The Final Technical Report for DFID-project R 7463

STRATEGIES FOR IMPROVED DIAGNOSIS AND CONTROL OF BACTERIAL DISEASE IN SMALL-SCALE FRESH WATER AQUACULTURE

July 1999 – March 2001

Dr K D Thompson and Dr M Crumlish

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Collaborators

- Dr Kim Thompson and Dr Margaret Crumlish, Institute of Aquaculture (IOA), Stirling, Scotland, UK
- Dr. Supranee Somsiri, Dr. Temdoung Somsiri and Ms. Yolprapa Dumrongphol, Aquatic Animal Health Research Institute (AAHRI), Dept. of Fisheries, Kasetsart, University Campus, Pahalyofhim Rd. Jatuyak, Bangkok 10900 Thailand
- Ms. T.T Dung and Ms N.T.N. Ngoc Aquaculture and Fisheries Science Institute (AFSI) College of Agriculture, Cantho University, CanTho, Vietnam

1.1 Executive Summary

Project R7463 represents a 21-month study, the results of which are presented in this report. The aims of the project were to establish the importance of bacterial disease to small-scale freshwater fish farming households in Thailand and Vietnam, to determine measurable biological indicators of disease, to promote fish health and in turn enhance fish production. Ultimately, the object of the project was to develop strategies to reduce disease outbreaks using the project findings, and establish guidelines to help farmers improve their husbandry management, so as to mitigate losses due to disease and increase harvest yields.

An initial 18-month study (R7054) was funded by DFID to examine the relationship between environmental conditions, bacterial load in the water and bacteria levels in tissue macrophages of a range of clinically healthy freshwater fish species, farmed in a range of culture systems in Thailand and Vietnam. A relationship was found between water quality, bacterial load in the pond and the occurrence of bacteria within macrophages. Also a field-based sampling technique was developed to provide a quick and simple method for isolating macrophages at the pond-side to be used to assess macrophages for the presence of bacteria. Preliminary studies were made to examine the significance of the bacterial load within fish macrophages and to determine the relationship between the ecosystem/bacteria /macrophage using a range of representative target systems. It was intended to develop guidelines from this work for management strategies and bacterial disease control. However, it was evident from the study that there was a fundamental lack of knowledge about the incidence of bacterial disease in the small-scale fresh water systems and the significance of bacterial disease on the livelihoods

of rural small-scale freshwater fish farmers in Thailand and Vietnam. This information is paramount if effective and useful strategies are to be developed.

These issues were addressed in project R 7463 by surveying fish farmers in Thailand and Vietnam, collecting information relating to their fish husbandry problems and fish disease experiences, trying to determine the frequency of disease related problems within their farming systems, and if possible establishing potential risk factors for disease outbreaks and water quality problems. From this it was hoped to gain a better understanding of the economic impact of bacterial disease in aquaculture on the livelihoods of these fish farming households. Attempts were also made to establish additional field-sampling techniques for assessing the health status of the fish, to be used as indicators of potential disease in fish. It was planned to use these in conjunction with the survey information to help develop strategies to improve disease control management, and thereby increase yields, reduce risks and improve the livelihoods of the small-scale fish farmer.

The research activities as specified in the logical framework of the project resulting in the following outputs.

- Data relating to the number of Aquaculture families in the provinces surveyed in Thailand and in the Mekong dealt of Vietnam established. Old Department of Fisheries lists of fish farmers were out of date and did not reflect current farmers involved in aquaculture.
- Data relating to the frequency of disease related problems within small-scale fish farming systems was collected together with information on their husbandry practices and disease control strategies used by the farmer. The etiological agents of the outbreaks were not identified however, as no biological samples were taken at the time of the interviews or during periods of disease or poor water quality.
- Some relative risks associated with disease were identified but these will be address further in DFID project "The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119)".
- In response to the findings of the surveys and the request of extension officers, a proposal has been submitted to the DOF of Thailand by AAHRI to set up central laboratories in each province to deal specifically with disease diagnosis and control/ treatments of disease.
- An important finding from the extension officers' questionnaire was that 75% of extension officers claimed that they felt that they did not provide an adequate service for the fish farmers. Reasons for this were thought to be due to their lack of technical knowledge, their lack of appropriate equipment for sampling and the lack of people to help conduct sampling work.
- This study was the first examination of the immune competence of fish at the pond-site combining two simple assays, which could be performed in the field; the isolation of macrophages stained for the presence of bacteria, and the ability of isolated macrophages to phagocytose yeast. The use of a combination of other simple field-based tests may provide a means of examining the immuno-competence of fish within these culture systems and provide potential indicators of disease.
- The clinical significance of the bacteria within the macrophages remains to be determined, but from studies in the laboratory, "live" *A. hydrophila* appears to inhibit the respiratory burst of macrophages, suggesting that the bacteria may in fact be immunosuppressive.
- New projects have directly resulted from DFID-project R 7463 which will further address the issues dealt with in the current project.

