Aquaculture 318 (2011) 277–282

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Longitudinal disease studies in small-holder black tiger shrimp (*Penaeus monodon*) farms in Andhra Pradesh, India. I. High prevalence of WSSV infection and low incidence of disease outbreaks in BMP ponds

Peter J. Walker [a,*], Nicholas Gudkovs [a], P.A. Padiyar [b], V. Stalin Raj [c], Balakrishnan Pradeep [d], Evan Sergeant [e], A.B. Chandra Mohan [f], G. Ravibabu [f], K.K. Vijayan [g], Indrani Karunasagar [d], T.C. Santiago [c], C.V. Mohan [b]

[a] CSIRO Livestock Industries, Australian Animal Health Laboratory, 5 Portarlington Road, Geelong, Victoria 3220, Australia
[b] Network of Aquaculture Centers Asia-Pacific, Suraswadi Building, Kasertsart University Campus, Ladyao, Jatujak, Bangkok, 10900, Thailand
[c] Central Institute for Brackishwater Aquaculture, 75 Santholme High Road, Chennai, 600028, Tamil Nadu, India
[d] Department of Microbiology, College of Fisheries, Karnataka Veterinary, Animal and Fisheries Sciences University, Mangalore, Karnataka 575 002, India
[e] AusVet Animal Health Services, Post Office Box 3180, South Brisbane Queensland 4101, Australia
[f] National Centre for Sustainable Aquaculture, 69-17-8, SBI Officers Colony, Rajendra Nagar, Kakinada 533003, Andhra Pradesh, India
[g] Central Marine Fisheries Research Institute, Post Box 1603, Ernakulam North, Kochi, Kerala, 682018, India

ARTICLE INFO

Article history:
Received 7 May 2011
Accepted 12 May 2011
Available online 19 May 2011

Keywords:
Shrimp
Penaeus monodon
Disease
White spot syndrome virus

ABSTRACT

A longitudinal study was conducted from January to August 2005 in small-holder black tiger shrimp (*Penaeus monodon*) ponds in the West Godavari District of Andhra Pradesh, India (16°25′ N, 81°19′ E). The study involved 457 ponds owned by low-income farmers participating in a better management practice (BMP) programme. Disease outbreaks occurred in 16.6% of ponds. There was significant spatial clustering of disease outbreaks with 31 (40.8%) of the 76 recorded disease outbreaks occurring in a single village block. Bivariate analysis indicated a 1.6-fold higher likelihood of disease outbreaks from nursery-stocked ponds but this was not significant in multivariate analysis due to the confounding effect of pond location. There was evidence of increasing prevalence of WSSV infection during grow-out. WSSV was detected in 5.9% of 119 batches of postlarvae tested at stocking, 38.2% of 34 juvenile batches collected at the time of transfer to grow-out ponds, and 47.0% of 336 pond stock tested at normal harvest or crop failure. WSSV was detected in 43 of 59 (72.9%) disease outbreak ponds tested and 115 of 277 (41.5%) non-outbreak ponds tested. Heavy WSSV infection was detected at harvest in 116 of the 336 (34.5%) of the ponds tested, including 78 ponds for which no outbreak was recorded. Duration of crop was recorded for 431 ponds with a mean of 117.0 days and a range of 20 to 176 days. Median duration was significantly shorter for disease outbreak ponds (68.5 days) compared to non-outbreak ponds (119.0 days). Duration of crop also varied according to WSSV detection levels at harvest, with median duration for ponds classified as heavy WSSV infection (108.5 days) significantly shorter than for ponds classified as either light WSSV infection (116.0 days) or WSSV-negative (116.5 days). The study indicated a high risk of WSSV infection during grow-out but a relatively low incidence of disease despite a high prevalence of heavy WSSV infection in non-outbreak ponds.

Crown Copyright © 2011 Published by Elsevier B.V. All rights reserved.

1. Introduction

Since the emergence of white spot syndrome virus (WSSV) in the early 1990s, disease has been a major obstacle to the sustainability and profitability of black tiger shrimp (*Penaeus monodon*) production in Asia (Bondad-Reantaso et al., 2005; Subasinghe and Phillips, 2002; Walker and Winton, 2010). As there are no effective vaccines or treatments, health management strategies are based primarily on risk reduction through pathogen exclusion, avoidance of stress and early detection and response to disease (Walker and Mohan, 2009). These strategies have been relatively effective in larger semi-intensive farming systems and the use of domesticated specific-pathogen-free (SPF) shrimp stock, primarily of the introduced Pacific white shrimp (*Litopenaeus vannamei*) has significantly improved the reliability of production (Lightner, 2005). However, for low-income, small-holder farmers who continue to cultivate the native black tiger shrimp produced from captured broodstock, there remains a very high risk of disease and associated poor yield and crop failure (Walker and Mohan, 2009). Improved health management for small-holder farmers in India and other Asian countries is being addressed through better management practice (BMP) programmes that provide recommended farming practices from pre-stocking to

* Corresponding author. Tel.: +61 3 5227 5400; fax: +61 3 5227 5000.
E-mail address: Peter.Walker@csiro.au (P.J. Walker).

