ELSEVIER

Preventive Veterinary Medicine 53 (2002) 117–132

PREVENTIVE VETERINARY MEDICINE

www.elsevier.com/locate/prevetmed

Problems and solutions with the design and execution of an epidemiological study of white spot disease in black tiger shrimp (*Penaeus monodon*) in Vietnam

F. Corsin[a], T.T. Phi[b], L.H. Phuoc[b], N.T.N. Tinh[b], N.V. Hao[b], C.V. Mohan[c], J.F. Turnbull[d], K.L. Morgan[a,*]

[a]*Department of Veterinary Clinical Science and Animal Husbandry, The University of Liverpool, Chester High Road, Leahurst, Neston CH64 7TE, UK*

[b]*Research Institute for Aquaculture No. 2, 116 Nguyen Dinh Chieu Street, Ho Chi Minh City, Viet Nam*

[c]*Fish Pathology Laboratory, Department of Aquaculture, College of Fisheries, UAS, Mangalore, India*

[d]*Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK*

Received 22 July 1999; received in revised form 22 October 2001; accepted 22 October 2001

Abstract

White spot disease (WSD) is caused by white spot syndrome virus (WSSV) and is an acutely fatal pandemic disease of crustaceans. It has resulted in massive losses to the shrimp-farming industry in Asia and has now spread to the Americas. This paper reports the problems and solutions associated with the design and execution of a longitudinal epidemiological study of shrimp (*Penaeus monodon*) health on farms practising a crop rotation of rice and shrimp in the Mekong Delta of Vietnam. The pre-sampling phase of the project involved selecting an appropriate site and sampling variables, obtaining permission and establishing the necessary laboratory and logistic facilities. At the start of the sampling phase, 40 farmers were selected and 32 of these were visited and interviewed. This resulted in the enrolment of only 17 farmers. A further seven had to be enrolled to obtain the maximum number of farmers that could be sampled by the study team. Compliance was enhanced through meetings, regular visits by senior members of the project team and ensuring that visits were punctual and that all information was treated confidentially. The production cycle began in January 1998 and lasted for approximately 5 months. An attempt was made to collect 500 post larvae (PL) before each pond was stocked to assess the health of the batch and to test for the presence of WSSV by one-step PCR. After stocking, the wild crustaceans also were sampled from the pond for PCR analyses. Information was collected on the management practices and samples of water, pond bottom, feed and shrimp collected throughout the production cycle. Water quality variables with predictable diurnal variation were sampled in the morning and afternoon, twice a week. Two months after

* Corresponding author. Tel.: +44-151-794-6026; fax: +44-151-794-6028.
E-mail address: k.l.morgan@liv.ac.uk (K.L. Morgan).

PII: S0167-5877(01)00265-3

stocking, the first outbreak of WSD occurred; subsequently, 18 farms conducted a complete emergency harvest due to the actual or perceived presence of a WSD outbreak. Detectable mortalities were reported from 19 farms, and moribund shrimps were collected from four of these for PCR and histological analyses.

Keywords: White spot disease; Aquatic epidemiology; *Penaeus monodon*; Rice–shrimp

1. Introduction

White spot disease (WSD) is an acutely fatal pandemic viral infection of crustaceans. The causative virus is most commonly known as white spot syndrome virus (WSSV). The disease was first identified in Japan in 1993 (Nakano et al., 1994). It spread rapidly through Asia and recently was reported in the Americas (Lightner et al., 1997). WSD has affected shrimp in all types of culture systems. Losses associated with WSD exceeded 0.5 billion US$ in Thailand during 1996 (Flegel and AldaySanz, 1998).

Most efforts to control WSD have relied on conventional studies of the pathogen (Durand et al., 1997; Inouye et al., 1996; Nadala et al., 1998), experimental infection (Chou et al., 1998; Kanchanaphum et al., 1998; Lightner et al., 1998) and uncontrolled field trials (Chanratchakool et al., 1998; Mohan and Shankar, 1997). These have resulted in only limited control of the disease. There have been relatively few epidemiological studies of farmed aquatic species and studies on shrimp have been even more limited (Nakano et al., 1994; Thompson et al., 1997).

The aim of the present project was to identify the risk factors associated with WSD in the Vietnamese rice–shrimp system, using a longitudinal study design. The rice–shrimp system is a form of crop rotation unique to the Mekong Delta. It involves rice culture during the rainy season and shrimp culture during the dry season. The rice is grown on a central plateau around which a ditch is dug to provide a pond for the shrimp.

As epidemiological studies in aquaculture are scarce, this paper describes the design of this observational study, the problems encountered in setting it up and the solutions used.

2. Materials and methods

2.1. Study design

The aim was to measure water quality, presence of WSSV infection and the health of *Penaeus monodon* on 24 farms over one 5-month (January–June 1998) production cycle in a rice–shrimp farming system in Vietnam. The maximum number of farms that could be visited and sampled twice weekly was estimated to be 24 (i.e. eight per day). This sample size allowed 80% power to detect a risk ratio of 3.5 with 95% confidence, assuming an attack risk in the non-exposed population of 25%.

