culture systems and/or biosecure ponds incorporating crab-proof fencing and bird-proof netting as well as disinfectant systems for workers' hands and feet (Corsin et al. 2005). Despite the availability of such risk mitigation methods, WSD still occurs widely and poses a major threat to shrimp farming industries throughout Asia and elsewhere (Flegel 2012).

Identifying disease risk factors can help devise management practices that are effective in reducing the impact of aquatic animal diseases (Peeler & Taylor 2011). Farm location and management practices can influence the incidence of WSD. For example, a study in the Philippines identified high stocking den-

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sities, the feeding of live mollusks, farm sharing of water sources, and increased pond size to be associated with increased WSD risks (Tendencia et al. 2011). Other studies identified increased mangrove to pond area ratios, the use of vitamin dietary supplements, water filtration through 300 µm mesh screens, and pond bottom dry-out and decontamination, including plowing and sludge removal between crops, as factors that reduce WSD risks (Corsin et al. 2005, Tendencia et al. 2011).

Risk analyses can be instructive as to what measures might assist in mitigating disease impacts in a particular farming region. The purpose of this study was thus to identify risk factors associated with the occurrence of WSD in Chanthaburi province, an intensive shrimp aquaculture region in Thailand, so that these might be used to guide government and farm WSD control policies.

MATERIALS AND METHODS

Study population and size

The study focused on Pacific white shrimp *Litopenaeus vannamei* farms located in the coastal area of Chanthaburi province, Thailand (Fig. 1) and was conducted between October 2011 and December 2013 in collaboration with the Chanthaburi Coastal Fisheries Research and Development Centre (CFRD), Department of Fisheries. Chanthaburi province is among the largest shrimp aquaculture regions in Thailand, producing >60 000 t yr^{-1} (Department of Fisheries 2014). Farmers generally purchase shrimp postlarvae age 10 to 12 d (PL10–PL12) from local or nearby (<300 km) hatcheries for grow-out in earthen ponds for 90 to 120 d.

The case–control study used a questionnaire to interview farmers (the full questionnaire is provided in the Supplement, available at www.int-res.com/articles/suppl_d117p145_supp.pdf). Farms were selected arbitrarily from 886 farms located in the CFRD study area. Farms were divided into WSD case and control groups based on their disease status in CFRD records. WSSV was detected using the nested PCR method endorsed by the World Organization for Animal Health (OIE 2014). Farms were assigned to the WSD case group when WSSV was detected in association with disease in at least 1 crop over the study period. Farms were assigned to the control group when monthly samples were WSSV-negative over the study period. Based on assuming an odds ratio (OR) of 4 with a 95% confidence interval (CI) and 80% statistical power with an expected proportion exposed amongst controls of 8%, at least 66 farms were required per group. A farm sample size of 100

