Optimising Emergency Harvest Strategy for White Spot Disease in a Semi-Intensive *Penaeus monodon* Culture System in Karnataka, India

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ABSTRACT

A longitudinal study of 70 semi-intensive shrimp farms was undertaken in Karnataka, southwest India. For the purpose of this study white spot disease (WSD) was defined as the observation of 5 or more moribund or dead shrimp at the side of the pond on a single day and detection of white spot syndrome virus (WSSV) by 1-step PCR or histopathology in harvested shrimp. Samples were collected from 62 ponds at harvest and 31 fulfilled the case definition. In this system WSD had a significant effect on the average length of production cycle, yield and weight of the shrimp at harvest. Farmers have tried to reduce losses from WSD through avoiding risks and harvesting in the face of an outbreak. However, the information available on which to base such strategies can be misleading. Neither stocking WSSV positive (2-step PCR) post-larvae nor the presence of WSSV (2-step PCR) in shrimp from cast net samples 6 weeks after stocking were significantly associated with the length of the production cycle, yield, average weight at harvest, the risk of either WSSV presence at harvest or WSD. The findings indicate that WSSV and shrimp with WSSV inclusions by histopathology can be present in the pond without progressing to a full pond level outbreak of WSD. A decision-making tool based on the incidence and clinical signs of dead or moribund shrimp at the side of the pond allowed WSD to be predicted with an estimated sensitivity of 93.8% and a specificity of 94.3%. The implication of these findings for informing decisions on harvest strategy is discussed.

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INTRODUCTION

Shrimp farmers have tried to limit the effects of white spot disease (WSD) by preventing white spot syndrome virus (WSSV) entering the farm or by avoiding circumstances that result in an outbreak of the disease. A considerable amount of research and practical experience has lead to a greater understanding of the biology of the disease and the risk factors for outbreaks. In spite of this outbreaks still occur and can cause devastating losses. In the face of an outbreak the farmers' main strategy to minimise losses is emergency harvest. However, the decision to harvest can be difficult as either premature harvest or inappropriately delayed harvest can dramatically reduce the profitability of the production cycle.

During 1999 and 2000 a longitudinal epidemiological study of WSD in ponds was conducted in Kundapur, Karnataka, India. This study was part of a project conducted in both Vietnam and India. Much of the data from this project have already been published (Corsin *et al*., 2001; Corsin *et al*., 2002; Thakur *et al*., 2002, Mohan *et al*., 2002, Corsin *et al*., 2003) or are in preparation for publications. This paper concentrates on the development of decision-making tool for emergency harvest derived from the Indian data set.

MATERIALS AND METHODS

The study was conducted in Kundapur, which was selected for its location, 110 km north of Mangalore, where the laboratories for sample analyses were located. The local farming history also made identification and selection of an appropriate sample population possible.

A random sample of 100 of the 150 farmers was selected stratified by three areas within the estuary system. These farmers were all visited individually and data on the date of stocking gathered. Seventy of the selected farmers were enrolled between September 1999 and January 2000 and were followed until harvest, which was completed by the end of April 2000.

Before the pond was stocked with *P. monodon*, wild animals (e.g. shrimp, crabs, fish, etc.) and plankton samples were collected. A structured interview based questionnaire was used to collect data on previous crops and pond preparation practices. At stocking, data on source of post larvae (PL) were collected by interviewing the farmer, while characteristics of the PL (e.g. activity and size) were measured by direct observation using a sample of 500 PL. Data on water quality i.e. dissolved oxygen, temperature (Oxygen and temperature meter YSI model 55) pH (pH meter Jenway 3071), and salinity (Refractometer CSP 1270) were also collected at stocking, together with samples of wild animals such as crabs, insects by net and polychaetes using a spade. From stocking till harvest farmers were supplied with sheets to record daily data on feeding regime, water exchange, other management practices, the presence, number and clinical signs of any moribund/dead shrimp observed at the side of the pond. The farmers removed a pleopod from each dead shrimp collected and fixed it in absolute methanol for analyses by polymerase chain reaction (PCR). The remaining shrimp were placed in a container of 10% formalin for processing and histopathological analyses. The recording sheets were collected and water quality measured during visits at a fixed time every week by the research assistant. During such visits samples of feed and moribund/dead shrimp were also collected.

