

# **FINAL TECHNICAL REPORT**

**Submitted to DFID**

*Project R No 7051*

**An epidemiological approach to aquatic disease control: a study of the risk factors associated with outbreaks of White Spot Disease in artisanal systems in Vietnam and India.**

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## **EXECUTIVE SUMMARY**

### **THE PROJECT**

This project adapted existing epidemiological techniques to the study of risk factors for White Spot Disease (WSD) shrimp in two vulnerable farming systems in the Mekong delta of Vietnam and South West India. It is one of the first major projects to apply existing epidemiological techniques to the study and control of aquatic disease. The focus on two different artisanal systems maximised the scientific and socio-economic benefits of the project and allowed the results of the research to be applied to a wider range of culture systems.

### **PROJECT PURPOSE – from project Log Frame**

The project purpose was to improve techniques for study and control of aquatic disease in the Asia through the development of knowledge, skills and protocols in aquatic epidemiology, focusing on shrimp WSD. The objectively Verifiable Indicators (OVIs) have been met in full. Details of the routes of dissemination, other projects that have adopted methodologies used and developed in this study and details of all publications are contained in **OUTPUTS and Appendix IV**.

### **OUTPUTS**

All the OVIs have been produced. Risk factors have been identified and reported to local stakeholders. They have also been disseminated by a variety of other routes. As planned potential interventions have been identified and field trials funded by the Vietnamese Government have been undertaken. Further funding has also been sought from DFID and an application submitted to the Wellcome Foundation. Epidemiological techniques have been used by all the participants outwith this project and by colleagues of the participants. Teaching material has been produced and used in Vietnam, India, UK, Australia and Mexico.

The volume and quantity of data produced has exceeded expectations. As a result the potential for data analyses is also greater than expected. All the analyses planned in the original proposal have been completed but some advanced cluster and survival analyses with time dependent variables is still ongoing. Scientific understanding of WSD and methodologies for studying aquatic disease in general have been improved and there is evidence of both dissemination and uptake. All agreements with farmers were fulfilled and the information on the farming system and WSD were reported back to them using a variety of techniques, within one year of the start of data collection in each country. Comments from farmers and responses to project evaluation forms suggested that they found the detailed description of their system very useful. It allowed them to compare their activities with those of farmers performing better and worse than themselves.

## **IMPACT ON DEVELOPMENT OBJECTIVES**

Risk factors were identified and decision-making tools supplied to vulnerable farmers. Although no formal intervention study has been initiated, anecdotal evidence from both study sites suggests that farmers have changed farming practices in response to the findings of this project. There is an ever increasing need for the dissemination of existing results and development of further strategies to control aquatic disease. At the start of the project it was estimated that 10,000 families relied on rice-shrimp farming in the Mekong delta. A recent communication from Can Tho University has suggested that there has been a dramatic increase in the number of rice-shrimp farmers as a result of the perceived success of existing farmers. However, in the last year it is estimated that more than 110,000ha of shrimp culture failed due to high mortalities. Given an average pond size of 0.33ha, this represents one third of a million families affected by severe losses.

## **THE FUTURE**

All obligations have been fulfilled. A concept note for a second phase has been submitted and a full project memorandum is in preparation. This will build on the scientific results of this project but also investigate more generic issues regarding uptake of information and generating influence and a variety of levels from the farm to government.

## **BACKGROUND**

### **This Report**

This report is a summary of the activities and outputs associated with project R7051. Further details of the activities are contained within annual and quarterly reports submitted to DFID. The outputs are described in detail and where appropriate material is appended to this report. Manuscripts still in preparation will be publicised through DFID web sites.

### **The project**

This project adapted existing epidemiological techniques to the study of risk factors for White Spot Disease (WSD) of shrimp in two vulnerable farming systems in the Mekong delta of Vietnam and South West India. It is one of the first major projects to apply and adapt existing epidemiological techniques to the study and control of aquatic disease. The focus on two different artisanal systems maximised the scientific and socio-economic benefits of the project and allowed the results of the research to be applied to a wider range of culture systems.

### **White Spot Disease**

WSD is a pandemic disease of shrimp caused by an unclassified virus commonly known as White Spot Syndrome Virus (WSSV). WSD outbreaks were first detected in *Penaeus japonicus* and *P.chinensis* in Japan and China in 1993 (Nakano et al, 1994; Zhan et al., 1998) and in the following 18 months the outbreak spread to the majority of the shrimp farming countries in Asia. Other shrimp species were infected including *P.monodon* which is by far the most economically important species of the continent (Anonymous 1997; Mohan et al., 1998; Park et al, 1998; Wongteerasupaya et al, 1995; Lo et al, 1998; Hao et al., 1997). WSSV has now also been reported in shrimp farms in Central and South America (Calderon, 2000).

### **The impact of WSD**

The massive social and economic impact of WSD has been widely reported and discussed. Estimates suggest that losses due to WSD exceeded 0.5 billion US\$ in Thailand during 1996 and over 2 billion in SE Asia in 1997 (Flegel and Alday Sanz, 1998). Reports have suggested that WSD has severely damaged small-scale coastal aquaculture systems throughout Asia. The effects have ranged from lost crops to abandonment of whole production areas. It is, however, difficult to accurately quantify the economic and social effect of this disease. WSD and the response of those depending on shrimp farming for their livelihoods are in a constant state of co-evolution. In such a relationship it is difficult to separate the effect of the disease from people's response to the disease. Methods were developed to calculate the effect of WSD during this project and information from

Vietnam has demonstrated that loss of a crop through WSD can result in extremely vulnerable farmers dropping to the lowest poverty levels, where their families are no longer food secure.