The criteria used to select the study site were: proximity to a laboratory in which labile samples could be analysed (i.e. Research Institute for Aquaculture No. 2, Ho Chi Minh City); a dense concentration of rice–shrimp farms and a high probability of a WSD outbreak occurring.

The site was located in Can Duoc District (10°30′N, 106°36′E), Long An Province, in the northern part of the Mekong River Delta, 50 km south of Ho Chi Minh City. Can Duoc was the largest of the three rice–shrimp farming districts in the province with 800 ha of farms in 1997. There were eight villages in the district but over half the total shrimp production area was in Tan Chanh, a village with 500 ha of ponds and almost 800 households in 1997. An address list of farmers producing shrimp in the district was provided by the district director of fisheries.

To visit and work at the study site it was necessary to obtain authorisation from the Ministry of Fisheries and the provincial and district administrative committees.

2.2. Data analysis

The statistical tests used to analyse categorical and continuous variables were Fisher's exact and Student's *t*-test, respectively (a Mann–Whitney test was used for ordinal data). Comparison between different people scoring post larvae (PL) quality was assessed using a 1-sample Wilcoxon signed-rank test. Repeatability of water analysis was tested with the coefficient of variation. Alpha was 0.05 (2-tailed).

3. Intentions, problems and solutions

3.1. Selection of the farmers

3.1.1. Intentions

The initial aim was to select a random sample of farms from the study area. Farms had to be accessible by motorbike or on foot. Our previous experience suggested that it would be necessary to select 40 farms initially to enrol 24 (French et al., 1994; Green et al., 1994).

3.1.2. Problems and solutions

In spite of the available address list, the village committee did not consider the selection of a random sample to be practical because farms in Tan Chanh were widely dispersed over a peninsula 3–4 km long and 3–4 km wide with some only reachable by boat. Therefore, they agreed to select a ''representative'' group of 40 farmers using the following criteria: size; degree of success; geographical distribution; presence of a shrimp crop and ease of access. They appointed a liaison officer to assist the project team.

3.2. Farmer enrolment

3.2.1. Intentions

Once the 40 farms had been selected, the aim was to visit individual farmers to explain the goals of the project, see if they were eligible for enrolment and discuss the details of data collection. To be eligible for enrolment, the farmers had to have grown rice during the previous season, be planning to stock shrimp in 1998 and be prepared to notify the research team of the time of stocking. Eligible farmers who agreed to participate were interviewed using a structured questionnaire and invited to a pre-study meeting involving all the selected farmers.

3.2.2. Problems and solutions

Four of the study team (with one Vietnamese researcher as an interpreter) and the village liaison officer visited each farm on foot or by motorbike over 3 days (15, 19, 20 December, 1997). Only 32 of the 40 farmers were visited. The remaining eight were either not available or were not visited because of lack of time. Of the 32 farmers interviewed, 97% (31/32) declared a willingness to participate and 88% (28/32) indicated that they would be available to attend a pre-study meeting.

A pre-study meeting was held, on the 21st of December, 1997. Only 57% (16/28) of the farmers who indicated that they were available attended and 25% (4/16) did not meet the enrolment criteria. Three had not cultivated rice during the previous season and one had decided not to farm shrimp during the current season. The meeting was also attended by the other farmers who had not been selected or interviewed.

A second pre-study meeting was held on the 10th of January 1998. Half of the remaining 16 farmers attended and all were considered eligible for enrolment. At this point, only 20 farmers had been selected. Only 17 (85%) of these were enrolled; three farmers (15%) failed to inform the research assistant of the time of stocking. These included all 12 selected farmers from the first pre-study meeting and 71% (5/8) of those who attended the second meeting.

A further seven farmers were enrolled. These were: one from the original group of 32 who did not attend any of the meetings because he thought he was already enrolled; three who, although not selected, had attended one of the pre-study meetings out of interest, and two who heard about the project from the liaison officer. All were interviewed, prior to enrolment.

At the end of the study, the farmers enrolled by different methods were compared with each other and with the general shrimp-farming population. The proportion of group leaders in the 18 farmers recruited from the 32 farmers originally selected was high: 17% (3/18) compared to 3% in the general population ($P = 0.02$). Group leaders were nominated heads of individual groups of farmers and might have been expected to be more skilled or experienced than other farmers. However, the proportion that harvested prematurely because of disease was similar to that in the whole village ($P = 0.34$). The remaining six farmers, although recruited by different methods, were similar to the first 18 in terms of the proportion of group leaders ($P = 0.57$), number and size of their ponds ($P = 0.92$ and $P = 0.57$, respectively), and number of shrimp stocked ($P = 0.57$).

3.3. Stocking date

3.3.1. Intentions

To be present in the village at the time of stocking, the study team needed to know the exact date of stocking. We tried to obtain this information by interviewing farmers, members of the village committee and Can Duoc district-extension workers.

3.3.2. Problems and solutions

Efforts to obtain an accurate prediction of the first stocking date proved unsuccessful. Half of the farmers (10/20) were unable to indicate when they would stock. Some said that they would stock before Tet (28 January—the Lunar New Year holiday), whereas

the village committee suggested that most farmers would stock after Tet. The research team was based in Ho Chi Minh city at this point, and to avoid missing stocking, kept in frequent telephone contact with the extension workers in Can Duoc. Those workers indicated that stocking would begin on the 15th of January and one research assistant moved to the study site. In spite of these efforts, one of the selected farmers had stocked by the time the research assistant arrived. However, he had other ponds and remained in the study.