Six weeks after stocking a sample of 100 *P.monodon* was collected from each pond by cast-net, examined for clinical signs and fixed for PCR and histopathological examination. Samples

of wild shrimp and plankton were also collected at this time. At harvest, 400 *P.monodon* were collected and fixed for PCR analyses, of these, 100 were also examined for size and clinical signs and 20 individuals fixed for histopathological examination. Wild animals were also sampled and data on the harvest were collected by interviewing the farmer.

A PCR laboratory was established at the College of Fisheries, Mangalore (Karnataka, India) and samples were processed in order to test for the presence of WSSV. A total of 1340 samples were tested including 382 *P. monodon* PL; 657 *P. monodon* collected 6 weeks after stocking; 56 moribund or dead *P. monodon*; 105 harvested *P. monodon*; 70 plankton during the production cycle and 70 feed samples. For PCR a rapid DNA extraction was used (Kiatpathomchai *et al*., 2001), the samples were then tested by nested PCR for the presence of WSSV DNA (Lo *et al*., 1996). Samples were recorded as 1-step positive if a PCR product was visible after the first step of amplification and as 2-step positive if re-amplification was necessary to visualise the presence of WSSV DNA. The PCR protocol was subjected to a full series of internal validations and results were also confirmed by parallel testing in other laboratories (manuscript in preparation).

Histopathological sections from moribund/dead and harvested *P. monodon* were also prepared and examined for the presence of WSD pathology and other pathological conditions (Mohan *et al*., 2002).

A previously developed case definition (manuscript submitted) was used to identify outbreaks of WSD at a pond level. This was:

> "the observation of 5 or more moribund/dead shrimp at the side of the pond in a single day and the detection of WSSV in the shrimp at harvest by 1-step PCR or histopathology."

The PCR status of the PL and the shrimp sampled at 6 weeks were compared with the productivity, the risk of an outbreak of WSD and PCR status at harvest.

Harvest decision-making tool

The mortality events were categorised in terms of :

- the number of sick or moribund shrimp observed at the side of the pond (< 5 or ≥ 5),
- the presence of gross white spot lesions (+ = present, – = absent, ? not reported),
- fulfilment of case definition (case or non-case)

A single mortality event was defined as a period of when dead shrimp were observed with less than 6 days between observations; if there were more than 6 days with no observed mortality then the next observed deaths were considered to be a new event. For example a single dead shrimp, with no signs of white spots, was observed in a pond and then no more mortalities were observed over the next 30 days. This was classified as "< 5-WS non-case". Subsequently in the same pond 8 dead shrimp were observed on one day with white spots therefore this was categorised as "≥ 5+WS case".

A similar categorisation process was followed for the second day of each mortality event. The distribution of cases and non-cases were examined in each category and a decision-making tool for emergency harvest developed.

In order to evaluate the value of decision-making tool the diagnostic sensitivity and specificity for WSD cases were determined (Win Episcope 2.0 Epidecon http://www.clive.ed.ac.uk/winepiscope/). Sensitivity was defined as the proportion of WSD cases identified by the decision-making tool and the specificity was the proportion of non-cases identified. If the advice would have been to harvest on the first day then the data from the second day was excluded from the analyses. This evaluation was conducted using the data set used to develop the tool since there were no other suitable data available.

Data analysis was conducted on Sigma Stat 2.0 (Jandel Corporation 1992-1995). Student-t test was used for univariate comparison of normally distributed data. Where possible non-normal data were transformed to a normal distribution or, if that were not possible, were analysed with a Mann Whitney Sum rank test.

RESULTS

WSD cases and productivity

Complete data and samples from stocking to harvest were collected from 62 of the 70 enrolled ponds. Thirty one of the ponds were WSD cases; at harvest 37 ponds were 1-step PCR positive and 59 ponds were 2-step positive. There was a significant difference in the production between the cases and non-cases but no significant difference in survival (Table 1).