### **Control of WSD**

The scientific community reacted promptly to this devastating pandemic. The virus was characterised by electron microscopic techniques (Takahashi et al., 1994; Durand et al., 1997) and molecular diagnostic methods such as PCR (Lo et al., 1996b; Takahashi et al., 1996; Kim et al., 1998) and in situ hybridisation (Chang et al., 1996; Durand et al. 1996) were developed. These allowed more sensitive diagnosis of the disease which had previously been based on the observation of white spots on the shell and other clinical signs such as red or pink discoloration (Nakano et al., 1994; Limsuwan, 1997a). Such highly specific and sensitive techniques were often combined with histopathological examination of specimens in order to identify intranuclear inclusion bodies in tissues of ectodermal and mesodermal origin which indicate WSSV replication (Lightner, 1996). Despite the range of sophisticated pathogen identification techniques available, there was still no agreed definition for an outbreak of WSD at a pond level. Laboratory techniques allowed the detection of the viral DNA, the virus itself or the pathology caused by the virus but it was still not possible to differentiate a pond suffering from a propagating outbreak of the disease from those that were infected but not suffering from disease.

Studies on the pathogen and the improvements in diagnostic techniques was followed by several studies based on experimental infection (Chou et al., 1998; Kanchanaphum et al., 1998; Lightner et al., 1998). In addition, WSSV was identified in shrimp of different ages from post-larvae to broodstock and in a wide range of crustaceans and other arthropods (Lo et al., 1996a)

Results of these largely laboratory based studies led several authors to suggest a series of possible risk factors for WSD outbreak. These were:

- ?? Stocking of infected post-larvae (Limsuwan, 1997b; Flegel and AldaySanz, 1998; Mushiake et al., 1999).
- ?? Addition of infected water to the pond (Nakano et al., 1994; Chou et al., 1998).
- ?? Presence of WSSV carriers in the pond or inflow water (Lo et al., 1996b; Kanchanaphum et al., 1998; Maeda et al., 1998).
- ?? Exposure to stress factors (Chou et al., 1995; Lo et al., 1996b; Sudha et al., 1998).
- ?? Ingestion of infected shrimp or fresh feed (Chou et al., 1995; Chou et al., 1998).

However, despite of the adoption of strategies to reduce or eliminate WSSV from the system and the use of immunostimulants (Song et al., 1997; Chang et al., 1999) only limited control of WSD had been achieved.

### **Aquatic Epidemiology**

During the latter half of the 1990s the scientific community identified the need for a more holistic, population-based approach to the understanding and control aquatic disease including WSD. Epidemiology was considered to be essential to practical aquatic health control by many organisations and individuals (FAO, DFID, ACIAR, NACA and others). Epidemiology combines the study of disease in populations and an inherent consideration of complex causes with effective participatory methods.

At the time this project was initiated there had been very few population based studies studying aquatic animal diseases and their impact on people. There are still very few examples of research investigating the risk factors for WSD in real populations (Hettiarachchi et al., 1999; Withyachumnarnkul, 1999). With the exception of a brief description of a WSD outbreak in Japan (Nakano et al., 1994) this project has generated the first epidemiological study of WSD (see **OUTPUTS**).

### **Participation**

As previously stated applied epidemiology requires a practical participatory component. Although the nature of such participation may be unfamiliar to social scientists it has a long history of effectively dealing with problems. Participation by farmers and local institutions was essential to the success of this project. The initial research plan was arrived at through expert consultation, however, implementation of the plan required extensive consultation. Stakeholders were introduced to the team, informed of the aims, the aims were discussed and appropriate changes made to the research plan. An essential part of this process was an agreement entered into with the farmers. The researchers promised to deliver findings back to farmers within one year. This promise was fulfilled in both phases of the project and produced a great deal of positive feedback from participating farmers.

### **Value for money**

The project has been extremely successful achieving all the initial objectives and far exceeding expectations in terms of data collected and advances in understanding. The success of the project was in large part due to the effort of the collaborators at all levels; farmers, research assistants and

senior scientific collaborators. The research was extremely cost effective with most of the funds being spent in developing countries. Farmers were compensated for all samples taken from their farms while the scientific collaborators did not charge for any of their time. Neither Prof. Morgan nor Dr Turnbull costed in any of their time and while Dr Mohan and Dr Hao were allocated funds in the proposal, they chose to reallocate those funds to support the research.

### **The future**

While all obligations regarding dissemination of information have been fulfilled, this project did not intend to implement full intervention studies. A concept note for a second phase has been submitted and a full project memorandum is in preparation. This will build on the scientific results of this project but also investigate more generic issues regarding uptake of information and generating influence and a variety of levels from the farm to government.

## **PROJECT PURPOSE**

### **From the Project Memorandum Logical Framework (Appendix III)**

*The project purpose was to improve techniques for study and control of aquatic disease in the Asia through the development of knowledge, skills and protocols in aquatic epidemiology, focusing on shrimp White Spot Disease (WSD) in artisanal systems in India and Vietnam.*

### **Objectively Verifiable Indicators**

*By the end of the project: epidemiological methodologies for the study and control of aquatic disease developed, information and techniques extended to participants and others.*

These have been met in full. Details of the routes of dissemination, other projects that have adopted methodologies used or developed in this study and all publications are contained in **OUTPUTS**.

### **Methodologies and techniques**

The two studies in Vietnam and India, allowed the development of practical epidemiological techniques for use in aquatic systems. Two different approaches were adopted, in Vietnam a small number of farms were involved with the focus on detailed examination pond level risk factors for outbreaks of disease. In India the study focused on routes of transmission of WSSV, involving a larger number of farmers and potential sources of the virus from outside the farm. The activities undertaken and the techniques developed during this project have contributed significantly to the development of aquatic epidemiology.



## **Information**

Information produced during the study has already provided farmers with decision-making tools. For example a robust and simple technique was developed in Vietnam to allow farmers to decide when emergency harvest was necessary. This was based on the number of dead shrimp observed at the edge of the pond. Details of the farming systems and the risk factors for WSD in both countries was also presented to and discussed with the farmers. The farmers considered such information valuable and preliminary evidence suggests that it improved their understanding of the disease problem.

Information produced has also significantly improved understanding of WSD in specific and the study of aquatic diseases in general.

## **Interventions**

With the results from this project we are in the process of applying for further funding to develop and test potential interventions to reduce the impact of WSD. This would be linked to a generic study of potential routes of dissemination and uptake of research findings.