0044-8486/$ – see front matter. Crown Copyright © 2011 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.05.029

harvest based on epidemiological studies to identify disease risk factors, experimental data, empirical observations and successful industry practices (Mohan et al., 2008; MPEDA/NACA, 2003; Padiyar, 2009; Subasinghe, 2005; Umesh et al., 2010). The use of PCR tests to eliminate WSSV-infected seed is a key BMP recommendation as there is evidence that PCR screening can significantly reduce risks of white spot disease during grow-out (Chanratchakool and Limsuwan, 1998; Peng et al., 2001; Withyachumnarnkul, 1999). PCR screening is usually conducted in hatcheries or as a service to farmers provided by local private sector or government laboratories but there is little or no regulation of this service and the effectiveness of testing for low-income small-holder farmers has not been adequately assessed.

Another strategy that is commonly employed by farmers to improve survival of shrimp during grow-out is the use of nursery ponds (Kungvankij et al., 1986; Lim, 1998). The nursery phase involves the culture of shrimp postlarvae in relatively high densities in earthen ponds prior to transfer to grow-out ponds. The use of nursery ponds has been promoted as a means of limiting loss due to predators, improving disease risk management and ensuring that only the strongest shrimp are selected for grow-out (Samocha and Lawrence, 1992). This commonly involves farmer-shared or commercial nurseries, some of which supply a large number of grow-out ponds.

In this paper, we report a longitudinal study conducted in BMP ponds in Andhra Pradesh, India, during January to August 2005 to determine the change in WSSV-infection status of stock from stocking to harvest, the association between WSSV infection and disease outbreaks in ponds, and the possible role of acclimation in nursery ponds in the amplification of disease.

2. Materials and methods

2.1. Study site and stocking procedure

The study involved a total of 457 ponds from 28 clusters in 15 villages in an area of 250 km^2 (16°25′ N, 81°19′E) on the western side of the Godavari River between Bhimavaram and the Bay of Bengal, in Andhra Pradesh, India (Fig. 1). The total pond area was approximately 250 ha. All ponds were owned by small-holder farmers participating in a BMP programme that was facilitated by extension officers of the Marine Products Export Development Authority (MPEDA) with assistance from the Network of Aquaculture Centers Asia-Pacific (NACA). Recommended procedures for pond preparation, stocking and grow-out employed in the BMP programme have been described (MPEDA/NACA, 2003; Padiyar, 2009). All 457 ponds involved in this study were stocked in early 2005 with PCR-screened postlarvae (PLs) that were obtained from local hatcheries; of these, 235 (51.4%) were stocked via nursery ponds and the remaining 222 (48.6%) were stocked directly from hatcheries. In total, 48 nursery ponds were employed, some of which were also subsequently used as grow-out ponds. Each nursery pond stocked between 1 and 32 study ponds with an average of ~5 grow-out ponds per nursery pond. Six nursery ponds each delivered seed to more than 10 grow-out ponds.

2.2. Shrimp sample and pond data collection

Samples of shrimp postlarvae (600 pooled PLs/pond) were collected from nursery ponds or grow-out ponds prior to stocking. Juvenile shrimp (20 shrimp per pond) were collected from nursery ponds at the time of transfer to grow-out ponds after approximately 14–16 days of culture. Shrimp samples (10 shrimp per pond) were also collected from grow-out ponds at the time of disease outbreaks, emergency harvests or planned harvests. For each juvenile or sub-adult shrimp, two pleopods were collected and stored separately as A and B replicates. All samples were collected in preservative (80% ethanol/20% glycerol) and stored at room temperature until required for PCR testing. In addition, shrimp samples (5 whole moribund shrimp and 2 clinically normal shrimp per pond) were collected from disease outbreak ponds for histological examination. Samples were collected in 10% neutral buffered formalin and stored at room temperature. Details of stocking dates, duration of the crop, yields,