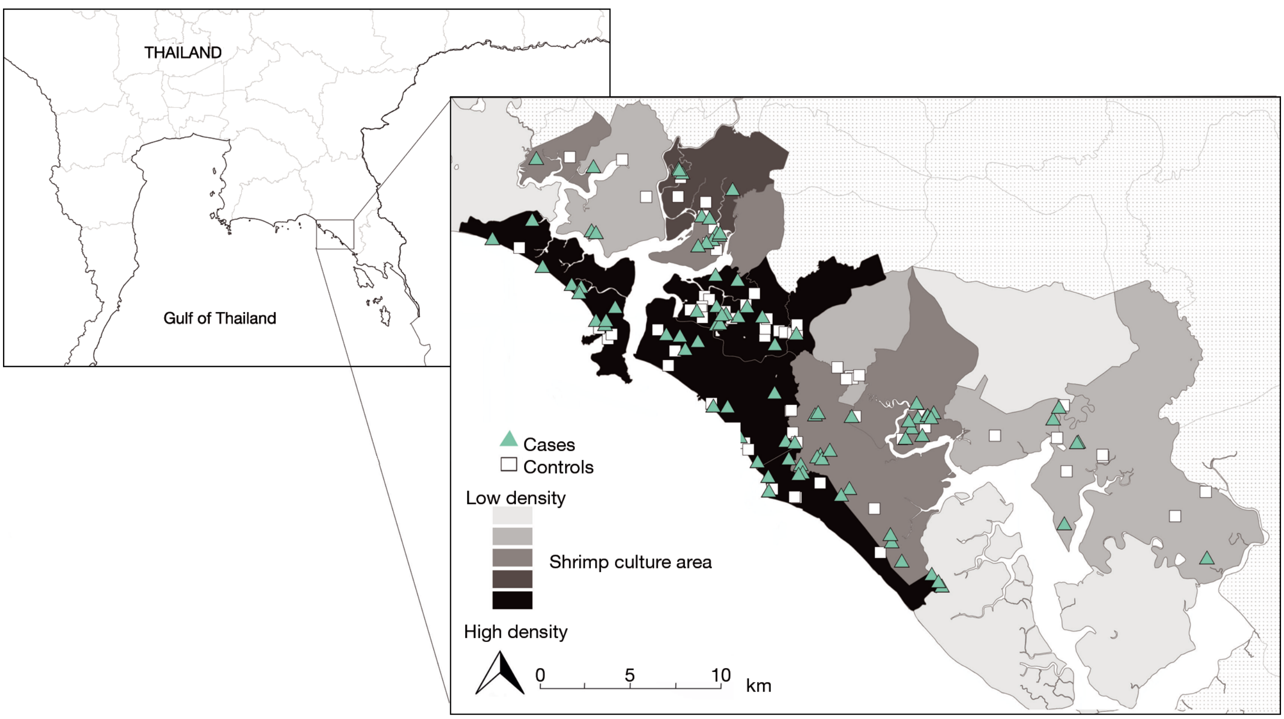

Fig. 1. Study area in Chanthaburi province, Thailand. Locations of case (▲) and control (□) farms are shown. Map shading shows farm densities from lowest (light grey) to highest (black)

for both the case and control groups was therefore selected for the interviews.

Information collection

Information on farming practices was collected using a structured farm owner questionnaire combined with interviewer observations (see the Supplement). The questions were approved by local government officers and farm personnel to ensure they were easily understood. For consistency, all farm owners and/or managers were interviewed by P. Piamsomboon on the basis that their responses would be anonymous. Questions on potential risk factor variables covered farm characteristics and management practices as well as various other factors described in studies in other Asian countries (Tendencia et al. 2011, Table 1).

Farm global positioning system (GPS) *x*,*y* coordinates were obtained using a Garmin eTrex®10 handheld GPS device. For each farm, distances to the nearest point on the coastline, public canal, national highway, and mangrove forest were determined using the Euclidean distance calculation function in the Spatial Analyst Tools, ArcToolbox, ArcGIS 10.0 software (ESRI). The relevant spatial data of road networks, coastline, canals, and mangrove forests were provided by the Royal Thai Survey Department, Ministry of Defense.

Statistical analysis

Statistical analyses utilized SPSS software (version 22). Continuous-scale risk factor variables were tested for normality and linearity of effect. If the distribution was not normal or linear, the variable was transformed to a categorical scale using quartiles. The WSD status of the farm was used as the dependent variable in each analysis. Univariate logistic regression was initially performed in order to identify a subset of statistically significant risk factor variables using $p < 0.1$. Spearman rank correlations were used to examine for collinearity between the significant risk factor variables. Correlation coefficients >0.4 were interpreted to indicate collinearity. Amongst pairs of collinear variables, a choice was made based on acceptability of potential biological cause–effect relationships to select the variable to include in the multivariable analysis. Subsequently, variables that were not collinear were included in a multivariable logistic regression analysis using a backward stepwise variable selection approach based on $p > 0.05$. ORs and their 95% CIs were calculated for statistically significant risk factor variables. Variables that changed the regression coefficient estimates of at least 1 other variable in the model by more than 20% were considered to be confounders and therefore included in the final model to adjust for the confounding effect of that risk factor.

RESULTS

Descriptive analysis

Of the 200 farms that had been in operation for at least 5 yr and were selected for study, 157 (comprising 88 case-group farms and 69 control-group farms) agreed to be interviewed. These included 87 (55.4%) small farms, 46 (29.3%) intermediate-sized farms, and 24 (15.3%) large farms (Table 1). The total culture area covered by the farms represented 25.1% of that in Chanthaburi province.

In terms of farm biosecurity, 26.2% were fenced and 6.4% had vehicle tire baths and personnel disinfection systems at their entrance. At most (77%) farms, water was released directly into the environment at harvest rather than being recycled. Very few (<1%) farms practiced water decontamination prior to release. Many (42%) farms had crab-proof fencing and 27.4% had bird-proof netting.