# **OUTPUTS**

## **From Project Memorandum Logistic Framework (Appendix III)**

- 1. Risk factors for WSD outbreaks identified.*
- 2. Rational intervention strategies designed.*
- 3. Project findings developed and disseminated, understanding and capacity in aquatic epidemiology in key regional organisations promoted.*

## **Objectively Verifiable Indicators**

- 1. By the end of the project: risk factors for disease identified and epidemiological methods and results published.*
- 2. The initiation of preliminary field trials in Vietnam and production of subsequent grant proposals for controlled application of further intervention strategies.*
- 3.- Participants use of epidemiological skills demonstrated, strategies being applied for this and other diseases, teaching material produced.*

The assumptions concerning the presence of the disease and the compliance of the participants were all met. All the OVIs have been produced. Risk factors have been identified and reported to local stakeholders. They have also been disseminated by a variety of other routes (see below). As planned, potential interventions have been identified and field trials funded by the Vietnamese Government have been undertaken. Further funding has also been sought from DFID and an

application submitted to the Wellcome Foundation. Epidemiological techniques have been used by all the participants outwith this project and by colleagues of the participants (see below). Teaching material has been produced and used in Vietnam, India, UK, Australia and Mexico.

## **Details of outputs**

### **Participating Farmers**

The 24 and 70 farmers in Vietnam and India participated in a successful project. It was anticipated that farmers might be reluctant to participate in an observational study, where no advice is provided during the data collection phase, particularly in the face of a devastating disease epidemic. However, by combining a spectrum of participatory approaches, compliance was maintained throughout the project. This was reflected by the excellent farmer attendance at the end of project meetings and the enthusiastic response to the presentations. In both Vietnam and India the meetings to present the project findings were attended by representatives from all the participating farms and significant numbers of other stakeholders including farmers and others. In India where 70 farmers were enrolled the final meeting was attended by over 250 people. At these meetings, descriptions of the local farming systems, risk factors and potential interventions were described both verbally and in written form to the farmers and extension workers with suggestions for implementation. In Vietnam all the information was presented in Vietnamese, whereas in India it was presented both in the local language of Kannada and English. From the questions and discussions at these meetings it would appear that the farmers not only understood the presented information but were able to appreciate its implications for disease control. After the group meetings all the farmers involved in the study were met individually, to provide them with a report in their own language describing the findings of the project in relation to their farm. According to Vietnamese authorities, the rate of failure of farms in the study site decreased from 90% in 1998 to 20% in 2000. However, it is currently extremely difficult to accurately evaluate the impact of a particular project recommendation. DFID has commissioned a study to examine the uptake of outputs from this project in Vietnam.

### **Participating Scientists**

All scientists involved in the project were part of a successful collaboration, the success of which was due to the knowledge, enthusiasm and effort of the research team. In particular Flavio Corsin, the research scientist primarily responsible for data collection and analysis undertook an exceptional workload. Organisational challenges were resolved, farmers were recruited, their compliance maintained and a complete data set collected. Excellent empathy and understanding was generated

between all members of the research team leading to exchange knowledge and skills in all directions. It is anticipated that this collaboration will continue in the future.

### **Understanding of WSD and aquatic disease**

The project provided a case definition for WSD, based upon mortality patterns and the presence of WSSV. It provided data on the spatial spread of the epidemic, the productivity and management practices of the rice-shrimp Vietnamese system and the semi-intensive Indian systems and allowed the financial loss associated with disease to be estimated. The project provided practical experience in the application and adaptation of epidemiological techniques to aquaculture in which the inevitable trade-offs between data quality and quantity were explored. A key component of this was the measuring instruments and tests used, including the development of population sampling protocols for PCR analyses. Some of the techniques developed were novel to epidemiology and the experience gained in this will be invaluable to future project design.

There follows a detailed list of the outputs generated by the project.

### **Training and Infrastructures**

Infrastructures and training were provided to the local research institutes allowing them to conduct further epidemiological studies leading to control strategies for aquatic diseases.

#### *Infrastructure*

PCR laboratories capable performing both 1-step and 2-step nested PCR tests were established in both RIA2 (Vietnam) and Mangalore Fisheries College (India). Water quality measuring instruments were also supplied to both research institutions.

#### *Training - Vietnam*

Two people were trained in PCR analyses Mr Trinh Trung Phi, currently attending a MSc course in Hanoi, Vietnam and Dr Tran Thi Minh Tam, currently working at RIA2 in aquatic disease control projects. Two people were trained in water quality analyses, Le Hong Phuoc and Nguyen Thi Ngoc Tinh. Both Trinh Trung Phi and Nguyen Van Hien were trained in the collection of epidemiological data.

#### *Training - India*

Five people were trained in PCR analyses. P.C.Thakur subsequently submitted an MSc thesis based on material from the DFID project and is currently employed in the Mangalore Fisheries College. Basavarajappa is currently attending a MSc course in Wageningen University in the

Netherlands. Pradhan is now undertaking a PhD project in the Department of Aquaculture, Mangalore Fisheries College. Guruchannabasavanna is currently attending a MSc course in the Department of Aquaculture, Mangalore Fisheries College and is using samples from the DFID project for his dissertation. Finally Arun Padiyar, who is still employed at the Mangalore Fisheries College analysing samples and data from the DFID project, also received training in histology, water quality analyses, data collection and entry.

Three people involved in the project were trained in histopathology processing and basic interpretation. These included Jagannath and Sasikala who both subsequently submitted MFSc theses based on samples collected by the DFID project and are currently employed at Mangalore Fisheries College.

Two other people received training in water quality analyses, Madhusudan now working in fish processing industry in the Middle East and Narayan currently employed by CP feeds in India. Both also received training in data collection.

### **Presentations to participating farmers and other stakeholders**

Meetings were held to initiate the project in both study sites. These have been reported in quarterly and annual reports.