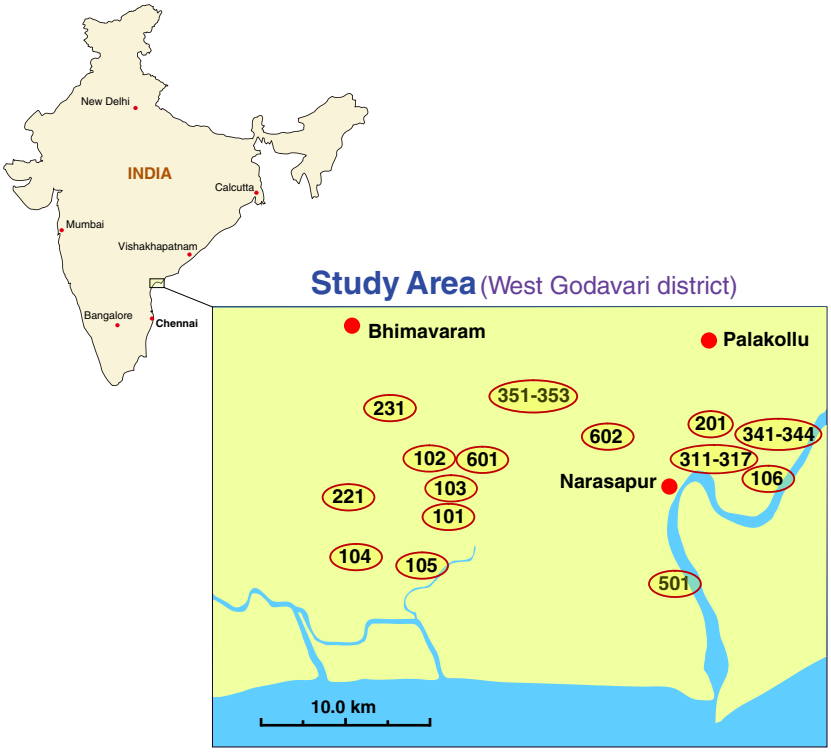

Fig. 1. Map illustrating the location the study site in the West Godavari District of Andhra Pradesh, India. The locations of 26 pond blocks owned by participating shrimp farmers are shown.

disease outbreaks, survival rates and feed conversion ratios were recorded for each pond.

2.3. PCR testing and histology

PCR testing of preserved shrimp tissue for WSSV was conducted using the IQ2000 WSSV PCR Kit (Farming IntelliGene Technology Corporation, Taiwan) according to the manufacturer's instructions and using the supplied DNA lysis buffer. Typically, pools of 30 PLs or pools of 5 pleopods from larger animals were used for DNA extraction. Because of the large number of samples, preliminary screening for WSSV was divided between laboratories at the Central Institute of Brackishwater Aquaculture (CIBA), Chennai and the Department of Fishery Microbiology, College of Fisheries, Karnataka Veterinary Animal and Fisheries Sciences University, Mangalore. WSSV infections were graded as very weak, weak, moderate or strong positive by using the banding pattern of PCR products according to the manufacturer's instructions.

Shrimp tissue fixed in 10% neutral buffered formalin was transferred to 70% isopropanol for subsequent processing by conventional histological techniques (Bell and Lightner, 1988). Sections (5–6 μm) were stained with hematoxylin and eosin, and examined using a Carl Zeiss Axiostar Plus microscope fitted with a ProgRess C-10 *Plus* CCD camera. Gill and cuticular epithelial cells were examined for histological lesions pathognomonic of WSSV infection including hypertrophied nuclei with basophilic inclusions.

2.4. Definition of a disease outbreak

For the purpose of this study, a disease outbreak was defined as: i) an abnormal reduction in feed consumption and increase in the number of shrimp with abnormal swimming behaviour and/or appearance; ii) an unusual increase in the number of dead or moribund shrimp at pond edges; or iii) a farmer-initiated emergency harvest (Padiyar, 2009).

2.5. Statistical analysis

Differences in outbreak incidence between groups were compared using relative risk (the ratio of cumulative incidences in two risk groups being compared). Statistical significance of apparent associations was evaluated by Yates' Corrected Chi-squared test, except where indicated otherwise. Differences in crop duration were compared using the Mann–Whitney U for two groups and the Kruskal–Wallis test for more than two groups. Logistic regression analysis was used to further investigate the associations between ponds and disease outbreak occurrence. All statistical analyses were undertaken in the R statistical environment (version 2.7.1, © 2008 The R foundation for statistical computing). Statistical significance was defined as $P<0.05$.

3. Results

3.1. Association of disease outbreaks with location and use of nursery ponds

Disease outbreaks were recorded in 76 (16.6%) of the 457 ponds in the study (Table 1). Outbreaks were recorded in 28 (12.6%) of the 222 ponds that were stocked directly and 48 (20.4%) of the 235 nursery-stocked ponds, corresponding to a relative risk of outbreaks in nursery-stocked ponds of 1.6 compared to direct-stocked ponds ($P=0.034$). Outbreak incidence also varied markedly between pond block locations (villages), ranging from 0% to 69.2% of ponds affected for locations with ten or more participating ponds (Table 2). However, 31 of the 76 disease outbreaks (40.8%) occurred in one village block (101) and 74 of the 76 study ponds in this village were stocked from nine nursery ponds. Of the 28 disease outbreak ponds stocked directly from hatcheries, 17 (60.7%) were in three pond block locations (201, 223 and 311) on the eastern side of the study site (Table 2; Fig. 1).