Univariate and multivariate analyses

The univariate analysis identified 15 risk factors for inclusion in the multivariable analysis (Table 1). The final model initially comprised 6 variables: water source, continuous culture throughout the year, owner of multiple farms, lime application, use of probiotics, and presence of a caretaker. Of these variables, significant correlations were found between caretaker and water source ($r = 0.54$) and between caretaker and continuous culture ($r = 0.46$). The water source and continuous culture variables were thus selected for the final model because they had previously been identified as potential WSD risk factors (Tendencia et al. 2011). The reintroduction of the 'distance to national highways' variable resulted in a >20% change in 1 category coefficient estimate for the water source variable, and it was thus included as a confounder in the model. The 95% CI, OR and level of statistical significance for each variable are detailed in Table 2.

Table 1. Results of the univariate logistic regression analysis of the association between white spot disease (WSD) occurrence in cultured Pacific white shrimp *Litopenaeus vannamei* and farm characteristics and farm management factors. Values in **bold** indicate $p < 0.1$; N: number of farms; OR: odds ratio; Ref.: reference groups. *Asterisks indicate risk factors ($p < 0.1$). Quartiles are ranked sets of continuous data equally divided into 4 groups

Variable	Explanation		N	p	OR
Farm area					
Farm size	Total farm area (ha)				
	<2.4		87	0.15	0.48
	>2.4–8		46	0.76	0.85
	>8		24	Ref.	Ref.
Culture area*	Total area used for shrimp culture (ha)				
	<0.88		39	**0.01**	0.31
	>0.88–4.32		80	**0.09**	0.49
	>4.32		38	Ref.	Ref.
Water reserve area*	Total area of reservoir pond (ha)				
	0		52	0.85	0.92
	>0–0.32		44	0.44	0.71
	>0.32–1.04		22	**0.01**	**1.50**
	>1.04		39	Ref.	Ref.
Sludge pond area*	Total area of sludge pond (ha)				
	0		47	0.88	0.93
	>0–0.16		42	**0.04**	0.39
	>0.16–0.48		31	0.57	1.33
	>0.48		37	Ref.	Ref.
Farm features					
No. of ponds farm^{-1}	Total no. of ponds used for shrimp culture farm^{-1}				
	≤10		127	0.17	0.57
	>10		30	Ref.	Ref.
Cul:res ratio*	Ratio between shrimp culture and reservoir pond area		Ref.	**0.03**	**1.03**
Caretaker*	Person responsible for farm operation:				
	Owner		80	**0.04**	**0.42**
	Owner and worker(s)		39	**0.07**	**0.42**
	Farm manager and worker(s)		38	Ref.	Ref.
Water source*	Source of water used in farm for shrimp culture:				
	Sea		23	0.36	1.8
	Public canal(s)		117	**0.01**	**3.98**
	Underground water		17	Ref.	Ref.
Owner of multiple farms*	Farmer operates more than 1 farm, each located	Yes	48	**0.06**	**1.89**
	in different areas	No	109	Ref.	Ref.
Water recycling	Reuse of water from the previous shrimp crop to	Yes	36	0.22	0.63
	culture the next crop	No	121	Ref.	Ref.
Adjacent farms	Presence of other shrimp farms next to the observed farm	Yes	138	0.19	1.8
		No	19	Ref.	Ref.
Limited access	No access of unauthorized personnel to farm	Yes	59	0.98	0.99
		No	98	Ref.	Ref.
Fence	Presence of barrier or other upright structure	Yes	41	0.27	1.5
	surrounding the farm	No	116	Ref.	Ref.
Pets in farm	Presence of other animals roaming freely in the farm,	Yes	69	0.45	1.27
	e.g. dogs and chickens	No	88	Ref.	Ref.
Vehicle disinfection	Disinfection processes used for vehicles entering	Yes	10	0.32	1.90
	the farm, e.g. tire baths and vehicle sprays	No	147	Ref.	Ref.
Separate workers	Different worker allocated to each pond	Yes	39	0.95	1.02
		No	118	Ref.	Ref.
Continuous culture*	Farm that stocks shrimp continuously year round,	Yes	86	**0.08**	1.73
	producing more than 2 crops per year	No	71	Ref.	Ref.
Pond preparation					
Sludge removal	Disposal of soil at the bottom of the pond after	Yes	82	0.52	0.81
	each crop is harvested	No	75	Ref.	Ref.
Lime application*	Application of lime to dried pond bottom for disinfection	Yes	57	**0.01**	0.49
		No	100	Ref.	Ref.