Farmers meeting. Long An Province, Vietnam. December 1998

The meeting was attended by 35 farmers participating in the study and other stakeholders including extension officers and farmers group leaders.

Meeting with extension workers and local scientists. Long An Province, Vietnam. December 1998

Attended by 45 extension workers and local researchers.

Visit to each of the 24 participating farmers, Long An Province, Vietnam, December 1998.

Farmers meeting. Kundapur, Karnataka, India. September 2000.

Preliminary findings of the study from 70 ponds was presented to about 250 shrimp farmers and other stakeholders from Kundapur and surrounding areas. Description of the system, study findings and associations with outcomes from the three major areas in the study, were presented in the morning in English and Kannada. The afternoon session was devoted to discussions. All participants received paper copies of all the material presented.

Five meetings in locations local to farms. Kundapur, India. September 2000.

Reports in English and Kannada of individual ponds in the study were presented and discussed with farmers.

Meeting with staff, post-graduate students and scientists from NIO (Goa), CMFRI (Cochin), CUST (Cochin), MPEDA (Karwar), Department of Fisheries (Mangalore) and others. Mangalore, Karnataka, India. September 2000.

**MFSc theses submitted to the University of Agricultural Sciences, Bangalore, Mangalore College of Fisheries.**

1. Prevalence of White Spot Syndrome Virus in *P.monodon* post larvae - P.C.Thakur 2001
2. Health status of cultured shrimp at harvest from low saline areas of Kundapur – Jagannath 2001
3. Health status of cultured shrimp at harvest from high saline areas of Kundapur – Sasikala 2001

**Peer review papers accepted with revisions for publication (see Appendix IV)**

Corsin, F., Turnbull, J.F., Hao, N.V., Mohan, C.V., Phi, T.T., Phuoc, L.H., Tinh, N.T.N. & Morgan, K.L. Problems and solutions with the design and execution of an epidemiological study of White Spot Disease in black tiger shrimp (*Penaeus monodon*). Preventative Veterinary Medicine.

Corsin, F., Turnbull, J.F., Hao, N.V., Mohan, C.V., Phi, T.T., Phuoc, L.H., Tinh, N.T.N. & Morgan, K.L. Risk factors associated with White Spot Syndrome Virus infection in a Vietnamese rice-shrimp farming system. Diseases of Aquatic Organisms.

**Peer review papers in preparation**

Corsin, F., Thakur, P.C., Padiyar, P.A., Madhusudhan, M., Turnbull, J.F., Mohan, C.V., Hao, N.V. & Morgan, K.L. Feeding farmed shrimp with shrimp waste – the lessons for aquaculture from BSE.

Corsin, F., Thakur, P.C., Padiyar, P.A., Madhusudhan, M., Turnbull, J.F., Mohan, C.V., Hao, N.V. & Morgan, K.L. Utility of dead samples in aquatic epidemiological studies.

Thakur, P.C., Corsin, F., Padiyar, P.A., Madhusudhan, M., Turnbull, J.F., Shankar, K.M., Mohan, C.V., Hao, N.V. & Morgan, K.L. Estimation of WSSV prevalence by Polymerase Chain Reaction in *Penaeus monodon* post-larvae stocked in shrimp farms: emphasis on the methodology used for detection.

Corsin, F., Turnbull, J.F., Hao, N.V., Mohan, C.V., Phi, T.T., Phuoc, L.H., Tinh, N.T.N. & Morgan, K.L. Spatial and temporal distribution of White Spot Disease during an outbreak in the rice-shrimp system of Vietnam.

**Conferences and meeting contributions**

Turnbull, J.F. & Chanratchakool, P. (1999) Field based approach to shrimp health problems. The annual international conference of the World Aquaculture Society. Sydney, Australia. 26 April-2 May 1999.

Turnbull, J.F., Corsin, F., Hao, N.V., Mohan, C.V. & Morgan, K.L. (1999) An epidemiological study of White Spot Disease in a rice-shrimp culture system in the Mekong delta. DFID/FAO/NACA Workshop on aquatic animal health care in rural aquaculture. 27-30 September 1999.

Aquaculture in the 3<sup>rd</sup> millennium, NACA/FAO meeting. Bangkok, Thailand. Disease Control and Health Management chaired by Rohan Subasinghe assisted by JF Turnbull. 21-25 February 2000  
200021-25 February 2000

### **Intra-departmental seminars**

Epidemiological Study of White Spot Disease. Marine Research Laboratory, Port Erin, Isle of Mann. May 1999.

Aquatic Epidemiology. University of Liverpool, Department of Veterinary Science. March and April 1999.

An Epidemiological Study of White Spot Disease in India. Research Institute for Aquaculture N<sup>o</sup>2, HCMC, Vietnam. April 2000

The preliminary findings of an epidemiological study of White Spot Disease in India. Bangalore Agricultural University, College of Fisheries, Mangalore India. September 2000.

An Epidemiological Study of White Spot Disease in Vietnam and India. University of Queensland, Australia. July 2000

Epidemiology of White Spot Disease in *P.monodon*. Institute of Aquaculture, University of Stirling, UK. October 2000.

Epidemiology of White Spot Disease in *P.monodon*. CIAD, Mazatlan, Mexico. January 2001.

### **Other routes of dissemination**

Prawn Health Management. Australian Prawn Farmers Association. Brisbane, Australia. May 1999.

Shrimp Health Management Course. AAHRI/NACA Bangkok Thailand. October 1999.

Lectures on disease control MSc/PG Diploma course in Aquaculture and Aquatic Veterinary Studies and Aquatic Pathobiology. Stirling, UK. October 1999, February 2000, October 2001 and February 2001

Lectures on disease control BSc courses Aquaculture. Stirling, UK. February 2000.

Workshop on PRA and RRA. Project R7051 was used as one of the case studies for this meeting. FAO/DFIA/ICLARM. Bangkok, Thailand. February – March 2000 and 2001.

Development of an aquatic Version of Survey Toolbox. Project R7051 was used as one of the case studies for this meeting. ACIAR, Bangkok, Thailand. June 2000

ACIAR meeting on development of an integrated health management package for small scale shrimp farming (Indonesia). CV Mohan acted as a resource person. July 2000.