1. Risk issues and management strategies for Bacillary Necrosis Disease (BNP) and other factors for Pangasius spp. farming in the Mekong Delta, Vietnam (R 8093)
2. The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119)

- This was originally a very technically based project, examining the incidence of bacterial disease in small-scale fresh water systems in Thailand and Vietnam and establishing strategies to control disease within these systems. However from the work so far, it is evident that there is a lack of understanding by the farmers as to what disease is and how it can affect their systems and livelihoods. We need to understand the farmers' perception of disease, verify true disease outbreaks and establish the impact of disease on the livelihoods of the farmers before we are in a position to establish the incidence of disease and develop strategies to alleviate it.

- It was difficult to define small-scale fish farms according to the DOF classification of fish farms i.e. whether the system was extensive, semi-intensive and intensive. In the opinion of the interviewer semi -intensive farming according to the questionnaire classification is large pond or a number of ponds where regular feeding is given. There appears to be two different levels of fish farming, especially in Thailand both of which could be found located in each province visited

  1. Farms where there is little input; fish farming is not the main source of household income and the farmer experiences few/no diseases or water quality problems possibly because the farmers don't have any concept of these and they are not important to them

  2. Farms where fish farming is the main occupation and disease appears to be a problem. In this case maybe the farmers have a better concept of disease.

This will be address further in DFID project “The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119)”.

Unanswered questions

- How small-scale fish farmers understand ‘fish losses’ and fish health management within their systems.
- What the farmer’s understanding of his farming system is and where it fits in with the families livelihood activities
- Determine the impact of fish losses on livelihoods of rural families involved in raising fish in Thailand and Vietnam.
- What is the farmers perception of problems within the systems specifically related to ‘fish losses’
- To understand the knowledge and language used by fish farmers to describe their systems and fish losses
- To establish the causal factor for the fish losses.

Some of these questions will be addressed in DFID project “The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119)” and will be put in context using key informant and group interviews in Thailand and Vietnam. Target groups will be identified from the information collected in surveys.

1.2 Background to the Project

Fresh-water aquaculture is a valuable contributor to nutritional and income demands of rural and peri-urban populations within developing countries. In the fresh water systems of South East Asia, continuous low level losses or poor growth of fish due to bacterial disease can be economically devastating for rural fish farmers and artisanal fishers. In small scale fish farming systems, widespread through the region, simple disease detection and management, supported by good diagnostic techniques and skills in local institutions may help to create substantial positive impact.

High bacterial loads have been reported within the aquatic environments of the fish farms in Thailand[1]. The levels of bacteria observed within the isolated macrophages were generally low (<1%), but were seen in a variety of fish species cultured in a range of freshwater farming systems in Thailand. Bacteria identified as Aeromonads and Pseudomonads were predominantly recovered and identified from the macrophage cell suspensions isolated from the anterior kidney of clinically healthy farmed fish[1,2,3].

An initial 18-month study (R7054) was funded by DFID to examine the relationship between environmental conditions, bacterial load in the water and bacteria levels in tissue macrophages[4,5,6,7] of a range of clinically healthy freshwater fish species, farmed in a range of culture systems in Thailand and Vietnam. A relationship between water quality, bacterial load in the pond and the occurrence of bacteria within macrophages was found. A field-based sampling technique was also developed to provided a quick and simple method for isolating macrophages at the pond-side[3,8]. Fish cultured in these systems co-exist with the systemic bacteria, appearing to be free from disease, though implications for incipient disease are not clear. Since many of the bacteria are opportunistic pathogens, they may pre-dispose the animal to disease.

