A longitudinal study of risk factors associated with white spot disease occurrence in marine shrimp farming in Rayong, Thailand

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Abstract

Background. A longitudinal study was conducted to analyze farm characteristics, farm practices, and biosecurity measures that influenced the occurrence of white spot disease (WSD) in shrimp farming in Rayong, Thailand.

Methods. Data were collected using a structured interview schedule administered between October 2017 and September 2019. A generalized estimating equations (GEE) model was used to identify risk factors. From the 270 responses, 86 possible risk factors were analyzed using univariate and multivariate analysis.

Results. We found that 17 possible risk factors were statistically significant with an alpha level of 1% and associated with WSD status. In the final model, multivariate analysis found that two independent variables were statistically significant. First, the absence of inclement weather during ponding was a statistically significant factor associated with WSD occurrence and it prevented WSD occurrence as the odds ratio (OR) was < 1 (OR = 0.196, P = 1.3 x 10^{-5}). Second, the separation of ponds into three specific types (cultured, water treatment, and pond for water reservoir) was significantly associated with WSD occurrence. Likewise, the presence of this ponding system was a protective factor against WSD (OR = 0.0828, P = 0.001).

Conclusions. The results of this study offer a reference for farmers and relevant authorities when addressing WSD occurrence in shrimp farming. In addition, our results can help relevant authorities in controlling WSD in other endemic areas.

Keywords:
Longitudinal study, risk factors, Thailand, white spot disease

Introduction

White spot disease (WSD), a severe viral disease affecting marine shrimp farming, is caused by the white spot syndrome virus (WSSV). This double-stranded DNA virus belongs to the genus Whispovirus within the Nimaviridae family. WSSV has a wide host range, including the marine shrimp, and it can be transmitted vertically and horizontally. The mortality rate of infected penaeid shrimp is high; however, the morbidity rate varies depending on various factors (OIE, 2009). In general, the cumulative mortality rate can reach 90%–100% within 3 to 10 days after infection (Wang et al., 1999; Millard et al., 2021). In infected shrimp with WSD, lesions that appear as white spots are commonly found within the exoskeleton. Furthermore, spots are present on the carapace of shrimp; while they are normally white, the colour of these spots can vary. Reddish or pinkish discoloration of shrimp is always present (OIE, 2019). Infected shrimp usually present abnormal behavior such as lethargy, slow swimming, swimming on the water surface, swimming along the edge of the pond, and/or reduced feed intake (OIE, 2019). Infrequent, infected penaeid shrimp present subclinical disease. Therefore, laboratory confirmation is needed to confirm WSSV in shrimp with suspected infection (OIE, 2009, 2019). In terms of economic impacts, previous studies have estimated the economic loss from WSD to be more than 8 billion USD (Stentiford, Bonami & Alday-Sanz, 2009; Verbruggen et al., 2016).

62   In Asia, the impacts from WSD were estimated to be 11 million USD in 2015 from production
63   and economic losses (Shinn et al., 2018).

64   Possible risk factors have been reported in several studies, including: wastewater from a
65   contaminated processing plant (Reddy, Jeyasekaran & Shakila, 2013), pond location near a sea
66   (Corsin et al., 2001), feeding infected carriers, sharing of the water source with infected farms,
67   high stocking density of shrimp (Tendencia, Bosma & Verreth, 2011), appearance of previously
68   infected crops and seawater use (Yaemkasem et al., 2017), owners who own multiple farms
69   (Piamsomboon, Inchaisri & Wongtavatchai, 2015), low atmospheric temperature and high daily
70   atmospheric temperature (Piamsomboon, Inchaisri & Wongtavatchai, 2016), presence of weed
71   on the farm site and inappropriate concentration of ammonia and oxygen (Talukder et al., 2021),
72   and insecure post-larvae (PL) provider (Worranut et al., 2018). However, only a few studies have
73   applied analytical techniques to understand factors that may vary over time. In this study, we
74   used the longitudinal analysis of a targeted study area to improve understanding of the behavior
75   of WSD.