Table 1 (continued)

Variable	Explanation		N	p	OR
Water preparation					
Water filter	Water in culture ponds is filtered using a trawling net	Yes	105	0.52	0.80
		No	52	Ref.	Ref.
Animal waste	Chicken/pig manure or cow dung used as pond fertilizer	Yes	53	0.92	1.03
		No	104	Ref.	Ref.
Inorganic fertilizer	Use of inorganic fertilizer to adjust water color before	Yes	99	0.87	0.94
	stocking shrimp	No	58	Ref.	Ref.
Insecticide	Use of insecticide to eliminate aquatic decapods	Yes	108	0.78	1.09
	during water preparation	No	49	Ref.	Ref.
Copper	Use of copper to eliminate shellfish before	Yes	101	0.54	0.81
	stocking shrimp	No	56	Ref.	Ref.
Tea seed*	Use of tea seed cakes or powder to kill small fish	Yes	138	**0.07**	**0.34**
	before stocking	No	19	Ref.	Ref.
Pond features					
PE-lined pond	Use of polyethylene (PE) to cover the pond slope	Yes	74	0.42	1.29
		No	83	Ref.	Ref.
Bird-proof netting	String or net installed above the pond to prevent	Yes	43	0.69	0.86
	access by birds	No	114	Ref.	Ref.
Crab-proof fencing	Nylon/plastic screen installed on dike surrounding the	Yes	66	0.74	0.90
	pond to prevent crabs from entering	No	91	Ref.	Ref.
Hand and foot baths	Containers placed at pond entrance that contain	Yes	24	0.18	2.12
	chemicals for disinfection of workers' hands and feet	No	133	Ref.	Ref.
Feed additive					
Vitamin C	Use of commercial vitamin C mixed into feed	Yes	22	0.75	1.27
		No	135	Ref.	Ref.
Probiotics mix in feed*	Use of commercial probiotics mixed into feed	Yes	136	**0.05**	**0.35**
		No	21	Ref.	Ref.
Postlarvae (PL)					
Source of shrimp PL*	Provinces from which farmers obtain PL				
	Province 1		63	**0.03**	**0.41**
	Province 2		47	0.50	0.73
	Province 3		43	Ref.	Ref.
Virus detection of PL	Test for abnormalities and the presence of important	Yes	88	0.91	0.96
	viruses in PL before stocking	No	69	Ref.	Ref.
Stocking density	No. of PL released to culture pond (PL m^{-2})				
	<62.5,		80	0.33	0.62
	>62.5-81.25		40	0.92	1.05
	>81.25		37	Ref.	Ref.
Distance variables[a]	Nearest distance categorized into quartiles from farms to the particular features (km)				
To coastline*	Quartile 1 (0.03–0.58)		39	0.17	1.86
	Quartile 2 (0.60–1.26)		39	**0.01**	**4.07**
	Quartile 3 (1.27–2.34)		39	0.72	2.30
	Quartile 4 (2.35–6.44)		39	Ref.	Ref.
To nearest national highway*	Quartile 1 (0.01–1.00)		39	**0.01**	**0.29**
	Quartile 2 (1.09–2.09)		39	0.99	0.44
	Quartile 3 (2.13–4.16)		39	**0.01**	**0.29**
	Quartile 4 (4.17–9.51)		39	Ref.	Ref.
To nearest public canal	Quartile 1 (0.06–0.38)		39	0.49	0.73
	Quartile 2 (0.39–0.85)		39	0.24	1.73
	Quartile 3 (0.86–1.85)		39	0.49	0.73
	Quartile 4 (1.86–5.68)		39	Ref.	Ref.
To nearest mangrove forest*	Quartile 1 (0.01–1.00)		39	0.29	0.61
	Quartile 2 (1.09–2.09)		39	0.74	1.16
	Quartile 3 (2.13–4.16)		39	**0.08**	**0.45**
	Quartile 4 (4.17–9.51)		39	Ref.	Ref.