For the logistic regression analysis, outbreak occurrence was the dependent variable and pond location (dichotomised as Block 101 and all other blocks) and stock source (nursery or direct stocked) were the independent variables. Pond location was dichotomised because the aim of the analysis was to determine whether the relationship between nursery ponds and disease outbreaks was confounded by the very high proportion of affected nursery ponds in Block 101. In this analysis, there was a highly significant association between pond location and occurrence of outbreaks (odds ratio = 5.4; $P<0.001$), whereas the apparent bivariate association between use of nursery ponds and disease outbreaks was no longer significant (odds ratio = 0.88; $P=0.69$).

3.2. Association of WSSV infection and disease outbreaks

WSSV testing was conducted by PCR on 119 batches of post-larvae collected from nursery or grow-out ponds at the time of stocking, juveniles from 34 nursery ponds or directly stocked ponds following the ~2-week period of grow-out. PCR and/or histology were also conducted on shrimp from 336 ponds at the time of disease outbreaks, emergency harvest or planned harvest. For simplicity, and to improve power of the analysis, the test results were collapsed into three categories: WSSV-negative (negative by PCR); light WSSV infection (weak positive reaction by PCR); and heavy WSSV infection (moderate or strong positive reaction by primary PCR and/or positive by histology). As shown in Table 3, there was a progressive increase in both the proportion of WSSV-positive samples and the proportion of samples graded as heavy WSSV infections as the crop progressed.

Of the 119 batches of postlarvae, 37 were obtained from nursery ponds and 82 from directly stocked grow-out ponds (Table 3). Only seven of 119 batches (5.9%) tested positive for WSSV, of which five were graded as light infections and two were graded as heavy. One batch graded as a light infection was used to seed a nursery pond for

Table 1
Performance of grow-out ponds according to use of nursery ponds.

Pond category	Total ponds	Disease outbreak ponds (%)	Total tested for WSSV	Total WSSV-positive (%)	Heavy WSSV-positive (%)	Ponds DOC[a] recorded	Mean DOC (days)	Median DOC (days)
All ponds	457	76 (16.6%)	336	158 (47.0%)	116 (34.5%)	431	111.2	117.0
All directly stocked ponds	222	28 (12.6%)	147	72 (49.0%)	49 (33.3%)	198	114.8	119.5
All nursery-supplied ponds	235	48 (20.4%)	189	86 (45.5%)	67 (35.5%)	233	108.2	116.0
All disease outbreak ponds	76	76 (100%)	59	43 (72.9%)	38 (64.4%)	76	72.7	68.5
All non-disease outbreak ponds	381	0 (0.0%)	277	115 (41.5%)	78 (28.1%)	355	118.7	119.0

[a] Duration of crop.

Table 2
Pond performance by location for pond blocks with 10 or more ponds.

Location code	Pond block	Total ponds	Disease outbreak ponds (%)	Nursery-stocked		Directly stocked		Mean DOC	Median DOC
				Total ponds	Disease outbreak ponds	Total ponds	Disease outbreak ponds		
101	Mogultur	76	31 (40.8%)	74	31	2	0	96.1	118.5
103	Kota	15	1 (6.7%)	14	1	1	0	115.9	112.0
105	Zilleditippa	10	0 (0.0%)	8	0	2	0	115.4	121.0
201	Dharbharevu	13	9 (69.2%)	3	2	10	7	75.9	67.0
223	Chinamamidipalli C	13	4 (30.8%)	0	0	13	4	110.2	121.0
311	Badava A	28	9 (32.1%)	14	3	14	6	109.0	110.5
312	Badava B	25	0 (0.0%)	11	0	14	0	115.3	114.5
313	Badava C	32	1 (3.1%)	19	0	13	1	110.3	113.5
314	Badava D	26	1 (3.8%)	14	0	12	1	111.1	113.0
315	Badava E	22	3 (13.6%)	7	2	15	1	123.5	124.5
316	Badava F	20	0 (0.0%)	14	0	6	0	112.3	110.5
317	Badava G	21	3 (14.3%)	10	2	11	1	114.2	119.0
341	YVL Society A	15	1 (6.7%)	3	0	12	1	116.4	118.5
342	YVL Society B	18	2 (11.1%)	0	0	18	2	112.4	113.0
343	YVL Society C	20	0 (0.0%)	2	0	18	0	120.8	124.0
344	YVL Society D	11	1 (9.1%)	1	0	10	1	112.6	111.0
351	YV Lanka A	12	0 (0.0)	1	0	11	0	127.3	127.5
352	YV Lanka B	25	1 (4.0%)	6	0	19	1	135.5	136.0
501	Matsyapuri	10	0 (0.0%)	10	0	0	0	140.6	138.5
602	Tundurra	19	5 (26.3%)	14	5	5	0	104.5	112.0
Total		430	72 (16.7%)	224	46	206	26	111.0	117.0
Other locations		27	4 (14.8%)	11	2	16	2	115.5	118.0
All locations		457	76 (16.6%)	235	48	222	28	112.2	117.0

which no subsequent data was recorded. The remaining six positive batches directly seeded grow-out ponds of which two were amongst ten ponds that developed disease outbreaks (20.0%) and four were amongst 72 ponds that did not record disease outbreaks (5.6%). The data indicated that ponds seeded with WSSV-positive post-larvae were 2.8 times more likely to develop a disease outbreak (95% CI: 0.77–10.45), with two of six (33%) ponds seeded from positive batches developing disease, compared to eight of 68 (11.7%) ponds seeded from negative batches. However, this result was not statistically significant (Fisher's exact test, $P = 0.18$).