Predicting stocking dates continued to be a problem even when the research assistant was at the study site and in regular contact with the farmers. They postponed stocking on at least two or three occasions for the following reasons: low salinity of the water, availability or poor quality of the PL, and personal reasons. Stocking started on the 29th of January and ended on the 14th of February.

3.4. Collection of wild crustaceans

3.4.1. Intentions

The aim was to collect samples of wild crustaceans from all the ponds before stocking and to store them in 95% ethanol for PCR analysis for WSD.

3.4.2. Problems and solutions

Wild crabs were not collected before stocking because of time constraints. Crabs were collected within 1 month of stocking by an individual with experience in catching crabs wading around the edges of the pond. In total, 478 crabs were collected, delivered to the laboratory and fixed in 95% ethanol for WSD PCR analysis.

3.5. Collection of data and PL at stocking

3.5.1. Intentions

The aim was to interview suppliers, to collect information on the source, age, transportation, storage and number of shrimp PL per bag and to record the total number of PL stocked (number of bags stocked $\times$ number of PL per bag) and the duration and method of acclimatisation at stocking. We planned to measure PL activity and homogeneity in size by examining a sample of 500 and assigning a group score of between -2 and $+1$ to each variable (Fegan et al., 1993). The length of the PL, chromatophore distribution, proportion of abdominal muscle in the shell, gut fill, presence of deformities, fouling organisms and histopathological changes in the hepatopancreas were determined by examining and scoring 25 individuals PL under a light microscope. To detect the presence of WSSV, 400 PL were stored in groups of five in 1.5 ml Eppendorfs containing 95% ethanol at room temperature for subsequent WSD PCR analyses. This sample size allowed 95% confidence of virus detection if present in more than 0.75% of the population.

3.5.2. Problems and solutions

To develop a questionnaire and sampling strategy for PL, full details of the source and supply routes were essential. Information about the delivery and distribution of PL obtained from farmer interviews and meetings was incomplete and to overcome this, a

meeting was held with one of the suppliers on the 15th of December. A *P. monodon* hatchery at Vung Tau, south-east of Ho Chi Minh City was also visited.

At stocking it proved difficult to obtain information from the supplier. It was not possible to trace the hatchery from which the PL had been collected and information about the number of days since metamorphosis was available only for 37% (10/27) PL samples.

The pre-study interviews suggested that a number of farmers would stock at the same time. This required the recruitment of five additional people (two to score PL and three to assist in collection). An attempt was made to reduce inter-observer variation in measurement by providing training and written guidelines. Inter-observer variation was measured. The three scorers examined the same batch of 500 PL and 25 individuals taking a maximum of 1 min for each PL. There was no significant difference between the individual PL scores of the trainees compared with those of the trainer ($P > 0.2$).

The PL were either sold directly to the farmer or stored in a tank at the village before purchase. Two different sampling strategies were used. PL from direct sales (85%; 23/27), were collected by taking a proportional sample from every bag at the pond side just before stocking. Stored PL (15%; 4/27) were sampled directly from the tank at the time of purchase.

One concern was the deterioration in PL quality between sampling and scoring. Fortunately, oxygen was available in the village and PL were transported to the field laboratory in plastic bags filled 1/3 with water and 2/3 oxygen. PL examination commenced within 2 h of collection. The PL were kept in an aerated bucket and scoring was completed within an hour. Assessment of the hepatopancreas proved time consuming and difficult to standardise; therefore, the 25 PL were fixed in 10% neutral buffered formalin for subsequent histological analyses. PL were not weighed at sampling but because of a later need to calculate the specific growth rate (SGR), their weight was estimated by weighing the drained formalin-fixed sample.

Some farmers stocked more than once. This had not been mentioned in pre-study interviews. Consequently, there were 32 stocking events on the 24 farms. Twenty-five per cent (6/24) farmers stocked twice and 4% (1/24) stocked three times. PL were collected from 84% (27/32) of these events. On the five remaining occasions, the farmers did not inform the research team of restocking. However, in three cases, shrimp stocked were from a batch already sampled on that day; therefore, only 6% (2/32) samples were missed. At restocking, only 200 PL were collected and information on their origin and the method and duration of acclimation was obtained from the farmer.

3.6. Collection of management data and shrimp samples

3.6.1. Intentions

During the production cycle, our intention was to record management activities and shrimp mortality at every visit by interviewing the farmer using a structured questionnaire. We planned to collect samples of *P. monodon* and other shrimp present in the pond monthly, using four throws of a cast-net at fixed sites, a number estimated to be sufficient to collect 20–25 shrimps. The aim was to record the appearance of individual *P. monodon* in this sample and then to fix them in neutral buffered formalin for subsequent histological examination. Species of shrimp other than *P. monodon* were stored in 95%

ethanol for future PCR analysis. In order to prevent the transfer of disease between ponds, the cast-net was disinfected after use by immersion in 2 g/l sodium hypochlorite for a minimum of 1 h.