76   Longitudinal studies can determine the association of risk factors over an extended
77   period. This kind of study can help researchers reduce recall bias, a major error in well-known
78   designs such as ordinary case–control studies (Lindberg et al., 2017; Cronin et al., 2020).
79   Likewise, a longitudinal study can help identify all possible changes in factors that may occur
80   over time (Fitzmaurice, Laird & Ware, 2012). Statistical testing with longitudinal studies must
81   consider numerous factors. Previously, common statistical methods such as the analysis of
82   variance (ANOVA), multivariate analysis of variance (MANOVA), mixed-effect regression
83   model (MRM), and generalized equation estimation (GEE) were used for analysis. In prospective
84   longitudinal data, the advantage is the ability to detect factors to exposures with regards to
85   presence, timing, and chronicity. However, there are issues about follow-up and missing data
86   (Caruana et al., 2015).

87   Using the GEE model in longitudinal data can reduce statistical problems such
88   underestimation of variability and type II statistical errors (Caruana et al., 2015). Tang et al.
89   (2022) used GEE to analyzed longitudinal changes in the lungs associated with asthma. They
90   found that patients with persistent asthma had lower forced expiratory indices than patients with
91   asthma resolution. Moreover, children with persistent asthma developed poorer lung function
92   growth. Santanasto et al. (2022) studied longitudinal changes in lean mass, fat mass, and grip
93   strength of African Caribbean men. They found that the acceleration in muscle strength decrease
94   lead the acceleration in lean mass decrease by 10–15 years. Gianfrancesco et al. (2019) examined
95   the relationship between smoking and rheumatoid arthritis, controlling for time-varying
96   covariates. They found evidence that smokers were associated with higher levels of disease
97   activity in rheumatoid arthritis.

98   Rayong province, located on the east coast of the Gulf of Thailand, is an important
99   shrimp farming area in Thailand; however, it suffers from continuously WSD occurrence. To
100   limit the economics loss caused by WSD, a longitudinal design was used to study farm
101   characteristics, farm practices, and biosecurity measures that influenced WSD occurrence over

102   time using a structured interview schedule. The results of this study provide new insight into the
103   presence of WSD, and offer a reference for authorities in their ateempts to improve the disease
104   control program of WSD in Thailand.

105

Materials and Methods

Study framework and ethical statement

108   A two-year longitudinal observational study was performed wherein data were collected
109   using a structured interview schedule administered between October 2017 and September 2019.
110   The study area was Rayong province, Thailand, located on the east coast of the Gulf of Thailand.
111   All shrimp farms were located in two districts: most were in the Klang district, with the
112   remainder in Mueang Rayong district. A total of 270 ponds from 22 marine shrimp farms were
113   included in this study.

114   Informed verbal consent was obtained before commencing the data and shrimp sample
115   collection. All procedures involving participants were performed following the principles of the
116   Declaration of Helsinki. In addition, this study was approved by the Institutional Animal Care
117   and Use Committee (IACUC) of the Department of Fisheries (DoF), Thailand. The approval
118   number is U1-05341-2559.

119

Structured interview schedule

121   The structured interview schedule contained both open- and close-ended questions asked
122   to respondents in face-to-face interviews. The structured interview schedule was reviewed by
123   aquaculture (n = 1) and epidemiology (n = 1) experts by proofreading. Before commencing the
124   data collection, the structured interview schedule was tested for corrections with the help of 20
125   farmers. Later, the approved structured interview schedule was discussed with local fishery
126   officers who performed the data collection. Each pond for the study was selected through a joint
127   decision between the owner and the authors. However, the same pond or a different pond on the
128   same farm was used for consecutive data collection. These ponds represent farms used as
129   cultivation sites. Moreover, each farm was studied for 2–23 sequential pondings that might
130   overlap with previous or subsequent cultivation (Supplement 1).