Of the 34 batches of juveniles, 20 were collected from nurseries and 14 were from directly stocked grow-out ponds (Table 3). A total of 13 of the 34 juvenile batches (38.2%) tested positive for WSSV, comprising 11 (65.0%) nursery-sourced batches and two (14.3%) pond-sourced batches. Nursery-sourced juveniles were 3.9 times more likely to be infected than those in directly stocked ponds (95% CI: 1.01–14.75, $P = 0.04$). No disease outbreaks were subsequently recorded in the 14 directly stocked grow-out ponds. Disease outbreak data was recorded for one or more ponds stocked from 15 of the 20 nurseries. These comprised nine nurseries from which the juveniles tested WSSV-positive and six nurseries for which the juveniles tested WSSV-negative. There was no significant correlation between disease outcome in ponds and the WSSV infection status of juveniles.

Of the 336 shrimp samples collected during disease outbreaks and/or during planned or emergency harvests, 189 were from nursery-stocked ponds and 147 were from directly stocked ponds (Table 3). There was no association between the occurrence of a disease outbreak and whether a pond was tested ($P = 0.37$). However, ponds stocked from nurseries were 20% more likely to be tested than directly stocked ponds ($P < 0.001$).

Table 3
WSSV testing of shrimp at different crop stages.

Production stage	Total samples	Total WSSV-positive (%)	Heavy WSSV-positive (%)	Light-WSSV positive (%)
Postlarvae	119	7 (5.9%)	2 (1.7%)	5 (4.2%)
Juveniles	34	13 (38.2%)	3 (8.8%)	10 (29.4%)
Disease outbreak or harvest	336	158 (47.0%)	116 (34.5%)	42 (12.5%)

There was evidence of WSSV infection at the time of harvest or crop failure in 158 (47.0%) of the ponds tested of which 116 (34.5%) were graded as heavy infections (Table 1). WSSV was detected in 43 of 59 (72.9%) disease outbreak ponds tested. There was evidence of heavy WSSV infection at the time of harvest in further 78 ponds that were not recorded as disease outbreak ponds. After collapsing the data into three WSSV infection categories (heavy, light and negative), there was a significant association between infection level and occurrence of disease outbreaks (Table 4). Disease outbreaks occurred in 38 of 116 ponds (32.8%) in which heavy WSSV infection was detected, five of 42 ponds (11.9%) in which light WSSV infection was detected, and 16 of 178 ponds (9.0%) that tested negative for WSSV. Ponds in which heavy WSSV infection was detected were 2.8 times more likely to have an outbreak than ponds classed as light WSSV infection and 3.6 times more likely than ponds classified as negative for WSSV ($P < 0.001$). However, there was evidence of WSSV infection in 115 of 277 ponds in which disease outbreaks were not recorded (Table 1). Heavy WSSV infections were detected in 38 of 59 outbreak ponds (64.4%), compared to 78 of 277 (28.2%) non-outbreak ponds.

3.3. Association of crop duration with disease outbreaks and WSSV infection

Crop duration was recorded for 431 ponds, with a range of 20 to 176 days (mean 111.2 days, median 117.0 days) (Table 1). Crop duration of 120 days or longer was recorded for 189 ponds (44.9%), only eight of which (4.2%) reported disease outbreaks. In contrast, 58

Table 4
Performance of grow-out ponds according to WSSV infection status of shrimp at harvest.

WSSV infection status	All ponds			
	WSSV	Disease outbreaks (%)	Mean DOC	Median DOC
Heavy	116	38 (32.8%)	101.3	108.5
Light	42	5 (11.9%)	112.3	116.0
Negative	178	16 (9.0%)	112.7	116.5
Total tested	336	59	110.1	116.0
Not tested	121	17	114.9	122.0
Total	457	76	111.2	117.0

ponds (13.5%) had duration of less than 90 days, 53 of which (91.4%) reported disease outbreaks. No crop duration data were recorded for 26 ponds but three of these were sampled as emergency harvest or disease outbreak ponds and six were sampled as planned harvest ponds.

Median duration was significantly shorter ($P<0.001$) for disease outbreak ponds (68.5 days) compared to non-outbreak ponds (119.0 days), and was marginally shorter ($P=0.003$) for nursery-stocked ponds (116.0 days) compared to those that were stocked directly (119.5 days) (Table 1). Crop duration of ponds also varied depending on WSSV infection level at harvest, with median duration for ponds classified as heavy WSSV infection (108.5 days) significantly shorter than for ponds classified as either light WSSV infection (116.0 days) or WSSV-negative (116.5 days) ($P<0.001$) (Table 4).