3.6.2. Problems and solutions

Management practices and shrimp mortalities were recorded in Vietnamese by an interpreter, using a standardised recording sheet. On a few occasions when the farmer was not at the pond, information was collected at the following visit. It became clear that there was a large weekly variation in the type of feed used by the farmer. Therefore, an attempt was made to collect feed samples during the production cycle and store them at −20 °C. Only a small proportion of feed samples were collected for each pond, and for three ponds no sample was available. This occurred because of language problems and misunderstandings.

Cast-net sampling proved more time-consuming than anticipated. It was possible to sample only two ponds a day; consequently, the first month's samples were collected 28–37 days after stocking. Further, at the time scheduled for the second monthly sampling, some ponds had experienced mortalities, farmers were carrying out emergency harvests and only 12 ponds were sampled. For similar reasons, only four were sampled on the third occasion.

The cast-net samples contained species of shrimp other than *P. monodon*. In total, 84% (863/1025) were *P. monodon* 14% (148/1025) were wild shrimp and 1.4% (14/1025) were *Macrobrachium rosembergii*. The *P. monodon* and wild shrimp were transferred to the study site laboratory in aerated containers, examined and fixed as soon as practicable. *M. rosembergii* were examined for the presence of WSD clinical signs and thrown back into the pond.

It proved difficult to standardise cast-net sampling. A standard mesh size of 1 cm was adopted throughout the study but this allowed small shrimp to escape. The same person threw the cast-net on all but 17% (27/160) of occasions. To overcome the problem of diurnal shrimp movement (from the ditch during the hottest part of the day to the plateau when sunset was approaching), ponds were always sampled between 8:00 and 10:00 a.m. or between 3:00 and 4:00 p.m.

3.7. Water volume

3.7.1. Intention

We planned to estimate water volume by measuring pond area and depth. Area was estimated from measures of the length of the dykes obtained by using a tape measure, and depth was measured using a calibrated stick placed permanently in each pond.

3.7.2. Problems and solutions

Attempts to measure pond area using a tape proved unsatisfactory. The irregular shape of the ponds made an approximation inaccurate. Therefore, pond measurements were contracted to the provincial surveying department. The same two people using the same equipment surveyed the area of all the ponds in July 1998. Almost all ponds were drained at the time of measurement.

To ensure the quality of pond depth measurements, we had to fix a horizontal board near the base of the calibrated stick at the 0 cm mark to prevent it sinking in the muddy pond bottom. We also became aware of differences in pond depth in different areas, and during the first month the water depth was also measured in several additional points in all the 24 ponds. This allowed what we assume was a more representative estimate of the mean water depth in the whole pond. In one farm, the measuring stick was moved between visits but was repositioned for measurements.

3.8. Water quality

3.8.1. Intentions

Water quality was to be estimated twice a week (eight ponds a day) at fixed sites in the ditch area. Owing to diurnal variation, dissolved oxygen (DO), temperature, pH were to be measured twice a day, around dawn and in the middle of the day. We estimated that the morning measurements would be possible only on four farms—allowing 15 min for measurements and journeys between ponds. In the remaining four ponds, these variables were to be measured only once. The sequence of the farms visited on any 1 day was reversed on the next visit day so that these variables were measured twice-daily on all farms each week. The measures of water quality, time of the day, location and methods used are summarised in Table 1. Bio-security was maintained by disinfecting every piece of equipment at the end of sampling, using hypochlorite solution (1 g/l).

3.8.2. Problems and solution

There was insufficient time to start sampling all farms on the day of stocking; however, all ponds were sampled within 5 days of stocking. Water samples were collected throughout the production cycle, between the 29th of January and the 9th of June—a total of 479 potential measurements. Ninety-five per cent or more of these were obtained

Table 1
Water quality variable recorded, time of the day, sampling site and the methods used for the steady of WSD in black tiger shrimp in Vietnam

Variable	Time[a]	Location	Method
DO	AM/PM	50 cm depth	Membrane oxygen meter (YSI Model 55)
Temperature	AM/PM	50 cm depth	Digital thermometer (YSI Model 55)
pH	AM/PM	Sample taken 15 cm from the pond bottom	pH-meter Jenway 3071
Salinity	AM	Sample taken 15 cm from the pond bottom	Refractometer CSP 1270
Total ammonia	AM	Sample taken at the surface	HACH kit HACH DR/2000 Spectrophotometer
Alkalinity	AM	Sample taken at the surface	HACH kit HACH DR/2000 Spectrophotometer
Planktonic pigments	AM	Sample taken at the surface	Parson et al. (1984)
Turbidity	PM	At sampling site	Secchi Disc Depth (Stirling, 1985)
Water colour	PM	At sampling site	Visual scoring

[a] AM: 6–7 a.m. and PM: 2–3 p.m.