British Council funded course on Aquatic Disease control. CIAD, Mazatlan, Mexico. January 2001.

## **Uptake of methodologies**

The application of epidemiology in aquaculture systems during this project has provided some useful methods and these have already been used by a number of other activities. These include:

Longitudinal study of production practise in semi-intensive system in Bac Lieu, Mekong Delta. Investigation into the risk factors and effects of WSD in the Mekong Delta of Vietnam. RIA 2, Vietnam. Funded by the Vietnamese Government. January 1999 to present.

A study of Red Spot Disease (RSD) of grass carp (*Ctenopharyngodon idella*) in Vietnam. MSc thesis conducted at RIA 1 and Institute of Aquaculture, Stirling. Partially funded by DFID. March to June 1999.

Strategies for improved diagnosis and control of bacterial disease in small-scale fresh water aquaculture. Institute of Aquaculture, Stirling; AAHRI, Thailand and University of CanTho, Vietnam. Funded by DFID-R7463. July 1999 to present.

Situation analysis of impact of Red Spot Disease on rural livelihoods Tuyen Quang, North Vietnam. RIA 1, Vietnam and Institute of Aquaculture, Stirling. Funded by the British council and The Government of Vietnam. November 1999.

Preliminary investigations into grouper aquaculture in Tamgiang lagoon, Vietnam. Hue University of Sciences, Vietnam and Institute of Aquaculture, Stirling. Funded by DFID. March 2000

Longitudinal study of WSSV in shrimp in Than Chan (previous study site). This involved 200 households and preliminary data was available by September 2000. Funded by the Vietnamese Government.

Longitudinal study of 75 households in Tra Vinh province, Mekong Delta. This study involved data on status if culture system, presence of WSSV and risk factors for outbreaks of the disease. A preliminary report has been prepared and farmer training has been organised through local extension services. Funded by the Vietnamese Government. November 2000.

## **ACTIVITIES**

### **From Project Memorandum Logistic Framework (Appendix III)**

*1.1. Study sites recruited in Vietnam and India; data collectors trained.*

*1.2. Questionnaires and sampling procedures developed.*

*1.3. Data collected and analysed.*

*1.4. Multivariate analyses for risk factors of WSD completed.*

*1.5. Farm level risk factors for WSD identified; results presented to participating farmers.*

*2.1. Control measures prioritised.*

*2.2. Intervention strategy field trials designed.*

*2.3. Project proposal for intervention study produced.*

*3.1. Reports, publications and teaching material prepared.*

*3.2. Epidemiological teaching material produced.*

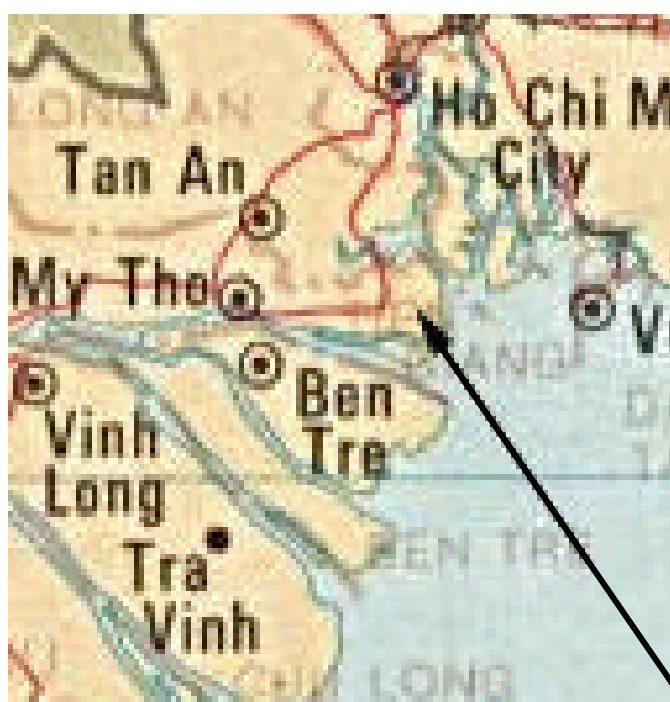
The volume and quantity of data produced has exceeded expectations. As a result the potential for data analyses is also greater than expected. All the analyses planed in the original proposal have

been completed but some additional analyses are still ongoing. Activities 1.1., 1.2., 1.3., 1.5. have all been completed. The analyses anticipated under Activity 1.4. has been completed but some advanced cluster and survival analyses with time dependent variables is still ongoing. Funds are being sought to allow these analyses to continue.

Both 2.1 and 2.2. have been achieved to a limited extent but as a result of the complexity of the data set these activities require further analyses. A concept note has been submitted and DFID has requested a full Project Memorandum. 3.1 and 3.2. have been completed.

### **Activities Vietnamese phase**

A longitudinal study on 24 ponds was conducted to identify the risk factors associated with WSD of *P.monodon* in a rice-shrimp farming system in Vietnam. The study site selected was Tan Chanh village, located in Can Duoc District (10° 30' North, 106° 36' East), Long An Province, in the northern part of the Mekong River Delta, 50 km south of Ho Chi Minh City, where RIA2 laboratories were sited. Tan Chanh was one of the 8 villages in the district that used the rice-shrimp culture system and was responsible for the production of over half of the total district production with 500 ha of ponds and almost 800 households involved in shrimp culture in 1997. Previous experience of RIA2 scientists suggested that a WSD outbreak was probable in this area during the study period.



**Can Duoc**



Meetings with the local authorities at village, district and provincial level were carried out in December 1997 and the district director of fisheries provided a list of all the names and addresses of farmers producing shrimp in the district. A convenience sample of 40 farmers was selected using the criteria of size; geographical distribution; presence of a shrimp crop and ease of access. Farmers were visited individually to explain the goals of the project, to determine if they were eligible for enrolment and to discuss the details of data collection. Eligible farmers who agreed to participate were interviewed using a structured questionnaire and invited to a pre-study meeting. Two pre-study meetings were held and resulted in the enrolment of the required 24 farmers.