131   Each farmer was interviewed twice using the structured interview schedule. Certain
132   questions were asked during the initial stage of cultivation regarding pond preparation and
133   activities during the stocking period to avoid recall bias. The remaining questions were asked
134   after harvesting a few days later. Furthermore, all respondents were farm owners or farm
135   managers. In the structured interview schedule, questions included those on farm information,
136   farm practices, farm biosecurity, and possible risk factors associated with WSD (Supplement 2).
137   All questions were designed based on literature reviews (Piamsomboon, Inchaisriand &
138   Wongtavatchai, 2015; Yaemkasem et al., 2017). In detail, the questions adopted from
139   Piamsomboon Inchaisriand and Wongtavatchai (2015) were those related to farm area, culture
140   area, number of pondings, water reserve area, source of farming water, owner of multiple farms,
141   adjacent farms, water management, and application of fertilizer. Questions derived from

142 Yaemkasem et al. (2017) included those on biosecurity measures, farm characteristics, stocking
143 density, feeding management, geographical factors, pond preparation, and water management.
144 Finally, questions based on our own experiences in the study area included those on modernizing
145 the water management system, the ponding system, factors related to weather and environment,
146 source of PL, vector control, disease status, and laboratory confirmation of WSD. Additionally,
147 the locations of the geographical centers of the farms were recorded and are illustrated in Fig. 1.
148

149 **Shrimp sampled status for WSD**
150 To confirm the status of WSD, we collected PL and shrimp samples twice from each
151 pond. First, PL was sampled before cultivating. In this study, only PL aged more than 10 days
152 was collected. At least 400 PL in batches of 50 were collected and tested for WSD using nested
153 polymerase chain reaction (PCR; OIE, 2009; OIE, 2019) at a local DoF laboratory or a certified
154 private laboratory by DoF. In addition, only half tail of PL was used for testing. Second, the
155 shrimp were sampled before harvesting. At least 60 shrimp in each pond were collected and were
156 tested using nested PCR. For testing, pleopod, gill, and hemolymph of shrimp were collected for
157 WSSV testing (OIE, 2009, 2019) at a local DoF laboratory. The number of samples collected
158 was according to the DoF requirement. A WSD-positive pond showed positive PCR results for
159 WSSV in PL and/or shrimp samples. In contrast, WSSV in PL and shrimp samples were absent
160 in ponds with negative PCR results.
161 For nested PCR, briefly, the pooled sampled of shrimp was homogenized in DNAzol®
162 reagent (Invitorgen, Thermo Fisher Scientific, Massachusetts, USA). The primers 146F1
163 (5'ACTACTAACTTCAG CCTATCTAG 3')/146R1 (5' TAATGCGGGTGTAAT
164 GTTCTTACGA3'), and 146F1/146R1 plus 146F2 (5' GTAACTGCCCCTTCCATCTCCA
165 3')/146R2 (5' TACGGCAGCTGCTGCACCTTGT 3') were respectively used for one-step and
166 two step PCR. The PCR products were visualized in 1.5% agarose gel electrophoresis containing
167 SYBR® Safe DNA gel stain (Invitorgen, Thermo Fisher Scientific, Massachusetts, USA). The
168 expected amplicon sizes were respectively 1447 and 941 bp, for first and nested step reactions
169 (OIE, 2009; OIE, 2019).
170

171 **Data analysis**
172 Univariate and multivariate analyses were performed using GEE with the R software
173 version 4.1.1 (R Core Team, 2021) and geepack package (Højsgaard, Halekoh & Yan, 2006).
174 This approach is a widely used statistical method in the analysis of longitudinal data. Here, the
175 variance function was used as a binomial family to fit the logistic model. A covariance matrix
176 was used as an exchangeable correlation structure. Through univariate analysis, possible risk
177 factors were calculated in succession as independent variables. The WSD status was set as the
178 dependent variable. Using multivariate analysis with backward elimination, the significant
179 variables with an alpha level of 1% from univariate analysis were input to the GEE model to
180 identify the association between possible risk factors and WSD. The final model reported