Table 2
Number of measurements recorded and those omitted from the recording sheet for the steady of WSD in black tiger shrimp in Vietnam, 1998

Variable	Recorded number (AM, %)	Recorded number (PM, %)	Omitted
DO	465 (97)	454 (95)	0
Temperature	465 (97)	454 (95)	0
pH	464 (97)	452 (94)	3
Salinity	478 (100)	–	1
Turbidity	–	468 (98)	11
Colour	–	430 (90)	49
Depth	462 (96)	348 (73)	20

(Table 2). Readings were missed because researchers were taking samples from emergency harvests at other farms (eight afternoon visits) and because readings were mistakenly omitted from the recording sheet. Water colour was not recorded regularly due to the considerable variability in different parts of the pond.

Towards the end of stocking, it became apparent that it would be possible to make twice-daily measurements of DO, temperature and pH in all eight ponds. This was done between 5:00–7:00 a.m. and 1:00–3:00 p.m. in 18 ponds by 10 days post stocking and in all the ponds by 15 days after stocking. Twice-daily water depth measurements were also introduced approximately 2 weeks after stocking to account for variation associated with tides.

To collect a representative water sample without disturbing the pond bottom, a sampling site was selected near pre-existing platforms in 29% (7/24) of ponds. In the other 71% (17/24), a site fixed half way from the main sluice-gate was selected. In 94% (16/17) of these ponds, the farmers subsequently built wooden platforms at the sampling site to facilitate collection. The same sampling sites were used throughout the whole production cycle.

Technical problems and physical problems were encountered in measuring both DO and pH. Calibration of the oxygen meter was time-consuming (it had to be adjusted for salinity between ponds). To overcome this, the instrument was calibrated at 0 ppt salinity before the morning and afternoon visits and the readings subsequently corrected for salinity (Eaton et al., 1995). It proved impossible to measure DO and temperature at the planned 50 cm depth on 33% (151/465) of occasions in the morning and 22% (101/454) in the afternoon because of the low water level. On these occasions, measurements were taken as close as possible to the pond bottom. The water level also influenced the turbidity measurements. It could not be measured on 29% (135/468) of occasions because the Secchi disk reading was greater than the water depth in the pond.

It proved impracticable to filter the samples for planktonic pigments at the pond side and to process water samples for alkalinity and ammonia at the study site laboratory. Filtration was carried out at the study site laboratory using Whatman GF/C for planktonic pigments analysis and 0.45 μm millipore filters before being stored at $-20\ ^{\circ}C$. Alkalinity and total ammonia were measured at the main laboratory in Ho Chi Minh City. Water samples were transferred there in a refrigerated box and analysed within 12 h of leaving the study site. Processing of planktonic pigments samples was initiated within 24 h. All but one of the samples (99.8%, 478/479) were analysed for total ammonia and alkalinity, but only 84.5%

(405/479) of the planktonic pigments samples were examined. Thirteen per cent (64/479) of the samples were not analysed because of a delay in receiving the necessary filters, 2% (8/479) samples suffered rodent damage and two samples were either not collected or not processed. One person carried out all the ammonia, alkalinity and planktonic pigments analyses. Repeatability of the measurements was determined by submitting unidentified duplicate samples and the results were satisfactory for ammonia and alkalinity (coefficient of variation <12.9 and 6.9%, respectively), but were highly variable for planktonic pigments (coefficient of variation sometimes higher than 100%).

3.9. Pond bottom

3.9.1. Intentions

The aim was to collect samples of the pond bottom monthly, and at harvest from two sites in the rice growing area, and two in the ditch. Pond-bottom pH was measured by using a soil pH meter (Model DM-I3 Takemura Electric Works); the samples were also scored for smell and colour.

3.9.2. Problems and solutions

Time constraints during the stocking period made it impossible to collect pond-bottom samples until 2 weeks after stocking. Thereafter, 55 samples were collected every month according to plan. Initially, samples were collected using an Ekman dredge; however, because of the muddy nature of the pond bottom and delicate structure of the wooden bridges, this proved unsuitable. The large volume collected using the dredge would have affected the characteristics of subsequent samples collected from the same location. Therefore, samples were collected with a scoop fixed to the end of a 3 m handle. This allowed 200 ml of sample to be collected from the top 5–10 cm of the pond bottom. In 58% (14/24) of the ponds the plateau could not be reached with the device used. Therefore, samples were collected from the small rice growing areas along the side of the dyke.

At harvest, pond bottom samples were only collected from one location in each pond because of time constraints. Four samples were collected within a 1 m radius of the calibrated stick and pooled to give a 50–100 ml sample. No variables were measured on site; samples were stored at $-20\ ^{\circ}C$ for subsequent analyses.

3.10. Collection of data and samples at harvest and during WSD outbreaks

3.10.1. Intentions

The farmers were asked to inform the research assistant of an intended harvest. The aim was to collect data on the weight of the different grades of shrimp harvested by interview and to purchase 410 individual shrimp. Ten of these were taken for histological examination and fixed in neutral buffered formalin at the pond side. Gill samples were taken from the remainder into a 1.5 ml Eppendorf containing 95% ethanol for PCR to detect WSSV.