Table 1. Comparison between the production in ponds that were cases and non-cases.

	WSD cases Median (Inter Quartile range)	WSD non-cases Median (Inter Quartile range)	P
Length of production cycle (days)	79 (64-89)	102 (95-112)	<0.001
Survival (%)	55.9 (45.8-69.8)	52.1 (34.8-68.6)	0.432
	Mean (Standard Deviation)	Mean (Standard Deviation)	
Yield (kg/ha)*	668.3 (334.2)	979.7 (571)	0.012
Shrimp weight at harvest (g)*	16.1 (6.2)	25.1 (6.7)	<0.001

*Normally distributed data. P < 0.05 indicates a significant difference between cases and non-cases.

Table 2. Comparison between the production in ponds stocked with 2-step PCR positive and negative PL.

	WSSV+ve PL Mean (Standard Deviation)	WSSV-ve PL Mean (Standard Deviation)	P
Length of production cycle (days)*	89.3 (22.2)	85.5 (23.1)	0.495
	Median (Inter quartile range)	Median (Inter quartile range)	
Yield (kg/ha)	726.4 (473.8-1001.5)	672.9 (388.9-1010.6)	0.843
Shrimp weight at harvest (g)	22.4 (12.7-26.0)	21.5 (14.6-27.0)	0.618

*Normally distributed data. P < 0.05 indicates a significant difference between ponds stocked with WSSV +ve and -ve PL.

Evidence to Inform Farmers' WSD Control Strategy

PL PCR status. The PCR status of the PL was not significantly associated with productivity (Table 2), the WSSV status at harvest or the risk of a WSD outbreak. Half of the ponds (31) were stocked with 2-step positive PL and only 3 ponds were stocked with 1-step positive PL. Of these 3 ponds, 2 were both 1-step PCR positive and WSD cases at harvest the other was neither 1-step positive nor a WSD case. Stocking with 2-step positive PL did not increase the risk of an outbreak of WSD (WSD outbreak relative risk (RR) 0.86, 95% confidence interval (CI) 0.58-1.26) or of harvesting 1-step PCR positive shrimp (WSSV at harvest RR 0.95, CI 0.67-1.36).

Table 3. Comparison between production in ponds with 2-step PCR positive and negative shrimp collected by cast net 6 weeks after stocking.

	Ponds =WSSV+ve Median (Inter quartile range)	WSSV-ve Median quartile range)	P
Length of production cycle (days)	87.5 (65.5-101.5)	96.5 (77.0-107.0)	0.135
Yield (kg/ha)	610.1 (361.0-1029.2)	763.1 (485.1-1008.7)	0.312
	Mean (Standard Deviation)	Mean (Standard Deviation)	
Shrimp weight at harvest (g)*	20.9 (9.0)	21.0 (8.9)	0.974

*Normally distributed data. $P < 0.05$ indicates a significant difference between WSSV +ve and -ve ponds.

PCR status of cast net sample at 6 weeks. When the shrimp collected by cast net 6 weeks after stocking were examined for PCR status, 24 ponds (40%) were 2-step positive and 36 (60%) were 2-step negative. Two ponds were 1-step positive at six weeks but neither supplied harvest data or samples and therefore could not be included in the analyses. The 2-step PCR status of the ponds did not have a significant effect on production (Table 3), the risk of being a case (WSD outbreak RR 1.0, CI 0.6-1.68) or the risk of 1-step PCR status at harvest (WSSV at harvest RR 0.97, CI 0.65-1.43).

Table 4. The sensitivity and specificity for WSD cases of data derived from moribund or dead shrimp observed at the side of the pond and randomly selected shrimp at harvest.