181   significant factors with an alpha level of 1%. In this step, we performed several analyses and
182   checked that each factor executed biological feasibility until a final model was obtained.
183
Results
General information
186   From the 270 responses, we found that most farms raised shrimp by the monoculture of
187   *Penaeus vannamei* (*P. Vannamei*). Most farms (217; 80.37%) had experience of over 10 years in
188   marine shrimp farming. All farms were operated by multiple workers, including owners. In
189   addition, 143 (52.96%) of owners have more than one farm. The number of crops per year
190   ranged between one to five crops. There were 126 (46.67%) and 123 (45.56%) farms that had
191   three and two crops per year, respectively. During the study, only four (1.48%) crops from two
192   (9.09%) farms were raised in a monoculture of *P. monodon*. We found that the PL source varied
193   from hatcheries within Rayong and neighboring provinces such as Trad, Chachoengsao, and
194   Chonburi. On average, the price per PL was 0.16 Baht (0.09–0.50 Baht; 1 Thai Baht is
195   approximately 0.03 US Dollar). Generally, most farmers raised PL at 12 days old (12–36 days).
196   For WSD status, 85 (31.48%) of crops from 269 farms were infected (Fig. 1). Only one farm
197   with nine crops did not have WSD during the study period. Example lesions of WSD in infected
198   shrimp and positive results of WSSV are shown in Fig. 2.
199
Univariate and multivariable analyses
201   After data management, 86 possible risk factors (categorical data = 71, continuous data =
202   15) were analyzed from univariate analysis using the GEE model. We found that 17 possible risk
203   factors were statistically significant and associated with WSD status (Table 1). Subsequently,
204   multivariate analysis was performed using the GEE model. We found that two independent
205   variables were statistically significant with biological sense, corresponding to WSD status. These
206   two categorical variables were: (1) inclement weather during ponding and (2) separation of the
207   ponds into a cultured pond, water treatment pond, and water reservoir for ponding.
208   From Table 2, we found that the absence of inclement weather during ponding was a
209   statistically significant factor associated with the occurrence of WSD. This factor prevents WSD
210   occurrence as the odds ratio (OR) was lesser than one (OR = 0.196, 99% confidence interval [CI]
211   of OR = 0.041–0.938, P = 1.3 x 10^{-5}). In contrast, inclement weather during ponding has been
212   identified as a risk factor for WSD by approximately five times (1/0.196 = 5.102) compared with
213   normal weather. Moreover, the separation of ponds into three specific types (cultured, water
214   treatment, and pond for water reservoir) was significantly associated with WSD occurrence.
215   Likewise, the presence of this ponding system was a protective factor for WSD (OR = 0.0828,
216   99% CI of OR = 0.0006–10.6688, P = 0.001). In contrast, other ponding systems have been
217   identified as a risk factor for WSD by approximately 12 times (1/0.0828 = 12.0773) compared
218   with pond separation into the three types described above.
219
Discussion

221   In this longitudinal study, we used GEE to analyze possible risk factors associated with
222 WSD from 85 infected crops and 185 non-infected crops in Rayong, Thailand. Additionally, we
223 found that the presence of inclement weather during ponding and the separation of ponds into a
224 cultured pond, water treatment pond, and pond for water reservoir were highly significant risk
225 factors corresponding to WSD occurrence.
226   In this study, most owners operated more than one farm and played an important role in
227 each ponding. We found that the same owner with different farms had WSD-infected and -non-
228 infected ponding in different crops. In addition, most farms were infected and non-infected in the
229 same pond at different times of ponding. This may indicate that WSD is not influenced by owner
230 activities. The results of univariate analysis were in agreement with this finding, but are
231 inconsistent with that of a previous study in Chanthaburi (a neighboring area of Rayong;
232 Piamsomboon, Inchaisri & Wongtavatchai, 2015). In this study, most of the farm owners
233 operated multiple farms. Of these, most managed the farms without infection of WSD. As such,
234 our univariate analysis results are not statistically significant. In the Chanthaburi study, most
235 owners operated a single farm. Theoretically, an area with a higher number of owners who
236 operate multiple farms should be at greater risk of disease. It is possible that there are additional
237 risk factors that were not measured in this study. Alternatively, we used a 1% chance of rejecting
238 the null hypothesis in the GEE model because we wanted to identify factors with a strong
239 association between independent and dependent variables; this may be why some factors (e.g.,
240 owners operating multiple farms) were dropped from our analysis. Another possible reason is
241 that the study area is major endemic area of WSD, and outbreaks spread rapidly. This could
242 influence owners with multiple farms to change their behavior to reduce the risk of disease
243 during an outbreak. Further study is needed to address each of these possibilities. Regardless,
244 owners and other workers need to be careful to prevent contamination or infection between
245 farming sites. Restrictions on biosecurity are a good way to control or limit WSD occurrence.
246 Furthermore, our results show that only one farm did not have WSD occurrence during our study
247 because this farm was operated with the highest biosecurity measures (complete closed-system
248 farming). However, notably, this farm had expensive biosecurity operations, which are difficult
249 to operate on general farms.
250   In the univariate analysis, we identified some significance factors with an alpha level of
251 1% that were inconsistent with the real world situation. For example, based on our repeated
252 calculation of thee GEE model, the source of PL (or the PL provider) had a negative value for
253 some sources. This suggests that the PL provider should have a preventive impact on the
254 occurrence of WSD, when in fact we actually identified a higher proportion of infections (data
255 not shown). Other confounding factors included the PL size and number of farms in a 1 km
256 radius of the studied farm. Therefore, we performed a backward elimination in which all non-
257 biological sense factors were removed from the final model. This ensured that our final model
258 had high accuracy.
259   The presence of inclement weather during ponding was a risk factor associated with
260 WSD (P = 1.3 x 10^{-5}), which is consistent with the study in Chanthaburi (Piamsomboon,