It was evident from the results of DFID project R7054, that there was a fundamental lack of knowledge about the incidence of bacterial disease in the small-scale fresh water systems and the significance of bacterial disease to the farmers' livelihood. This information is essential if effective and useful strategies are to be developed.

The intention of the present study was therefore to address these issues by surveying farmers in Thailand and Vietnam to find out more about their husbandry problems and their experiences with fish disease, to try to determine the frequency of disease related problems within their farming systems and if possible to establish potential risk factors for disease outbreaks and water quality problems. From this it was hoped to gain a better understanding of the economic impact of bacterial disease in the region. Further attempts were made to establish new field-sampling techniques for assessing the health status of the fish, to be used as indicators of potential disease outbreaks. It was planned to use these in conjunction with the survey information to help develop strategies to improve disease control management, and thereby increase yields, reduce risks and improve the livelihoods of the small-scale fish farmer.

1.3 Project Purpose

The aim of the proposal was to establish the importance of bacterial disease within the small-scale freshwater systems in Thailand and Vietnam, and determine measurable biological indicators for the control of disease outbreaks, promote fish health and enhance production. Ultimately, the object of the project was to develop strategies to reduce disease outbreaks using the project findings, and establish guidelines to help farmers improve their husbandry management, so as to mitigate losses due to disease and increase harvest yields.

1.4 Research Activities[1] and Research Outputs

1.4.1 Activity (1)

Three surveys, executed through local institutions, extension officers and farmers, were carried out to establish the incidence of bacterial disease in small freshwater aquaculture systems in Thailand and the Mekong Delta in Vietnam. These were used to examine how disease outbreaks were related to husbandry management and environmental conditions in Activity (3). A diverse range of fish species and culture systems were surveyed. Fish farms of varying productivity or that have experienced periodical disease outbreaks were included in the survey. Because of the problems fish farmers had recalling information a record keeping study was carried by farmers in the Mekong Delta.

1.4.1.1 THAI FISH FARMER SURVEY DATA: GENERAL FINDINGS (See Appendix 1)

A survey of small-scale freshwater fish farmers was conducted in 2000 by staff at the Aquatic Health Research Institute (AAHRI), Bangkok, to assess farmers' problems associated with husbandry and fish disease and to try to establish the frequency of disease related problems. The households selected represented farms in the North, North East, Central and Southern regions of Thailand and were selected because they carried out freshwater aquaculture as one of their household activities. In total, 304 households in 24 different provinces were surveyed.

The target population of the survey was 'rural poor fish farmers'. However, after pilot testing the questionnaire around Bangkok in Central Thailand and in the Northeast region of Thailand it was found to be more difficult than expected to reach the 'rural poor fish farmers '. This was because they lived in remote areas, which were difficult to reach, especially in the Northeast region of Thailand. Hence, the survey sample frame was extended to include extensive, semi-intensive and intensive fish farm systems. A structured questionnaire was designed in English using Microsoft Word and then translated into Thai. The survey consisted of sections on fish disease outbreaks, clinical signs of disease and treatments applied. The questionnaire was pilot tested and adapted as required for Thailand as recommended by the Thai research team. It was later found that the word for "disease" in English means "health" in Thai so when the research team asked questions about "fish disease" they were actually asking about "fish health".

The farms were randomly selected using a devised survey sample frame, which was designed to include only families currently involved in small-scale freshwater fish farming. Overall, the research attempted to visit a total of 3,614 households, while the number successful visited was 304 farms. The level of compliance was therefore 8%.

Relative risks and potential risk factors were identified from different exposure variables associated with disease or water quality as outcome variables from data collected from the fish farms (discussed under Activity 3 below). Analysis has also been partially carried out comparing the farming practices and disease issues between the four regions of Thailand, but the results of this will be presented under DFID project "The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R

[1] As stated in the Logical Frame of the RD1

8119)"; a new DFID project (PI: Jimmy Turnull) in which some of the issues arising from the current project will be address.