[a]One of the farm locations was missing (i.e. information not collected), resulting in a total of 156 farms for the distance variables

Table 2. Final logistic regression model of white spot disease (WSD) risk factors in intensive Pacific white shrimp *Litopenaeus vannamei* culture systems in Chanthaburi province, Thailand. N: number of farms; β: estimated coefficient; CI: confidence interval; Ref.: reference group of each variable

Parameter Variable	N	β	Odds ratio Value	 95% CI	p
Constant		0.23	1.26	0.2–7.83	0.8
Water source					
Sea	23	−0.68	0.5	0.08–3.09	0.46
Canal	117	1.15	3.16	0.95–10.57	0.05
Underground	17	Ref.	Ref.	Ref.	Ref.
Lime application					
Yes	57	−0.99	0.37	0.16–0.86	0.02
No	100	Ref.	Ref.	Ref.	Ref.
Probiotic used in feed					
Yes	136	−1.54	0.21	0.06–0.72	0.01
No	21	Ref.	Ref.	Ref.	Ref.
Owner of multiple farms					
Yes	48	1.4	4.05	1.41–11.64	0.01
No	109	Ref.	Ref.	Ref.	Ref.
Year round continuous culture					
Yes	86	0.83	2.29	0.99–5.28	0.05
No	71	Ref.	Ref.	Ref.	Ref.
Distance from the nearest national highway					
Quartile 1	39	−1.02	0.36	0.12–0.96	0.05
Quartile 2	39	−0.89	0.40	0.12–1.30	0.12
Quartile 3	39	−1.11	0.33	0.1–1.08	0.06
Quartile 4	39	Ref.	Ref.	Ref.	Ref.

DISCUSSION

WSSV has been a cause of serious disease in shrimp farmed in Thailand and has impacted both black tiger shrimp *Penaeus monodon* and Pacific white shrimp in hatcheries as well as in grow-out ponds (Withyachumnarnkul et al. 2003, Flegel 2012). Control of WSD in Thailand has been challenging because small- to medium-scale farms make up a large part of the industry. These farms are usually clustered, share common water sources, and disregard recommended disease management practices. Despite strict biosecurity measures being used actively at the large-scale study farms, WSD remains an issue because biosecurity is neglected at neighboring small-scale farms. Better management practices (BMPs) have been introduced to small-scale aquaculture farms in many developing countries (Mohan et al. 2008, Phan et al. 2009, Umesh et al. 2010). These BMPs aim to provide small-scale farmers with knowledge of inexpensive and practical farm management and feeding procedures to assist them in improving yields and minimize losses caused by infectious diseases (Padiyar et al. 2003). In India, BMPs have been implemented in shrimp culture communities affected seriously by WSD, and risk factor analyses have been pivotal to identifying and quantifying the value of various BMPs (Padiyar et al. 2003, Mohan & De Silva 2010).

In the Chanthaburi province study area, WSD occurrence was associated profoundly with the sourcing of pond water from communal canals conveying water to a cluster of farms from either the sea or a river. This has also been identified as an important WSD risk factor in the Philippines, particularly when the canal is used for both farm inlet and outlet water, and more so when used as a water outlet during emergency harvests (Tendencia et al. 2011). At all of our study farms, however, canal inlet water was typically chlorinated before being used to fill ponds, and all employed zero water exchange grow-out systems. However, not all farms applied pond biosecurity systems to prevent entry of WSSV carriers likely to reside in the communal canals, and thus such carriers might represent a source of disease (OIE 2014). WSD outbreaks in farms in Vietnam have been associated with the introduction of WSSV-infected decapods and WSSV-contaminated zooplankton (Corsin et al. 2001). The use of communal canals also increases the likelihood of ponds receiving poor-quality hypertrophic or eutrophic water with potential to cause stress that could in turn induce disease (Lyle-Fritch et al. 2006, Huang et al. 2011).

In the study area, shrimp farms most distant from highways tended to be those either using or located nearest to communal canals. Therefore, this variable was also correlated with higher farm densities and WSD occurrence. The higher risk of WSD at farms where the owner operated several farms might be due to increased movements of staff and vehicles between the farms together with inadequate biosecurity precautions.