3.10.2. Problems and solutions

In March–April, an outbreak of a disease with clinical signs typical of WSD occurred. Samples of moribund shrimp seen prior to harvest were collected only from four ponds. It

was not possible to sample the other ponds because the farmers sold or ate the dead shrimp. The frequency with which farmers harvested posed logistic problems. Seventy-five per cent (18/24) of the farmers conducted an early harvest and on one occasion seven farmers harvested within 3 h.

Two farmers failed to inform the research team of the time of harvest. In one case, no shrimp was collected for histology; in the other farm, 10 shrimps were collected from the few shrimps remaining in the pond after harvest. On the other farms, 10 shrimps were collected for histological examination.

Samples for PCR analysis were collected from all ponds at either early or normal harvest. In ponds harvesting large number of shrimps, 400 gills were collected. It proved impossible to collect all these gill samples at the time of harvest, so gills from 100 shrimps were collected within 24 h of harvest. The remaining shrimps were stored at $-20\ ^{\circ}C$ for a period of up to 1 month and gill samples collected when practicable. In ponds from which small number of shrimps were harvested, the sample size needed for the set limit of detection was recalculated using the Cannon and Roe formula (Martin et al., 1987). In three ponds (12%), farmers only harvested a proportion of the shrimp. In these cases, gills from a sample of 60 shrimps were collected for PCR to allow a limit of detection of 5% within 95% confidence intervals.

Some of the shrimps showed signs of bacterial infection at emergency harvest, therefore, the protocol was modified to examine and record the clinical signs seen in 100 shrimps at harvest.

Information about the weight and number of shrimps harvested was collected from every farmer. Since fish, wild shrimp, cultured *M. rosembergii* and, occasionally, mud crabs were also harvested, the protocol was modified to collect data on their presence, species and number.

At the end of the study, it became clear that there were still a number of variables for which no data had been collected. These included variables such as movements of equipment between ponds, family composition, people working at the pond and unusual changes in insect population. Therefore, a supplementary interview-based questionnaire was used. This was prepared in English, translated into Vietnamese and retranslated orally into English to double-check the translation. The final version of the questionnaire included 451 questions. The questionnaire was pre-tested on a Can Duoc extension worker who was a shrimp farmer and it was administered to the 24 farmers by the Vietnamese research assistant within 3 months of harvest. The overall management skills of the farmer were also scored independently by the two research assistants at the end of the data collection period.

3.11. Farmer communication and compliance

3.11.1. Intentions

To maximise participation and compliance, farmers were visited individually prior to the start of the study and the project was explained in detail. They were invited collectively to attend a pre-study meeting. The aim of these meetings was to establish a dialogue, allow farmers to contribute to the study design and to ensure that practical data collection fitted in with their management practices and day-to-day activities. In this way, ownership of the

project was encouraged. The confidentiality of all data collected by the research team was stressed throughout these meetings.

It was anticipated that one of the major difficulties would be in explaining the need for an observational rather than an intervention study. Farmers were aware that failure of a successful harvest was a common occurrence, and we predicted that they would be reluctant to participate in a study in which no advice was offered throughout and from which there was no immediate benefit. Another anticipated problem was obtaining permission and co-operation to sample PL and the cast-net catches of growing shrimp.

Sample collection was carried out punctually on the scheduled days and there was twice-weekly contacts with most of the farmers. Senior members of the study team also visited all the farmers during the production cycle to encourage their continued participation. It was important that farmers inform the research team of the time of stocking, shrimp mortality, intention to harvest or of major changes in management. To facilitate this, a telephone was installed in the research assistant's accommodation. There was a telephone at the village committee headquarters which all farmers had access to, and some farmers also had telephones or close access to other phones. Farmers also were given a timetable of the activities of the research assistants. During the first 2 weeks of February, the farmers were informed of the date and time of the following visit. Afterwards, the farmers were given timetables of the sampling schedule so that they could contact the research assistant at any time of the day. The timetable also included telephone numbers of the laboratory and in Ho Chi Minh City.

3.11.2. Problems and solutions

It appeared that all the farmers grasped the importance of understanding WSD prior to any intervention. They accepted that although frequent monitoring of shrimp health and water variables would be carried out by the project team, no information, intervention, assistance or advice would be given before or during the production cycle. They were assured that the study results would be presented to them collectively at an end-of-project meeting. Farmers were also promised written reports on the events which took place on their own farms with advice on any management changes that might prevent disease or improve productivity.

At the pre-study meetings, farmers suggested that the quality of the PL was the most important factor influencing the health and productivity of *P. monodon*. They were aware of differences in appearance of PL between bags and saw the importance of obtaining a representative sample. They agreed to order 500 extra PL and to allow sampling from each of the bags. The PL samples were purchased from farmers at market rates. The need to collect 20–25 shrimps by cast-net, from 1 month after stocking until harvest was explained, and to facilitate co-operation full harvest price was offered for these shrimp. All farmers agreed.

Despite the efforts of the research team and the apparent agreement and understanding by the farmers, there were some problems with compliance. The research assistant was not informed of five stocking events and as a result no samples were collected from two of them. Further, this information came to light only at an advanced stage of sampling. Two farmers (8%) failed to notify the research assistant of their intention to harvest. Fortunately, these events were detected and samples collected, although in one case the shrimp had

already been graded. Farmers also sought advice from the Vietnamese member of the research team during data collection. His refusal to supply advice did not appear to cause any resentment.