261 Inchaisri & Wongtavatchai, 2016) and with those in other countries (Hanson et al., 2008;
262 Tendencia et al., 2010). Millard et al. (2021) reported that abiotic factors (e.g., extreme weather
263 events) can increasee the severity of WSD in shrimp ponds without careful management.
264 Another study reported that climate change factors (e.g., increasing temperature and acidification
265 in marine systems) positively affects hosts' susceptibility to WSSV (Shields, 2019). Sun et al.
266 (2014) reported that WSSV overworked the cellular machinery used for its own propagation
267 after the culture temperature rose from 18°C to 25°C. However, this factor needs further
268 investigation. In our structured interview schedule we asked all respondents about extreme
269 inclement weather during each ponding to prevent error and uncertainty when an owner could
270 not decide when the weather was good or bad. In general, owners uses the principle of the
271 presence or absence of monsoon during ponding. Many factors related to climate, such as
272 atmospheric temperature, humidity, atmospheric pressure, wind speed, and amount of rainfall are
273 measured by relevant authorities, but the study region covers an extensive area. Although the
274 results of this study are sufficient to inform farmers and relevant authorities about the
275 relationship between WSD occurrence and inclement weather, we highly recommend that further
276 investigations be conducted on the weather at the pond level.
277   Some farmers raise shrimp by separating specific ponds during cultivation, resulting in a
278 protective factor (P = 0.001). In this system, for each crop, the farm comprises an intensive
279 grow-out pond, water treatment pond, and reservoir pond for adding water during cultivation,
280 which is often used to reduce the risk of WSD and contamination through carriers. This is a
281 closed system that has higher biosecurity than an open ponding system. According to a previous
282 study, in terms of operational cost, farmers can invest and profit in this system (Karim et al.,
283 2014). In other systems, most farmers have only cultured ponds and reservoirs. Water treatment
284 is performed during pond preparation. Generally, farmers need to add water during cultivation;
285 hence, they use water directly from natural reservoirs or reservoir ponds, increasing the risk of
286 WSD occurrence through untreated water. This result is consistent with our previous study,
287 indicating that water added without treatment during ponding is a major risk factor (Yaemkasem
288 et al., 2017). In contrast, a closed recirculation system can reduce the risk of WSD more than an
289 ordinary closed system; however, the operation of this system is more expensive. In our opinion,
290 water management using a closed system is sufficient to limit the occurrence of WSD. However,
291 the restrictions on biosecurity along with this system must be consistent.
292   In this study, the final model and univariate analysis using GEE showed a strong
293 relationship with an alpha level of 1% between the dependent and independent variables,
294 indicating that our analysis provides high-precision results. However, concerning the separation
295 of ponds, we found a high variation of 99% confidence interval of odds ratio (OR), indicating
296 that an uncertainty level is present around this factor. To prevent this possible error in the field,
297 farmers and relevant authorities need to focus on the water added during cultivation. Every time
298 the water is added, farmers must ensure the safety and efficiency of the treated water for WSSV.
299 The results of this study can be applied to other areas in which WSD is present; however, shrimp
300 farming in different geographic areas may have different geographical risk factors. We suggest

301   that longitudinal data specific to a given area are needed to prevent potential losses from the
302   outbreak of WSD.