Experienced shrimp farmers in Thailand and the Philippines generally avoid stocking ponds during colder weather due to higher risks of WSD (Withyachumnarnkul et al. 2003, Tendencia et al. 2010). Higher WSD risks do occur at farms growing 3 or more crops each year compared to farms growing fewer than 2 crops during the warmer months. WSD becomes less problematic in grow-out water temperatures above 30°C, and WSSV has been identified to replicate more effectively in Pacific white shrimp at water temperatures of ~26°C compared to ~32°C (Vidal et al. 2001). These findings are supported by WSSV gene expression in subcuticular epithelial cells being higher among Pacific white shrimp in

~26°C water compared with 33°C water (Reyes et al. 2007). Cell apoptosis caused by WSSV infection is also lower at water temperatures below 32°C (Granja et al. 2003). While growing shrimp at colder water temperatures poses higher risks of WSD, several farmers in the study region disregarded this due to attractive shrimp prices during cooler periods. In addition, farmers who produce >2 crops per year would have less time for pond drying. WSSV has been shown to remain infectious for up to 19 d in the sediment of a pond being sun-dried and up to 35 d in an undrained pond (Satheesh Kumar et al. 2013). Considering that each production cycle of Pacific white shrimp in the study area usually takes 145 to 165 d (including 30 d pre-stocking for pond preparation, 100 to 120 d for shrimp grow-out, and 15 d for post-harvest pond drying), only those farmers growing 1 or 2 crops a year can set aside adequate time for pond drying.

The multivariate regression analysis showed that the application of lime to disinfect the bottoms of fallow ponds is useful in preventing WSD, as also reported from findings in India (MPEDA/NACA 2003) and Bangladesh (Rakibul Islam et al. 2014). After each harvest, farmers usually removed the sludge pile and dried the pond bottom before applying lime, as is standard practice in shrimp aquaculture (Cruz-Lacierda et al. 2008). Farmers in the study region also applied lime at concentrations sufficient to generate a pond bottom soil pH >10, which has proven to be an effective disinfectant (Boyd & Massaut 1999, Boyd 2003).

The final logistic model indicated that the use of probiotic feed supplements was a preventive factor against WSD. *Bacillus* spp. probiotics from either commercial or government sources were used commonly in the study region. Many farmers used pineapple or banana as probiotics because they contain substantial amounts of vitamin C (Klimczak et al. 2007), which has been shown to improve stress and non-specific defense responses in shrimp (Lee & Shiau 2002, Qiao et al. 2011). Some lactic acid bacteria have been reported to enhance growth of shrimp and fish (Kesarcodi-Watson et al. 2008, Lara-Flores 2011, Tuan et al. 2013, Aguilera-Rivera et al. 2014). Probiotics have been suggested to enhance the resistance of cultured shrimp to WSSV by mechanisms involving competitive exclusion and/or immune stimulation (Li et al. 2009). For example, probiotic organisms such as *Staphylococcus hemolyticus* and *Pediococcus pentosaceus* have been found to protect Pacific white shrimp against WSSV and infectious hypodermal and hematopoietic necrosis virus (Leyva-Madrigal et al. 2011). However, it is possible that the use of probiotics at a farm was simply an indicator of the farmer having the financial ability to better manage crop grow-out, which might confound the survey data correlating probiotics use with lowered WSD risks.

With *P. monodon*, stocking ponds with WSSV-infected PL has been reported to be useful in mitigating WSD risks (Limsuwan 1997, Withyachumnarnkul 1999), and PCR screening of PL for WSSV is generally recommended for shrimp cultured in Thailand (Flegel 2012). However, PCR data on PL screened over the study period identified no correlation between WSSV detection and WSD occurring during grow-out. Other WSSV infection entry routes into ponds such as intake water or carrier species thus appear to have overridden any benefits of PL screening. While this finding supports PL screening for WSSV being non-mandatory in Thailand, our findings are unlikely to dissuade farms with the capacity to accommodate screening from continuing with this practice as part of their disease risk management strategy.

The primary findings of the shrimp farmer survey and PL testing undertaken in the Chanthaburi province study region were that the use of communal water sources by many independent farms was the major WSD risk factor. Thus, mitigating WSD needs to be a shared responsibility of shrimp farming communities using such water sources and should be supported by appropriate government incentives. The study also identified a need for farms utilizing communal water canals to exercise care with water and pond management practices, including the use of lime at concentrations adequate to disinfect the pond bottom soil during dry-out. Farms undertaking continuous culture cycles need to consider employing an adequate period for pond drying and avoiding shrimp culture during cooler months. While surveillance for WSD carriers in communal water canals and mandatory PL testing might also be considered to reduce the risks of WSD, key to the success of such measures will be the active support and participation of local shrimp farming communities.

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