Crop duration also varied significantly between locations ($P<0.001$), with the two worst affected locations, 201 (69.2% of ponds affected) and 101 (40.8% of ponds affected), having mean durations of 75.9 and 96.1 days, respectively (Table 2). Mean duration for other locations ranged from 109.0 to 140.6 days. At location 101, the mean crop duration (96.1 days) was significantly lower than the median duration (118.5 days).

4. Discussion

This study was undertaken to improve understanding of the disease risks associated with WSSV infection in a selection of small-holder BMP shrimp farms in the West Godavari District of Andhra Pradesh. Shrimp farming in India is undertaken primarily by small-holder farmers, with 90% of the total area utilised for shrimp culture comprising farms of less than 2 ha, contributing approximately 80% of total shrimp production (Umesh et al., 2010). Organised farmer groups ('aquaclubs'), employing BMPs-based on a disease risk factor study, were initiated in the West Godavari District in 2002 with 10 demonstration ponds, extended in 2003 to 108 ponds one village, subsequently leading to adoption in 2007–08 by over 5600 farmers across five states of India (Umesh et al., 2010). The BMP programme has led to significant gains in the economic sustainability of small-scale farming through reduced costs and reduced disease risks. However, disease has continued to impact on production from BMP ponds and has remained a major issue for the many small-scale farmers not enrolled in the BMP programme.

The study identified a strong association between the location of pond blocks and both the risk of disease and the mean duration of crop. This was primarily due to one location (Mogultur — block 101) which included 17% of all ponds and 41% of outbreak ponds. The reason for the higher outbreak incidence in Mogultur could not be determined in this study. One distinguishing feature of Mogultur was that all except two of the study ponds were stocked from nurseries, suggesting that the nursery ponds might have been the source. However, this was not supported by observations at other locations. Although other factors may have contributed to the high incidence of disease in Mogultur, there was no indication of a lower level of adoption of BMPs than in other locations and there were few identifiable differences in geography, structure of the farms or water management that could account for the high disease prevalence. It was observed that, to avoid the early monsoon season, Mogultur stocked one month earlier than some areas with lower disease prevalence (e.g., Bavada and YV Lanka pond blocks). It was also observed that ponds in Mogultur and parts of Kota and Tundurra drew water from a single source (Gontheru Creek). However, the disease incidence and mean duration of crop varied widely between these blocks. Therefore, although the widespread use of commercial nurseries was the most distinguishing characteristic of Mogultur, we were unable to determine the cause of the increased outbreak incidence in at this location. The apparent increased risk for nursery-stocked ponds compared to direct-stocked ponds in the bivariate analyses was not evident in the multivariate analysis due to confounding by the high incidence observed in Mogultur ponds.

WSSV is a major cause of crop loss during grow-out in shrimp ponds in India and elsewhere in Asia, and WSSV-infected seed has been identified as a common source of infection and disease (Flegel and Alday-Sanz, 1998; Lo et al., 1996; Shankar and Mohan, 1998). PCR screening to eliminate WSSV-infected seed can be effective in reducing disease risks during grow-out (Chanratchakool and Limsuwan, 1998; Peng et al., 2001; Withyachumnarnkul, 1999) and the BMP programme in which the farmers in this study participated recommends PCR screening prior to stocking (MPEDA/NACA, 2003; Padiyar, 2009). Subsequent PCR testing of postlarvae collected by the study team at the time of stocking confirmed that the prevalence of WSSV infection was relatively low (5.9%) and, of the seven WSSV-positive batches detected by nested PCR, five were graded as weak or very weak reactions. As a previous study in this region of Andhra Pradesh had indicated a high prevalence of WSSV infection in unscreened seed batches (Padiyar, 2009), this analysis suggests that there was a good level of compliance by farmers in obtaining PCR-screened seed and a good level of proficiency in PCR testing laboratories. However, as the postlarval samples collected by the study team were preserved in alcohol fixative and stored for a significant period prior to testing, some loss in detection sensitivity may have occurred and so the true prevalence may have been somewhat higher. Nevertheless, the prevalence of WSSV infection in juvenile and sub-adult shrimp samples preserved and stored under similar conditions was relatively high. Therefore, the observed increase in both WSSV prevalence and viral load from stocking to harvest appears to indicate exposure to WSSV infection during grow-out and the relatively high prevalence (38.2%) in juveniles collected after only ~2 weeks of culture suggests that exposure to WSSV infection commenced very early in the crop. It is also possible that some postlarval batches may have been infected with WSSV at levels below the sensitivity of the PCR test and the increase in detected prevalence in juveniles was due to a subsequent amplification of viral loads. Clearly, the increase in the prevalence of heavy WSSV infection from the juvenile stage (8.8%) through disease outbreak or harvest (34.5%) is indicative of ongoing amplification of viral loads as the crop progressed and the associated risk of the onset of white spot disease.