303

304 **Conclusion**

305   This study showed two statistically significant factors associated with WSD occurrence
306 in Rayong, Thailand: inclement weather during ponding and separation of ponds during
307 cultivation. The findings of this study offer a reference for farmers and relevant authorities
308 focused on possible risk factors during the shrimp cultivation period. In addition, the results
309 could be advantageous in limiting WSD occurrence in Rayong, which is an endemic area for this
310 disease. Finally, the results of this study could be applied to other areas in which WSD is present.

311

312 **Conflicts of interest**

313   None.

314

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318

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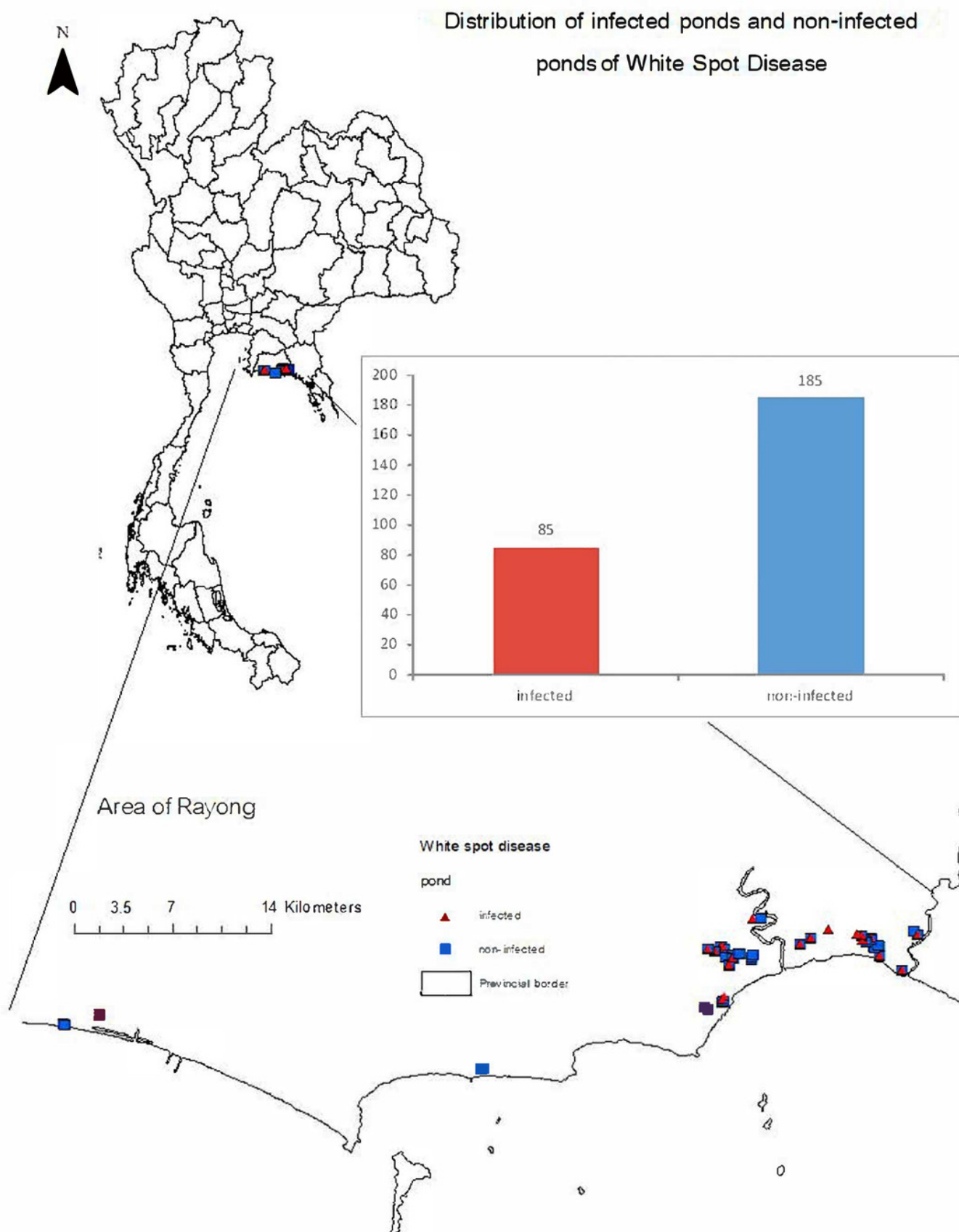
426   Figure 1. Distribution of infected (symbol: red triangle) and non-infected (symbol: blue square)
427   ponds associated with white spot disease (WSD) in Rayong, Thailand.
428   Figure 2. Comparison of (a) normal whiteleg shrimp and (b) infected whiteleg shrimp discolored
429   by white spot disease (WSD). (c) White spot lesions on carapace of shrimp. (d) Agarose
430   gel electrophoresis of positive white spot syndrome virus (WSSV).
431   Table 1. Univariate analysis of significant risk factors associated with white spot disease (WSD)
432   in marine shrimp farms in Rayong, Thailand.
433   Table 2. Significant risk factors associated with white spot disease (WSD) in marine shrimp
434   farms in Rayong, Thailand (n = 270), using multivariate analysis.