Sampling started in January and finished in June 1998. At stocking, management practices were recorded, a sample of 500 PL was collected in order to assess shrimp health and size. Whole batch scores (i.e. PL activity and homogeneity in size) and individual PL scores (i.e. chromatophore distribution, proportion of abdominal muscle in the shell, gut fill, presence of deformities, fouling organisms and histopathological changes in the hepatopancreas) were analysed. During the production cycle water quality variables were measured and farmers interviewed twice weekly. Salinity, turbidity, total ammonia, alkalinity and planktonic pigments were measured. Variables subjected to diurnal variation (i.e. Dissolved Oxygen, pH and temperature) were measured twice daily on two days each week. Data was collected on number of dead *P.monodon* detected, the frequency and amount of feed, lime or fertiliser applied, the frequency and amount of water exchange. *P.monodon* and wild shrimp inside the pond were sampled by cast net at monthly intervals, the total number collected, external clinical signs, weight and length of individual shrimp were recorded. In addition, fixed management practices were measured at the end of study by questionnaire. Crabs were collected from the pond within a month after stocking. Pond bottom quality was assessed monthly by measuring pH, colour and smell. At harvest, presence and prevalence of clinical signs was recorded from 100 shrimp. Data on the weight, grades and number of *P.monodon* within each grade were collected by farmer interview. Variables on the presence of wild crustaceans and fish were also recorded.

More than 95% of the planned 950 farm visits were conducted on time. Communication with the farmers was maintained throughout the project and an excellent partnership developed between farmers and research assistants. The volume and quality of the data produced far exceeded expectations.

At the end of the data collection period a PCR laboratory was established at RIA2, where samples collected were analysed to detect WSSV. A total of 346 pooled samples were tested including 192

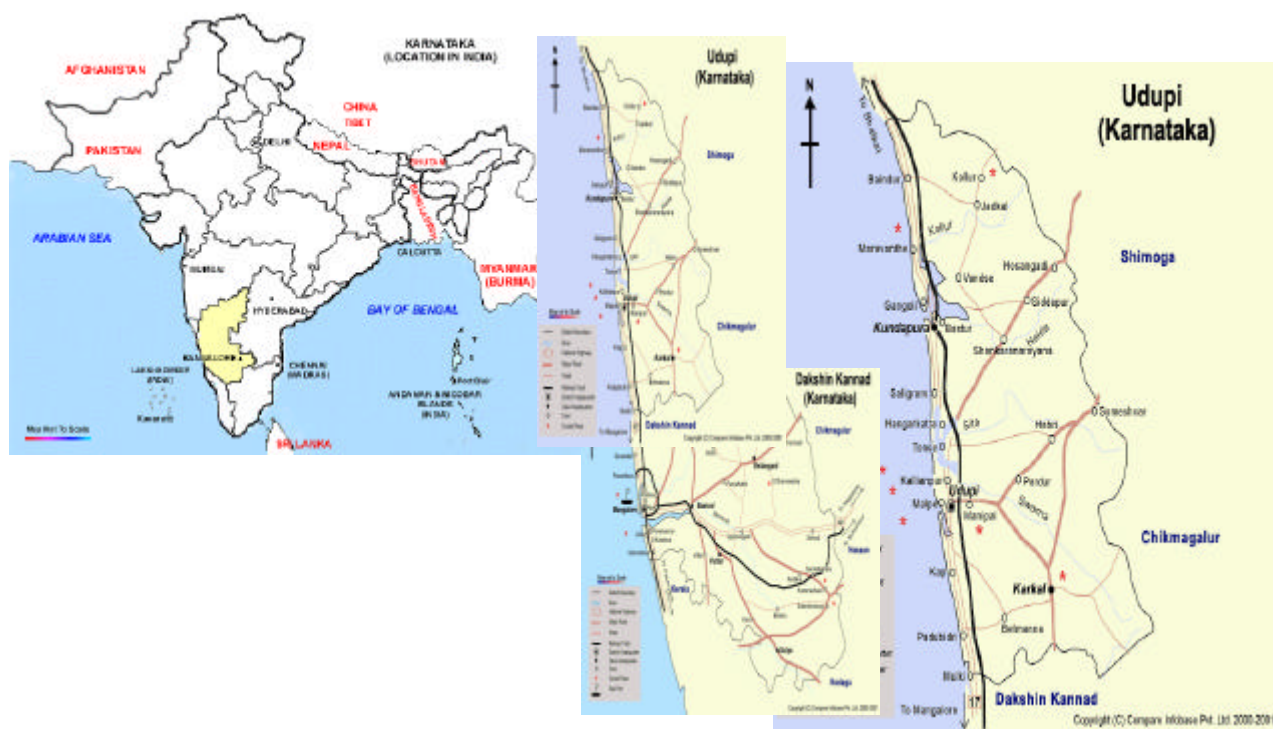
*P.monodon* PL; 46 *P.monodon* collected during the production cycle; 24 harvested *P.monodon*; 38 wild shrimp and 46 crabs. Histological sections of all *P.monodon* samples collected from stocking to harvest were also prepared.

Data collected were analysed using both univariate and multivariable methods. The presence of WSSV by PCR was used as outcome.

As a follow up of the longitudinal study since the end of 1998 the local coordinator (Dr NV Hao) carried out 3 projects. These have largely been funded from local Vietnamese sources but have also received some support from this project, e.g. supply of chemicals, supervisory advice and assistance with travel (see **OUTPUTS - Uptake of methodologies**). Preliminary results have been analysed and further analyses was conducted by KL Morgan, during the meeting in Mangalore in May 2000.

### Indian phase

The Indian phase of the study started in August 1999. Meetings between the project team and other staff from the Mangalore Fisheries College took place in order to select the area for the study. There are 3 shrimp farming areas in Karnataka, the state where the Mangalore Fisheries College is situated. Of these Kundapur was selected for its location, 110 km north of Mangalore, where the laboratories for sample analyses were located. It was also suitable because the local farming history made identification and selection of an appropriate sample population possible.