Notification of intended changes in management was given on two occasions. One farmer opened a canal to connect the study pond with a neighbouring pond 1 month after stocking. The second pond was sampled for water quality within 4 days of connection and on eight other occasions. A shrimp sample was also collected within 3 weeks.

The second farmer added shrimp to his pond from a pond outside the study half way through the production cycle. In this case, 105 shrimps were collected from both ponds by cast-net. Five were preserved at the pond side for histopathology and the remainder were examined and gills collected for PCR analysis.

The occurrence of disease did not appear to affect the attitude or compliance of the farmers in the study; however, some farmers outside the study suggested that the research team had infected the ponds.

4. Discussion

This paper describes the framework of a longitudinal epidemiological study of WSD in Vietnam. The starting date of this project was particularly important because there is only a single shrimp crop in most years. Stocking was expected to start in January and February 1998 and the success of the project depended on being fully prepared and present on site at stocking. A 3-month lead-in time was planned but funds were not made available until the end of October 1997. This short lead-in time influenced farm selection and the collection of pre-stocking data.

Although the planned number of 24 farms was recruited, the selection was not random and was incomplete at the start of stocking. Random sampling is essential to allow study results to be generalised to the target population. Sampling required the permission of the village committee and although the importance of obtaining a random sample was explained, they were prepared to provide a list of only 40 potential participants based upon our selection criteria. When compared with the whole Tan Chanh village, this sample contained a higher proportion of group leaders. However, there was no difference in the percentage of farms emergency harvesting.

Of the 40 farmers originally selected, only 32 were visited; this was partly due to time constraints but also because the enthusiastic response of all but one farmer suggested that it would be possible to recruit 24 farmers from this group. Their willingness to participate during the original interview may have been influenced by the presence of the village liaison officer, because in spite of their apparent enthusiasm, only 56% (18/32) of the farmers selected by the village committee were recruited—supporting our original estimate that 40 farmers would have had to be visited to enrol 24 farmers.

Only 16 farmers attended the first meeting. From previous experience, farmers who fail to attend such meetings often show poor compliance and our intention was to omit them from the study. However, it was suggested that the poor attendance reflected the short notice and so a second meeting was held. Repeated postponements of this meeting resulted in it being held at one day's notice and attendance was poor then too. The compliance of

farmers attending this meeting was lower than the first; 63% (5/8) gave notice of stocking compared with 100% (12/12) from the first meeting ($P = 0.05$)—supporting our original premise.

Considerable effort was devoted to achieving and maintaining compliance. The research assistants gave all the farmers a timetable of visits and were punctual throughout the study. Further, senior members of the team visited all the farmers at the start, in the middle and at the end of the production cycle to thank them for participating. The only financial inducement offered was the purchase of shrimp at full market price regardless of size. The only failures of compliance were failures to notify the study team of restocking, mixing of shrimp from different ponds and of emergency harvest.

Multiple stocking or mixing of shrimp from different ponds had not been mentioned at any of the project planning meetings, and the study team was not aware that it took place. It is also possible that the farmers did not appreciate the importance of collecting PL from every batch stocked. In the case of mixing, sufficient notice was given for contingency sampling strategies. At emergency harvest 2, farmers considered that harvest had to be conducted without delay and there was insufficient time for notification.

In spite of these failures, we consider that the level of co-operation achieved from the farmers was good. At pre-project meetings, the farmers were given the opportunity to contribute to the practical design of the project, to voice their concerns and to provide information on husbandry techniques. The major concern of the research team was that farmers would be reluctant to participate in a project in which they would be given no assistance until the end of the study. However, this topic was discussed and the farmers accepted the concept. Other issues such as sampling by cast-net and at harvest did not cause any significant problems. Compliance exceeded the requirement of the project team in some instances—allowing the data to be collected on all the planned variables. However, data collection was often impeded by the harsh working conditions and communication problems. A regular presence on the farm was essential and two research assistants had to cope with the onerous work schedule. The working day was sometimes 18–20 working hours per day; therefore, twice-daily sampling was not possible until 2 weeks after stocking. In these cases, only the morning sample was taken to allow the detection of the lowest DO and pH values. The many partial and emergency harvests during disease outbreaks also resulted in eight afternoon readings being missed and in a restriction of the pond bottom sampling sites at harvest.

High concentration of partial and emergency harvests, like multiple stocking and mixing of shrimp, had not been foreseen by the project team. Such activities required additional sampling protocols which were time-consuming. Communication problems between farmers and research assistants created other difficulties.

Misunderstandings arose during the pre-study interview. Three of the 32 farmers selected and interviewed did not grow rice (therefore were not eligible for selection). Some farmers said that they had only one pond even if they owned more than one because they had understood that the project team was interested in studying only one pond. The lack of any advice or intervention during the study was a source of disagreement with the local authorities during disease outbreaks. However, relationship between local authorities and the project team improved dramatically after a series of meetings was held to disseminate the preliminary results. These meetings were well attended by farmers,

extension workers and local authorities and provided a forum to plan the future control of WSD outbreaks in the study area.