	Sensitivity % (95% confidence limits)	Specificity % (95% confidence limits)
Histopathological evidence of WSD inclusions	92.9 (83.3-100)	75.0 (53.8-96.2)
White spots under the cuticle	65.5 (48.2-82-8)	94.1 (82.9-100)
1-Step PCR	66.7 (47.8-85.5)	85.7 (67.4-100)
2-Step PCR	87.5 (74.3-100)	53.8 (26.7-80.9)
White spots observed on a random sample of 400 shrimp at harvest		
1-step PCR	68.3 (54.0 - 82.5)	81.0 (64.2 - 97.7)

Data from moribund/dead shrimp. The data derived from the dead or moribund shrimp collected by the farmer during the production cycle was associated with the subsequent disease status of the pond. The clinical signs, histopathological findings and the PCR results were all associated with the subsequent WSD status of the pond. The sensitivity and specificity of the various parameters as diagnostic tests for WSD cases are summarised in Table 4.

There was evidence of WSSV and WSD in individual shrimp within ponds that did not progress to WSD cases. Dead/moribund shrimp that were both 1-step PCR positive and had histological evidence of WSD were observed in 2 ponds that did not become cases and shrimp that were 1-step negative but positive by histopathology were observed in a further 2 non-case ponds.

Harvest decision-making tool

A harvest decision-making tool was developed and is represented in Table 5. The sensitivity and specificity of the tool for predicting an outbreak of WSD in the pond within 6 days from the last day of observed mortalities was determined (Table 6). If the tool suggested that the pond should have been harvested then the risk of being a case was 16.4 times greater than in those ponds that that would not have been harvest (RR 16.4, CI 4.26-63.20).

Table 5. Harvest Decision Making Tool: Observations made from counting and examining moribund or dead shrimp from the side of the pond and the suggested management decision.

Observation	**Action** (cases/non-cases following the course of action in this study)
No mortalities	Continue production (0/12)
First day of observed mortalities	
White spots observed under the cuticle of the shrimp	Harvest (17/1*)
> 20 Mortalities (The mortalities in this study were 30/50/100).	Harvest (3/0)
Other observations	Wait for a further day
Second day of observed mortalities	
No mortalities	Continue production (1/28)
White spots observed under the cuticle of the shrimp	Harvest (2/0)
≥ 5 mortalities	Harvest (7/1#)
< 5 mortalities	Continue production (1⊗/5)
Summary of suggested action	
Do not harvest = 2 cases and 33 non cases	Harvest = 30 cases and 2 non cases

* There was some doubt regarding the validity of the data from the pond that was not a case.
\# The one pond that was not a case had up to 60 mortalities observed in a day.
⊗ The case experienced another mortality event 6 days later that would have resulted in harvest being recommended.

Table 6. The sensitivity, specificity and 95% confidence limits of the harvest decision-making tool for predicting a case of WSD in the pond within 6 days.

Outcome of DM Tool	WSD case	Non-case
Harvest	30	2
Continue production	2	33
Sensitivity %	Specificity %	
93.8 (85.4-100)	94.3 (86.6-100)	

DISCUSSION

Much of the information available to farmers regarding WSD is either difficult to interpret or potentially misleading. This paper describes an attempt to derive simple management decision-making tools from a large and complex longitudinal data set. The data available to the farmer at the pond side were examined to identify those that were most predictive of an imminent outbreak of WSD. Many experienced shrimp farmers have strategies to decide when emergency harvest is necessary. However, the information contained within this paper may lead to simple strategies for less well informed or less experienced farmers.

Despite the progress that has been made in understanding the biology and risk factors for outbreaks of WSD (e.g. Nakano *et al*., 1994; Chou *et al*., 1995; Lo *et al*., 1996; Limsuwan, 1997a; Flegel and Alday-Sanz, 1998; Chou *et al*., 1998; Kanchanaphum *et al*., 1998; Maeda *et al*., 1998; Sudha *et al*., 1998), outbreaks still occur and evidence presented here demonstrates the continued impact of this disease on shrimp farm productivity. In the face of an outbreak, farmers' only option is to conduct an emergency harvest. There were farmers in this study that either harvested before there was an outbreak of WSD in the pond or after the outbreak had progressed for some time, both responses resulting in lost productivity. However, many farmers appeared to have very good strategies for dealing with outbreaks of WSD and none of the outbreaks of WSD were allowed to follow their entire natural course.

The similarity between survival in WSD cases and non-cases may be an indication of successful emergency harvests since rapid response to an outbreak reduces the detrimental effect on survival; however, the data was biased since cases harvested significantly earlier than non-cases.