Research Outputs from Thai fish farming survey

Main Findings of Survey

- Only 69 households out of 304 households visited claimed to have aquaculture as their main source of income and only 39 of these claimed to had experienced disease problems on their farms
- Many of the families interviewed owned the land on which their farm was based, and tended to use only one pond, even if they had access to additional ponds.
- Although some of the families interviewed had been involved with fish farming for many generations, there appeared to be an increasing number of people who were starting to practice aquaculture.
- Many of the families interviewed had a diverse portfolio of livelihood activities and very few carried out only fish farming as their main source of income.
- Although some of the families interviewed had been involved with fish farming for many generations, there appeared to be an increasing number of people who were starting to practice aquaculture.
- Although many families regarded it as source of income, it is not regarded as important as other sources of income, rather one that could be used sporadically.
- Of the farms visited, 288 households used ponds to farm their fish, while the reminder used small and large cages and pens. The most common pond size was 400 m^2
- The most common type of small-scale freshwater system used in inland Thailand was polyculture ponds.
- More households cultured tilapia compared with any other fish species
- Most families harvested their fish for family consumption as well as for local markets
- Generally, households involved in small-scale fish farming stocked and harvested their fish ponds throughout the year.
- The average size of fish at harvest ranged between 0.11-0.3 kg and the size appeared to be related to the species that they farmed, the size of the pond used and the market purpose of their harvest.
- The majority of households fed their fish with pellets, but a proportion also used rice bran and vegetables
- Relatively few families reported ever having had experienced poor water quality
- Less than 40 % of farmers had encountered health related problems and these tended to occur mostly during November to February, but the highest number of farmers reported problems December.
- The information generated from this survey suggested that families involved with fish farming were aware of the importance of good pond preparation, high quality nutrition at strategic points and also the importance of good water quality.
- Most of the fish farming families relied on other farmers or their own experiences for diagnosing and treating their stocks.

The primarily purpose of the survey was to gather information to assess the problems associated with husbandry and fish disease and to try to establish the frequency of disease related problems within small-scale fish farming systems. Further work is required to establish factors, which influence families to make the decisions they do about their fish farming livelihoods. The species cultured by farmers were locally found species, and a higher number of families interviewed produced fish for local market and for family consumption. Therefore, it may be market influences, which dictate the species of fish cultured and the size of fish harvested for sale at the market. Generally, households involved in small-scale fish farming stocked and harvested their fish ponds throughout the year. Fish could be harvested as and when required depending on whether the fish were to be used to feed the family or sold at the market. Although many families regarded it as source of income, aquaculture was not regarded as the main source of income, rather as a resource to be used when the family required additional money.

The information generated from this survey suggested that families involved with fish farming were aware of the importance of good pond preparation, high quality nutrition at strategic points and also the importance of good water quality. It appeared that most of the families understood the importance of providing good quality/high protein diets at the juvenile/fingerling stage, since most families fed pelleted diets at this time. At other times throughout the production cycle most households used household/animal waste and agricultural by-products such as rice bran. Further work is needed to evaluate the farmers understanding of poor water quality, as relatively few families reported ever having had experienced poor water quality. This may reflect the farmers' awareness of water quality, since those who reported having poor water quality provided detailed lists of the problem and the time the problem occurred. Since no water samples were taken at the time of the survey, there is no substantiation as to whether farmers had in fact experienced water quality problems or not.

Just under half of the families involved in the survey experienced and described diseased fish from a range of indicators provided. Interestingly, the majority of households thought that their fish were most susceptible to disease problems during the winter months and also again when most of the families were stocking their ponds. Daily water temperature fluctuate during Thai winters, and this in turn can lead to immunosuppression in fish, which has severe implications for the health status of the animal. Stocking is a critical period in the production cycle for which farmers need an adequate knowledge of fish husbandry to ensure a suitable environment for the culture of the young fish.

The results of this work highlighted that most of the fish farming families relied on other farmers or their own experiences for diagnosing and treating their stocks. This was emphasised by the fact that many of the families obtained their own chemicals and drugs to treat the fish without asking advice and guidance for either extension services or research/diagnostic laboratories.