The data also indicated that, although there was a high prevalence of WSSV infection in shrimp collected during disease outbreaks or at harvest, WSSV was not the only cause of disease in study ponds. Heavy WSSV infection, which may be indicative of a causal association, was detected in only 64.4% of outbreak ponds and it is likely that other viral, bacterial, fungal or parasitic diseases may have contributed to losses. It is also likely that poor survival in some ponds was due to poor water quality and or unsuitable pond management practices. However, as disease outbreaks occurred in only 32.8% of ponds in which heavy WSSV infection was detected, on the whole, good pond management practices promulgated by the BMP programme may have contributed to successful crop outcomes despite progressively increasing WSSV prevalence and viral load. It is also of interest that there was no significant difference in the incidence of disease outbreaks or the duration of crop between ponds that tested negative for WSSV and those in which light WSSV infections were detected. A study conducted in non-BMP small-holder shrimp (*P. monodon*) ponds in Karnataka on the West Coast of India from September 1999 to April 2000 reported a significant negative correlation between the detection of WSSV in ponds and the duration of crop, and an association between moderate or heavy levels of WSSV detected in ponds and the yield per hectare and survival rate, suggesting that examination of the white spot disease status at mid-crop could assist decision-making by farmers (Mohan et al., 2002; Sahoo et al., 2010). As we have observed disease outbreaks in only 32.8% of ponds in which heavy WSSV infection was detected, we suggest caution in the use of this approach, particularly in BMP ponds.

The potential risks associated with use of commercial nurseries are recognised in contemporary BMP programmes which recommend the use of in-pond, farmer-operated nurseries for the initial stages of grow-out (ADB/ACIAR/AwF/BRR/DKP/FAO/GTZ/MMAF/NACA/WWF, 2007; Mohan et al., 2008; MPEDA/NACA, 2003; Padiyar, 2009). In this study, only ten in-pond nurseries were employed, each of which supplied juveniles to a maximum of three other nearby ponds. Disease outbreaks were recorded in only two of a total of 26 ponds (7.7%) supplied from these in-pond nurseries and heavy WSSV infection was detected in six of 15 ponds tested (40.0%), including the two disease outbreak ponds. These outcomes were similar to those of other nursery-stocked ponds (excluding ponds from Mogultur), in which 17 of 162 (10.5%) ponds experienced outbreaks. However, the crop duration for the 26 ponds (mean = 126.0 days; median = 131.5 days) was significantly higher than any other pond category analysed. This suggests that in-pond nurseries may offer significant advantages, particularly if used as recommended to stock only single ponds. However, the number of ponds involved here was relatively small and other factors, such as careful pond management by this group of farmers may have contributed to achieving a long duration of crop. Nevertheless, the results are encouraging and further studies should be conducted to assess the merits of in-pond nurseries in a farming area where they are more commonly used.

This study identified significant spatial clustering of disease outbreaks in study ponds, although the reason for this clustering could not be determined. It also demonstrated that, although there was a high prevalence of WSSV in outbreak ponds, this was not the only cause of disease as some affected ponds remained WSSV negative. Conversely, not all ponds with evidence of high WSSV infection levels experienced outbreaks, suggesting that the BMP measures implemented in study ponds may be alleviating the impact of infection in some instances.

Acknowledgements

This work was conducted as part of an international cooperative project sponsored by the Australian Centre for International Agricultural Research and the Marine Products Export Development Authority (MPEDA), Government of India. We would like to thank Dr. Jeff Cowley and Dr. Peter Durr of CSIRO Livestock Industries for critical review of the manuscript. We would also particularly like to thank MPEDA Director, Mr. B. Vishnu Bhat, for his strong support and encouragement and Dr. Michael Philips of NACA for his advice during the developmental stages of the project.

References

ADB/ACIAR/AwF/BRR/DKP/FAO/GTZ/MMAF/NACA/WWF, 2007. Practical Manual on Better Management Practices for Tambak Farming in Aceh. Asian Development Bank ETESP, Australian Centre for International Agricultural Research, Food and Agricultural Organization of the United Nations, International Finance Corporation of the World Bank Group, Banda Aceh.

Bell, T.A., Lightner, D.V., 1988. A Handbook of Normal Penaeid Shrimp Histology. World Aquaculture Society, Baton Rouge, La. v, 114 p.

Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z., Shariff, M., 2005. Disease and health management in Asian aquaculture. Veterinary Parasitology 132, 249–272.

Chanratchakool, P., Limsuwan, C., 1998. Application of PCR and formalin treatment to prevent white spot disease in shrimp. In: Flegel, T.W. (Ed.), Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok, pp. 287–289.

Flegel, T.W., Alday-Sanz, V., 1998. The crisis in Asian shrimp aquaculture: current status and future needs. Journal of Applied Ichthyology-Zeitschrift Fur Angewandte Ichthyologie 14, 269–273.