There was no significant relationship between the presence of WSSV by 2-step PCR in either the PL or the shrimp collected at 6 weeks post stocking and WSD or WSSV at harvest. The lack of association with PCR positive PL does not support previously published information (Limsuwan, 1997a; Flegel and Alday-Sanz, 1998; Mushiake et al., 1999). There are various hypotheses to explain the differences between our data and other reports. We are confident in the PCR results since they were validated by external laboratories as well as by internal controls. PCR could have detected non-viable viral DNA, or PCR could have detected viable virus that did not have a sufficiently large basic reproductive number to sustain an epidemic. Alternatively in this system other larger effects could have obscured the effect of infection in either PL or shrimp at 6 weeks. While we have no data to support or refute the first two options the third is unlikely since there was no significant association

in multivariable or multivariate analyses (manuscript in preparation). It is also probable that there are inherent differences between the systems examined in this study and those reported elsewhere.

There were insufficient data to allow inferences to be drawn regarding the association between the presence of WSSV by 1-step PCR in the PL or at 6 weeks and WSSV at harvest or WSD. Withyachumnarnkul (1999) published information suggesting a relationship between stocking PCR positive PL and crop failure in more intensive systems.

The data presented here further support the previously published opinion that individual shrimp infected by WSSV or even suffering from WSD may be present in the pond that does not progress to a full outbreak of WSD (Lo *et al*., 1998; Tsai *et al*., 1999).

The evidence from the dead shrimp collected at the side of the pond had the potential to inform the harvest decisions but the reported sensitivities and specificities require some additional consideration. It would seem counterintuitive that histological diagnosis of WSD would be more sensitive than specific, since it is well documented that histology is very specific at an individual animal level. However, the cumulative effect of examining larger numbers of animals may well increase the sensitivity and since individuals with WSD are not necessarily indicative of an outbreak at a pond level the specificity for a pond level outbreak would not necessarily be high.

The presence of the widely reported white spots under the cuticle in dead or moribund shrimp was found to be highly specific for WSD at a pond level. A previous publication by Corsin et al. (2001) reported that white spots were 77.3% sensitive and 77.8% specific for the presence of WSSV by 1-step PCR, based on the presence of white spots and 1-step PCR product in 400 harvested shrimp. Analyses in this study produced similar sensitivity (68.3%) and specificity (81%).

Limsuwan (1997b) reported white spots associated with a variety of environmental conditions and pathogens. Based on extensive experience of the industry he described four types of clinical picture, the key observation was that the presence of shrimp (<12 g) with white spots at the side of the pond was usually associated with WSD. White spots on otherwise healthy shrimp from cast net samples were not usually associated with WSD. Linsuwan did refer to a syndrome characterised by both white spots and brown gills, this clinical picture was not reported as a distinct syndrome in this study.

This study based on farmer observation in a semi-intensive Indian system produced findings that are consistent with our previous study in a Vietnamese rice-shrimp system (Corsin *et al*., 2002) and those of Limsuwan (1997b) based on the intensive Thai systems. All these studies would suggest that white spots in random samples from the pond are not a good indicator of WSD but white spots in moribund or dead shrimp from the side of the pond are a highly specific but not sensitive indication of WSD in the population. The lack of sensitivity indicates that not all affected shrimp demonstrate these signs.

While the results presented here are based on univariate analyses the conclusions were supported by multivariate and multivariable analyses (survival analyses, logistic and multiple regression – manuscript in preparation).

The decision-making tool described here was very effective at predicting outbreaks of WSD. This efficacy is undoubtedly dependent on the accuracy of the case definition. Development of a pond level case definition when management practices prevent full expression of an outbreak has proved very challenging. The development and evaluation of the case definition involved a variety of approaches (manuscript submitted) but has produced a definition that appears to be sensitive and specific in the system studied. The sensitivity and specificity of the decision making tool was tested on the data from which it was derived; this will have inevitably overestimated sensitivity and specificity. The tool should be further evaluated on other data sets or a prospective study in the same system.

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