1.4.1.2 VIETNAMESE FISH FARMER SURVEY DATA: GENERAL FINDINGS (See Appendix 2)

A similar survey was also conducted of families involved in freshwater aquaculture in the Mekong Delta, in Vietnam. This was performed by research staff from AFSI in 2000 and covered 7 different provinces within the Mekong Delta. including Cantho, Vinh Long, An Giang Dong Thap, Tien Giang, Long An and HoChiMinh. In total, 201 families were interviewed using a similar structured questionnaire, which had been adapted for the Vietnamese fish farmers. Again the target population of the survey was 'rural poor fish farmers'.

<u>Main Findings of Survey</u>

- Farms were generally been established from between 1945 and 1999, with 3 main periods of development identified [1990 (n=21), 1995 (n=21) and 1998 (n=24)].
- Of the households interviewed, 83% (n=166) had previous farming experience and of these families 71% (n=141) had previous experience in fish farming.
- The other types of farming practised on the farms visited included growing rice, fruit vegetables and animals.
- More families used polyculture systems to culture their fish, compared with the other types of farming system quoted. A number of farmers used more than one type of culture system on their farm.
- Only 14% (n=25) of the families interviewed had fish farming as their main source of income. The other 86% (n=149) households had a wide range of other livelihood activities
- Nearly all of the families interviewed cultured fish in ponds rather than cages.
- A high number of families owned between 1 or 2 ponds
- The size of the fishponds varied from 12.5 to 14,000m^2 (n=197), however, the most common size was 100, 200 and 500m^2.
- A higher number of families claimed to stock their fish farm during April, May and June compared with any other time.
- The main months in which fish were harvested tended to be March, April and December
- Farmers in the Mekong Delta cultured a wide range of fish species with more than half of the households interviewed culturing *Pangasius hypophthalmus* either in monoculture or polyculture systems. The six most popular species cultured were *P. hypophthalmus*, tilapia, silver barb, common carp, kissing gourami and giant gourami
- Farmers obtained their fry/fingerlings from a variety of different sources with the most common source of fish seed being from middlemen/traders or hatcheries.
- Fifty two % of families observed fry mortalities on arrival.
- The stocking density of fingerlings ranged from 0.5 fish to 82 fish m^3 with most families stocking at either 2, 4, or between 10-20 fish m^3.
- More families knew the individual weight per fish at harvest time rather than the total weight at harvest
- The fish produced by a large number of households tended to be either for household consumption or for selling at the local market. A high number of families only produced fish for family consumption.
- Eighty four percent of farmers prepared their pond before stocking.
- Eighty eight percent of families interviewed fed their fish with only 12% of households not feeding their fish at all
- Half of the families interviewed said that they did not experience any water quality problems
- Water quality problems were found to occur throughout the year, but a higher number of families reported poor water quality in January, March and April compared with any other time
- In total 70% of families always exchanged water in their ponds irrespective of whether there was a water quality problem.
- Only 11% households kept some form of written records related to their fish farming activities, relating mainly to production costs.
- Forty nine percent of fish farming families claimed to have experienced fish disease on their farms at some point during fish farming which tended to occur throughout the year, although a high number of families reported disease outbreaks in November and

- Fish diseases appeared to occur throughout the year, but a higher number of families reported disease outbreaks in their stocks from October to January compared with any other time of year.
- Six percent of families claimed to have received some form of training in fish health and disease outbreaks mainly from the extension officers.
- Only 10% of farmers claimed to contact the extension officers with most families relying on their own experience or advice from neighbouring farmers to diagnose disease.
- Treatment given ranged from providing antibiotics mixed in the feed, to giving antibiotics and chemical treatments such as lime, malachite green and salt.
- Twenty-nine percent farmers did not give any treatment, as they were unsure of what treatments to give or how to apply them.
- Most fish farmers interviewed purchased treatments from pharmacies.
- Information and advice on diseases, and treatment were mostly from families' previous experience or other farmers.