Kungvankij, P., Chua, T.-E., Pudadera, B.J., Corre, K.G., Borlongan, E., 1986. Shrimp culture: pond design, operation and management. FAO Training Series No. 2. FAO, Rome.

Lightner, D.V., 2005. Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. Journal of the World Aquaculture Society 36, 229–248.

Lim, C.E., 1998. Feeding penaeid shrimp, In: Lovell, T. (Ed.), Nutrition and Feeding of Fish, Second ed. Kluwer Acanemic Publishers, Boston, pp. 227–246.

Lo, C.F., Leu, J.H., Ho, C.H., Chen, C.H., Peng, S.E., Chen, Y.T., Chou, C.M., Yeh, P.Y., Huang, C.J., Chou, H.Y., Wang, C.H., Kou, G.H., 1996. Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. Diseases of Aquatic Organisms 25, 133–141.

Mohan, C.V., Corsin, F., Thakur, P.C., Padiyar, P.A., Madhusudan, M., Turnbull, J.F., Hao, N.V., Morgan, K.L., 2002. Usefulness of dead shrimp specimens in studying the epidemiology of white spot syndrome virus (WSSV) and chronic bacterial infection. Diseases of Aquatic Organisms 50, 1–8.

Mohan, C.V., Phillips, M.J., Bhat, B.V., Umesh, N.R., Padiyar, P.A., 2008. Farm-level plans and husbandry measures for aquatic animal disease emergencies. Revue Scientifique et Technique Office International des Epizooties 27, 161–173.

MPEDA/NACA, 2003. Shrimp Health Management Extension Manual. Prepared by the Network of Aquaculture Centres Asia-Pacific (NACA) and the Marine Products Export Development Authority, India, in cooperation with the Aquatic Animal Health Research Institute, Bangkok, Thailand; Siam Natural Resources Ltd., Bangkok, Thailand; and AusVet Animal Health Services, Australia. MPEDA Cochin.

Padiyar, A.P., 2009. Epidemiology and management of *Penaeus monodon* farms in Andhra Pradesh, India., PhD Thesis. Deakin University, Australia.

Peng, S.E., Lo, C.F., Lin, S.C., Chen, L.L., Chang, Y.S., Liu, K.F., Su, M.S., Kou, G.H., 2001. Performance of WSSV-infected and WSSV-negative *Penaeus monodon* postlarvae in culture ponds. Diseases of Aquatic Organisms 46, 165–172.

Sahoo, A.K., Mohan, C.V., Shankar, K.M., Corsin, F., Turnbull, J.F., Thakur, P.C., Hao, N.V., Morgan, K.L., Padiyar, A.P., 2010. Clinical white spot disease status in *Penaeus monodon* during the middle of the culture period — its epidemiological significance. Journal of Fish Diseases 33, 609–615.

Samocha, T.M., Lawrence, A.L., 1992. Shrimp nursery systems and management. In: Wyban, J. (Ed.), Proceedings of the Special Session on Shrimp Farming. World Aquaculture Society, Baton Rouge, pp. 87–105.

Shankar, K.M., Mohan, C.V., 1998. Epidemiological aspects of shrimp viral diseases in India — a review. Journal of Aquaculture in the Tropics 13, 43–49.

Subasinghe, R., 2005. Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. Preventive Veterinary Medicine 67, 117–124.

Subasinghe, R.P., Phillips, M.J., 2002. Aquatic animal health management: opportunities and challenges for rural, small-scale aquaculture and enhanced-fisheries development: workshop introductory remarks. In: Arthur, J.R., Phillips, M.J., Subasinghe, R.P., M.BR., MacRae, I.H. (Eds.), Primary Aquatic Animal Health Care in Rural, Small-scale, Aquaculture Development, FAO Fisheries Technical Paper No. 406. Food and Agricultural Organization of the United Nations, Rome, pp. 1–5.

Umesh, N.R., Chandra Mohan, A.B., Padiyar, P.A., Phillips, M.J., Mohan, C.V., Vishnu Bhat, B., 2010. Shrimp farmers in India: empowering small-scale farmers through a cluster-based approach. In: De Silva, S.S., Davy, F.B. (Eds.), Success Stories in Asian Aquaculture. Springer, NACA, Bangkok, pp. 41–66.

Walker, P.J., Mohan, C.V., 2009. Viral disease emergence in shrimp aquaculture: origins, impacts and the effectiveness of health management strategies. Reviews in Aquaculture 1, 125–154.

Walker, P.J., Winton, J.R., 2010. Emerging viral diseases of fish and shrimp. Veterinary Research 41, 51.

Withyachumnarnkul, B., 1999. Results from black tiger shrimp *Penaeus monodon* culture ponds stocked with postlarvae PCR-positive or -negative for white-spot syndrome virus (WSSV). Diseases of Aquatic Organisms 39, 21–27.