Stake holders including shrimp farms, hatcheries and feed companies were visited in order to improve understanding of the system. Subsequently all the farmers from Kundapur (150), surrounding areas (50), hatchery and feed staff were invited to a meeting. The meeting it was held in the centre of the study area and repeated 4 times during a day to accommodate the participants. The study team was introduced, the proposal explained and discussed and a short questionnaire was distributed in order to gather general data on previous crops, intention to stock and contact address.

In the Indian phase the team required a larger number of ponds (see **PROJECT PURPOSE - Methodologies and techniques**), 100 of the 150 farmers were selected using stratified random sampling. These 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 PL were collected by interviewing the farmer, while variables on the PL (e.g. activity and size) were measured by direct observation on a sample of 500 PL. Data on water quality (i.e. DO, pH, temperature and salinity) were also collected at stocking, together with samples of wild animals such as crabs, insects and polychaetes. From stocking till harvest farmers were supplied with sheets to record data on feeding regime, water exchange, addition of substances to the pond and presence and number of any dead shrimp. The sheets were collected and water quality measured during visits at a fixed time every week. During such visits feed samples and dead shrimp fixed by the farmer during the preceding week were also collected.

Six weeks after stocking a sample of 100 *P.monodon* was collected by cast-net, examined for clinical signs and fixed for PCR and histopathological examination. Samples of wild shrimp and plankton were also collected. At harvest, 400 *P.monodon* and 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. In order to test if the study population was representative of the whole population, at the end of the data collection period a questionnaire concerning all ponds in the Kundapur estuary was used to collect data on degree of success and some management practices.

In addition to the selected ponds, wild shrimp and plankton samples were also collected from the estuary twice weekly from 6 locations. Shrimp were also purchased each month from local fishermen (10 locations) and crabs were collected from outside the study ponds.

A PCR laboratory was established at the Fisheries College and samples were processed in order to test for the presence of WSSV. A total of 1340 pooled 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.

Histopathological sections from PL, moribund and harvested *P.monodon* were also prepared and examined for the presence of WSSV infection and other pathological conditions.

Data collected were analysed by univariate and multivariable techniques against the outcomes: presence of WSSV, yield per unit area, length of production cycle and a composite outcome including presence of WSSV, mortalities and length of production cycle.

## **CONTRIBUTION OF OUTPUTS TO DEVELOPMENTAL IMPACT**

### **Project goal as stated in Project Memorandum Logical Framework (Appendix III)**

*Impact of disease on aquaculture production in Asia reduced.*

As stated in **BACKGROUND** WSD is one of the most damaging aquatic animal diseases ever encountered in both economic and social terms. However, it is extremely difficult to accurately determine the impact of any aquatic disease. Therefore quantification of the impact of a research project on livelihoods is very difficult. As initially anticipated evaluation of the impact of this project is outwith the scope and resources available. However, a concept note has been submitted to DFID, if funded will in addition to continuing the research initiated in this project also examine methods to evaluate uptake and impact of research projects such as this.

As already described the project fulfilled or exceeded its original objectives in all areas. Scientific understanding of WSD and methodologies for studying aquatic disease in general have been improved and there is evidence of both dissemination and uptake. All agreements with farmers were fulfilled and the information on the farming system and WSD were reported back to them using a variety of techniques, within one year of the start of data collection. Comments and responses to project evaluation forms suggested that the farmers found the description of the system

information very useful. Allowing them to compare their activities with those of farmers performing better and worse than themselves.

Risk factors were identified and decision-making tools supplied to vulnerable farmers. Although no formal intervention study has been initiated, anecdotal evidence from both study sites suggests that farmers have changed farming practices in response to the findings of this project. According to Vietnamese authorities, the rate of failure of farms in the study site decreased from 90% in 1998 to 20% in 2000. It would, however, be inappropriate to attribute any specific proportion of this improvement to this project.

In terms of contact and dissemination those stakeholders contacted directly can be quantified while the broader dissemination of findings through teaching and other materials is more difficult to assess. Below direct contact with farmers and other stakeholders is detailed at the end of the Vietnamese and Indian phases of the project.

<b>Activity</b>	<b>Number of people involved</b>	<b>Nature of participants</b>
Farmers meeting Vietnam	35	Farmers and farmers group leaders
Extension workers meeting Vietnam	45	Extension workers, local researchers and government officials
Individual meetings Vietnam	48 (average of 2 per meeting)	Farmers and family
Farmers meeting India	265	Farmers and families, workers, hatchery employees, feed company employees, researchers and others
Individual meetings India	68	Farmers, families and workers

There is an ever increasing need for projects of this nature and dissemination of existing results. At the start of the project it was estimated that 10,000 families relied on rice-shrimp farming in the Mekong delta. A recent communication from Can Tho University has suggested that there has been a dramatic increase in the number of rice-shrimp farmers as a result of the perceived success of existing farmers. However, in the last year it is estimated that more than 110,000ha of shrimp culture failed due to high mortalities. Given an average pond size of 0.33ha, this represents one third of a million families affected by severe losses.

The issues associated with this increase and failure of a farming system are complex. There has been encouragement from the Vietnamese Government to engage in shrimp farming which is a high risk form of farming and may be inappropriate for vulnerable families. However, the expansion of the industry suggests that there are methods by which changes in farming practice can be

encouraged. Such issues will be addressed in the project memorandum for the next phase of this project.