Fish farming appeared to be only one of many livelihood activities that the households were involved in. A higher number of households had polyculture systems compared with monoculture or other types of systems, and this probably reflected the market purpose of the fish being produced. Most of the families interviewed not only sold their fish locally, but used the fish to feed their family, and so having a diverse range of fish species in their farming system ensured that they always had food available or something to sell.

The most common species cultured was the indigenous Asian freshwater catfish (*P. hypophthalmus*), which has been farmed in Mekong delta for a number of decades. However, a wide range of fish species were cultured and again this was probably influenced by the types of farming systems used and the market purpose of the fish produced. Smaller sized fish were more popular with the local consumers, and indigenous species are more frequently purchased, because they are recognised by the local market-goers.

Half of the farmers interviewed had experienced water quality problems and disease problems at some point. No biological samples were taken at the time of the survey to confirm the farmer's interpretation of these problems. However, it was interesting to observe from the frequency data that fish farmers appeared to rely on their own experience and the knowledge of other farmers for advice on the management of water quality problems and disease outbreaks. The fish farmer commonly applied treatments when diseases were recognised, and again this was usually after self-diagnosis of the problem. It was often the opinion of the AFSI research team that many households did not report problems on their fish farms because they lacked understanding of the concept of disease and water quality problems. Households were frequently unaware that fish could have disease problems or that poor water quality could affect fish and result in fish mortalities. This was reflected in the fact that few families had received any form of training on fish health and disease management.

1.4.1.3 SURVEY ON THE AQUACULTURE ACTIVITIES OF EXTENSION OFFICERS IN THAILAND (See Appendix 5)

A survey of aquaculture activities of extension officers in four different regions of Thailand (the North, North East, Central and Southern regions) was conducted by the

Aquatic Animal Health Institute (AAHRI) in Bangkok. A structured questionnaire was sent to the extension services of 26 different provinces in these regions. Personal interviews were conducted in provinces more accessible to the institute, while postal questionnaires were sent to the extension services in provinces further away from AAHRI and more difficult to access i.e. Nakornpathom, Ayutha, Pattalung, Chaingrai, Trang, Pijit, Nakhonna-yok and U-Bonratchathani.

A similar survey was conducted in the Mekong Delta of Vietnam, but the results of that particular survey still need to be translated from Vietnamese into English.

Research Outputs from Extension Officers survey

Main Findings of Survey

- Sixty-nine percent (n= 57) of the extension officers believed their main activities, which related to aquaculture, were providing advice to fish farmers and promoting small-scale freshwater aquaculture within their region. The majority of extension officers questioned said their knowledge of fish farming was obtained from "on-job" training and previous experience, although a relatively high number of them had also had attended university. Eighty-three percent (n= 57) of extension officers had received some form of training from the Department of Fisheries (DOF). No information was obtained about the contents of the training courses.
- Of the extension officers who replied to the questionnaire, it was found that they had responsibility for between 34 to 800 villages, and most of the officers were responsible for more than 20 fish farms in total. Sixty two percent of these officers (n=50) claimed to visit fish farms monthly and 15% (n=12) of officers claimed to visit fish farms weekly. Twenty percent (n=16) visited when the farmer contacted them only, 1% (n=1) did not know when they visited and 1% (n=1) did not never visited fish farms.
- Extension officers were asked to rank the importance of different issues that the farmers seemed most concerned about. A higher number of extension officers thought that anti-theft strategies, water exchange rates and use of lime were the most import questions most frequently asked of them. The least important issues as judged by the extension officers were stocking density, treatments, diseases and disease prevention.
- Forty-three percent (n=36) of extension officers had received some form of training predominantly from the DOF and 63% (n=20) of these officers had received training in fish health and disease prevention.
- Extension officers claimed to use various methods to recognise diseased fish. A greater number of extension officers used a combination of signs to recognise disease including change in the fish's appearance, fish not feeding, farmers' description of abnormal fish, clinical signs and the number of mortalities, which occur in the pond. According to the extension officers, the main cause of health problems in fish farm was related to poor husbandry.
- Ninety-nine percent (n=81) of extension officers interviewed claimed that they recommended treatments to fish farms were asked. Most of the officers claimed to use their own experience or previous knowledge to determine which treatment to recommend. Twenty-three percent of officers did claim to contact research staff to ask advice and guidance before recommending treatments.
- Ninety-eight percent (n=79) of extension officers interviewed claimed to recommend prophylactic treatments. However, very few (n=3) officers claimed to recommend antibiotics. The most common prophylactic treatment recommended was related to

good farm management, such as preparation of ponds, water quality, and guidance on choosing fingerling and stocking density. More that half of the extension officer who replied to the questionnaire said that they returned to the fish farm after the farm had experienced a disease outbreak.