Figure 1

Distribution of infected (symbol: red triangle) and non-infected (symbol: blue square) ponds associated with white spot disease (WSD) in Rayong, Thailand.

Distribution of infected ponds and non-infected ponds of White Spot Disease

Area of Rayong

0   3.5   7   14 Kilometers

White spot disease

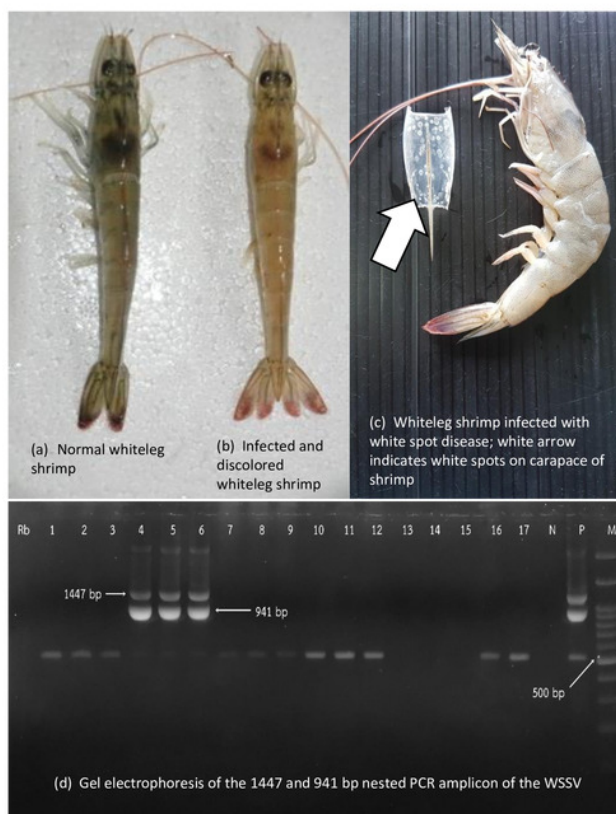
pond

▲ infected

■ non-infected

▢ Provincial border

Figure 2

Comparison of (a) normal whiteleg shrimp and (b) infected whiteleg shrimp discolored by white spot disease (WSD). (c) White spot lesions on carapace of shrimp. (d) Agarose gel electrophoresis of positive white spot syndrome virus (WSSV).

Univariate analysis of significant risk factors associated with white spot disease (WSD) in marine shrimp farms in Rayong, Thailand.