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**Appendix II**  
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## Appendix III

### LOGISTIC FRAMEWORK FROM PROJECT MEMORANDUM

Hierarchy of Objectives	Objectively verifiable indicators	Mean of Verification	Important Assumptions												
<p><b>Goal</b> Impact of disease on aquaculture production in Asia reduced.</p>	<p>By the end of the project : - improved health management and disease investigation techniques in use.</p>	<p>- National production statistics and - research project reports</p>	<p>- Climatic and enabling environmental conditions remain favourable to aquacultural production and - aquatic disease resources in the region maintained.</p>												
<p><b>Purposes</b> Improved techniques for study and control of aquatic disease in the Asia through the development of knowledge, skills and protocols in aquatic epidemiology, focusing on shrimp White Spot Disease (WSD) in artisanal systems in India and Vietnam.</p>	<p>By the end of the project : - epidemiological methodologies for the study and control of aquatic disease developed - information and techniques extended to participants and others</p>	<p>Project and meeting reports.</p>	<p>Existing regional disease control resources maintained.</p>												
<p><b>Outputs</b> 1. Risk factors for WSD outbreaks identified.  2. Rational intervention strategies designed.  3. Project findings developed and disseminated, understanding and capacity in aquatic epidemiology in key regional organisations promoted.</p>	<p>1. By the end of the project : - risk factors for disease identified, - epidemiological methods and results published.  2. The initiation of preliminary field trials in Vietnam and production of subsequent grant proposals for controlled application of further intervention strategies.  3.- Participants use of epidemiological skills demonstrated, - strategies being applied for this and other diseases, - teaching material produced.</p>	<p>Peer review publications. Conference presentations. Project reports.  Project reports. Meeting/workshop reports. Proposals for future research projects.  Extension and other material. Project reports. Skills/capacity analyses.</p>	<p>Access to field sites obtained. Diseases occur at different times on selected sites. Key participants remain available and comply with obligations.</p>												
<p><b>Activities</b> 1.1. Study sites recruited in Vietnam and India; data collectors trained; 1.2. questionnaires and sampling procedures developed, 1.3. data collected and analysed, 1.4. multivariate analyses for risk factors of WSD completed, 1.5. farm level risk factors for WSD identified; results presented to participating farmers.  2.1. control measures prioritised 2.2. , intervention strategy field trials designed, 2.3. project proposal for intervention study produced.  3.1 Reports, publications and teaching material prepared. 3.2 Epidemiological teaching material produced.</p>	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 60%;">Staff</td> <td style="text-align: right;">72,271</td> </tr> <tr> <td>Overheads</td> <td style="text-align: right;">35,175</td> </tr> <tr> <td>Equipment</td> <td style="text-align: right;">20,378</td> </tr> <tr> <td>Travel + overseas costs</td> <td style="text-align: right;">13,691</td> </tr> <tr> <td>Miscellaneous</td> <td style="text-align: right;">41,000</td> </tr> <tr> <td><b>Total</b></td> <td style="text-align: right;"><b>£182,515</b></td> </tr> </table>	Staff	72,271	Overheads	35,175	Equipment	20,378	Travel + overseas costs	13,691	Miscellaneous	41,000	<b>Total</b>	<b>£182,515</b>	<p>Quarterly, annual and final reports.</p>	<p>Farmers participate in study. Political environment allows field work to progress. Key participants remain available and comply with obligations.</p>
Staff	72,271														
Overheads	35,175														
Equipment	20,378														
Travel + overseas costs	13,691														
Miscellaneous	41,000														
<b>Total</b>	<b>£182,515</b>														

## **Appendix IV**

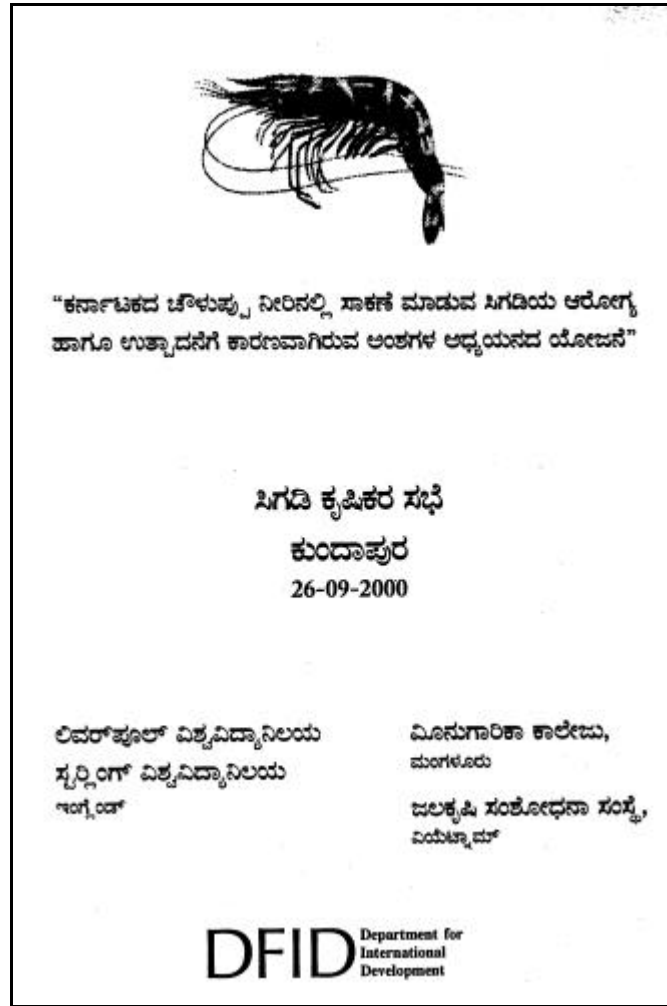
### **DRAFT MANUSCRIPTS ACCEPTED WITH REVISION OR IN PREPARATION**

This appendix contains the latest available draft of publications from this project. These should not be taken as the definitive version and are confidential since they have not yet been published.

## Appendix V

### SPECIMENS OF FARMERS REPORTS AND INFORMATION

Four examples of the information supplied to and discussed with farmers are included here. The individual confidential report (A) was supplied to farmers in Kundapur in English and Kannada. The notes supplied to accompany the farmers meeting in Kundapur (B) were supplied in English with an expanded set of notes covering all the presentations in Kanada. The general report to participating farmers in Long An (C) and the individual reports (D) were supplied only in Vietnamese.



Front page of notebooks supplied at Indian farmers meeting