Acknowledgements

We are grateful to Richard Callinan, Pornlerd Chanratchakool, Michael Phillips, Ian MacRae and Rohana Subasinghe who helped during the preliminary planning of the study. We thank Nguyen Son Nam and Mai Van Chinh of the Agriculture-Rural Development Service of Long An Province, the officers of Can Duoc district and the Tan Chanh village committee for their collaboration. A special thanks goes to Nguyen Van Hen who acted as liaison officer. We also thank the Can Duoc extension workers for their co-operation and for sharing important information with the project team. We thank all the members of RIA2 who contributed to the project for their help and co-operation, and Thao, Hoa and Hao for helping in assessing PL quality. This study was supported by the Department for International Development through project R7051 of the Strategy for Research on Renewable Natural Resources Program.

References

Chanratchakool, P., Turnbull, J.F., Funge-Smith, S.J., MacRae, I.H., Limsuwan, C., 1998. Health Management in Shrimp Ponds, 3rd Edition. Aquatic Animal Health Research Institute, Bangkok, 152 pp.

Chou, H.Y., Huang, C.Y., Lo, C.F., Kou, G.H., 1998. Studies on transmission of white spot syndrome associated baculovirus (WSBV) in *Penaeus monodon* and P-japonicus via waterborne contact and oral ingestion. Aquaculture 164 (1–4), 263–276.

Durand, S., Lightner, D.V., Redman, R.M., Bonami, J.R., 1997. Ultrastructure and morphogenesis of white spot syndrome baculovirus (WSSV). Dis. Aquat. Org. 29 (3), 205–211.

Eaton, A.D., Clesceri, L.S., Greenberg, A. (Eds.), 1995. Standard Methods for Examination of Water and Wastewater, 19th Edition. American Public Health Association, Washington, DC.

Fegan, D.F., Flegel, T.W., Nietes, A., Waiyakhruttha, M., Rojsuwan, S., 1993. A practical method for determining quality of post larvae of *Penaeus monodon* Fabricius. Poster Presented at Asian Fisheries Society, Fish Health Section Meeting, Phuket, Thailand.

Flegel, T.W., AldaySanz, V., 1998. The crisis in Asian shrimp aquaculture: current status and future needs. J. Appl. Ichthyol. 14 (3–4), 269–273.

French, N.P., Wall, R., Morgan, K.L., 1994. Lamb tail docking: a controlled field study of the effects of tail amputation on health and productivity. Vet. Rec. 134 (18), 463–467.

Green, L.E., Berriatua, E., Morgan, K.L., 1994. Problems and some solutions in the collection of data when investigating diseases of lambs in early lambing (housed) flocks. Prev. Vet. Med. 18, 275–285.

Inouye, K. et al., 1996. The Penaeid rod-shaped DNA virus (PRDV), which causes Penaeid acute viremia (PAV). Fish Pathol. 31 (1), 39–45.

Kanchanaphum, P. et al., 1998. Experimental transmission of white spot syndrome virus (WSSV) from crabs to shrimp *Penaeus monodon*. Dis. Aquat. Org. 34 (1), 1–7.

Lightner, D.V. et al., 1997. Risk of spread of penaeid shrimp viruses in the Americas by the international movement of live and frozen shrimp. Rev. Sci. Tech. 16 (1), 146–160.

Lightner, D.V., Hasson, K.W., White, B.L., Redman, R.M., 1998. Experimental infection of western hemisphere penaeid shrimp with Asian white spot syndrome virus and Asian yellow head virus. J. Aquat. Anim. Health 10 (3), 271–281.

Martin, S.W., Meek, A.H., Willeberg, P., 1987. Veterinary Epidemiology: Principles and Methods. Iowa State University Press, Ames, IA, 343 pp.

Mohan, C.V., Shankar, K.M., 1997. White spot viral disease management in shrimps: need for scientific approach. Fish. Chimes 17, 41–43.

Nadala, E.C.B., Tapay, L.M., Loh, P.C., 1998. Characterization of a non-occluded baculovirus-like agent pathogenic to penaeid shrimp. Dis. Aquat. Org. 33 (3), 221–229.

Nakano, H. et al., 1994. Mass mortalities of cultured kuruma shrimp, *Penaeus japonicus*, in Japan in 1993—epizootiological survey and infection trials. Fish Pathol. 29 (2), 135–139.

Parson, T.R., Maita, Y., Lalli, C.M., 1984. Determination of Chlorophylls and Total Carotenoids: Spectrophotometric Method, A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford, pp. 101–104.

Stirling, H.P., 1985. Water transparency by Secchi Disc Depth. In: Stirling, H.P. (Ed.), Chemical and Biological Methods of Water Analysis for Aquaculturalists. Institute of Aquaculture, University of Stirling, Stirling, pp. 100–101.

Thompson, J.A., Davidson, T.J., Frelier, P.F., Lawrence, A.L., 1997. Epidemiology: what causes shrimp mortality? In: Flegel, T.W., MacRae, I.H. (Eds.), Diseases in Asian Aquaculture, Vol. III. Fish Health Section Asian Fisheries Society, Manila, pp. 195–201.