1 Table 1. Univariate analysis of significant risk factors associated with white spot disease (WSD) in marine shrimp farms in Rayong,
2 Thailand

Possible risk factors	Type of variable (number of factor levels)	Mean (SD)	Number of exposures without WSD infection	Number of exposures with WSD infected	P-value
Farm size (unit: acres)	Numeric	138 (252)	-	-	0.00072
A number of shrimp ponds	Numeric	13 (16)	-	-	0.0034
Source of post-larvae (source Z)	Categorical (21)		11	3	Reference
Source A			0	1	$< 2 \times 10^{-16}$
Source E			2	0	$< 2 \times 10^{-16}$
Source G			3	0	$< 2 \times 10^{-16}$
Source H			0	2	$< 2 \times 10^{-16}$
Source I			1	0	$< 2 \times 10^{-16}$
Source K			0	2	$< 2 \times 10^{-16}$
Source L			2	0	$< 2 \times 10^{-16}$
Source M			2	0	$< 2 \times 10^{-16}$
Source O			0	8	$< 2 \times 10^{-16}$
Source T			2	0	$< 2 \times 10^{-16}$
Size of post-larvae (larvae aged 12)	Categorical (6)		168	82	Reference
Larvae aged 15 days			9	0	$< 2 \times 10^{-16}$
Larvae aged 22 days			1	0	$< 2 \times 10^{-16}$
Larvae aged 25 days			1	2	$< 2 \times 10^{-16}$
Larvae aged 30 days			5	1	1.7×10^{-5}
Larvae aged 36 days			1	0	$< 2 \times 10^{-16}$
Number of farms within 1 kilometer radius from studied farm (one farm)	Categorical (5)		45	27	Reference
Four farms			13	1	0.0042
Five farms			0	1	$< 2 \times 10^{-16}$
Size of sludge pond (unit: acres)	Numeric	8.28 (16.7)	-	-	0.0042
Inclement weather during ponding (found)	Categories (2)		93	68	Reference

Not found		92	17	7.3×10^{-5}
Usage of formalin during ponding (used)	Categories (2)	176	83	Reference
Not used		9	2	$< 2 \times 10^{-16}$
Usage of benzalkonium chloride during ponding (used)	Categories (2)	180	85	Reference
Not used		5	0	$< 2 \times 10^{-16}$
Usage of chemical fertilizers during ponding (not used)	Categories (2)	175	83	Reference
Used		10	2	0.0015
Separation of water reservoir, usage of potassium permanganate, cultured pond and water treatment for ponding (not used)	Categories (2)	182	81	Reference
Used		3	4	5.6×10^{-16}
Separation of cultured pond, water treatment pond and water reservoir for ponding (not used)	Categories (2)	160	83	Reference
Used		25	2	0.0075
Added water during ponding (added)	Categories (2)	170	61	Reference
Not added		15	24	0.0079
Cultivated water treatment before released into public resource (treated)	Categories (2)	175	60	Reference
Not treated		10	25	0.0078
Usage of chlorine during ponding (not used)	Categories (2)	185	82	Reference
Used		0	3	$< 2 \times 10^{-16}$
Usage of trichlorfon during ponding (not used)	Categories (2)	183	64	Reference
Used		2	21	0.00052
Partial reusing of water for ponding (not reused)	Categories (2)	124	74	Reference
Reused		61	11	0.0014

3   Note: P-value reported as $< 2 \times 10^{-16}$ when the P-value produced by the software is too small to be significant.

Significant risk factors associated with white spot disease (WSD) in marine shrimp farms in Rayong, Thailand (n = 270), using multivariate analysis.

1 Table 2. Significant risk factors associated with white spot disease (WSD) in marine shrimp farms in Rayong, Thailand (n = 270),
2 using multivariate analysis

Factors (type of variable/number of factor levels) and significance factor level	Number of exposures without WSD infection	Number of exposures with WSD infected	Estimate	SE	OR	99% CI of OR	P-value
Intercept	-	-	0.0411	0.2778	-	-	0.882
Inclement weather during ponding (categories/2)							
Found	93	68	-	-	-	-	Reference
Not found	92	17	−1.6286	0.373	0.196	0.041–0.938	1.3 x 10^{-5}
Separation of cultured pond, water treatment pond and water reservoir for ponding (categories/2)							
No	160	83	-	-	-	-	Reference
Yes	25	2	−2.4916	0.7559	0.0828	0.0006–10.6688	0.001

3 SE = standard error, OR = odds ratio, 99% CI of OR = 99% confidence interval of odds ratio. Degrees of freedom is 267.