- Nearly all extension officers (87%) said that they would like to have additional training relating to disease issues, and 86% of them also wanted to have additional resources for disease control i.e. training, technical information, documentation and data. Some also wanted central laboratories in each province to deal specifically with disease diagnosis and control/ treatments of disease.
- An important finding from the questionnaire was that 75% of extension officers claimed that they felt that they did not provide an adequate service for the fish farmers. Reasons for this were thought to be due to their lack of technical knowledge, their lack of appropriate equipment for sampling and the lack of people to help conduct sampling work.

One of the main findings of the study was the size of the area and number of fish farms that individual extension officers were responsible for. These tended to be very large and therefore it was difficult for many of the extension officers to visit their designated farms on a regular basis. However, it was apparent that the officers did attend training courses and they did appear to pass on information to the fish farmers. Nevertheless, it was interesting that a high percentage of officers who replied, thought that they did not provide an adequate service to fish farming communities felt that they needed greater support in the form of training and access the literature.

1.4.1.4 FARMER RECORDING KEEPING STUDY (see Appendix 4)

It was evident from the results of a fish farmers' survey carried out in the Mekong Delta, Vietnam (Appendix 2), that many small-scale farmers were unable to recall information related to the fish farming practices that they carried out either on a day to a basis, or generally. For example many rural families did not known the number of fish in their pond or were unable to calculate the productivity of their farm, since they did not have the relevant information to do so.

It was found from the fish farmers survey (Appendix 2), that 11% (n=22) of farmers interviewed kept some form of written record about their fish farming activities. The remainder of farmers interviewed found it difficult, if not impossible, to recall information relating to their farming activities.

The information recorded by these farmers was related to their costs associated with fish production. Only one farmer recorded information relating to disease outbreaks in his pond. Information relating to fish losses due to disease is necessary to help establish the economic impact of disease outbreaks on the livelihoods of the household.

A record keeping study was initiated to establish if keeping written records was compatible with farmer's lifestyles, how useful these were to rural small-scale farmers producing fish for the local market and whether they thought that such an exercise would help increase their overall productivity. Two groups of fish farming families located in the Mekong Delta region of Vietnam were invited to participate in the study. The first group of farmers were visited by the research team at the beginning of the study, and again at the end of the study. Half of the farmers were chosen from the list of farmers who had participated in the fish farming survey and who had reported having problems with disease in their stock (Appendix 2). The remaining half of the group was unknown to the research team, and they were randomly selected from lists supplied by the Department of Fisheries and the provincial extension services listing families who had fish farming as part of their livelihood activities. The second group of farmers consisted of a much smaller group (n=9), who were visited by the research team throughout the study period. These farmers were selected from the previous fish farmer survey that had previous disease problems on their farms (Appendix 2). The research team visited each farm in this group at monthly internals to take biological samples (see Appendix 3), to see how the farmers were coping with the records keeping activity, and to advise and encourage them to continue with this activity.

Research Outputs from record keeping study

Main Findings of Study

- At the onset of the study, a high number from both groups of farmers said that they were willing to participate in the study (97% of families asked), irrespective of whether the farmers had had previous contact with the research team.
- However, their willingness to participate in the study was not reflected in their ability or commitment to maintain the records throughout the six-month study period.
- They tended to be involved in many other livelihood activities, which contributed towards the total household income.
- Of a total of 37 farmers in Group 1, only three farmers kept detailed records for the duration of the study and had had no previous contact with the research team.
- The nine farmers from Group 2 recorded very little information apart for one family.
- The results of the record keeping study show that previous contact with the research team had no bearing on the Vietnamese fish farmers' willingness to participate in the study.
- Regular contact with the research team (through monthly visits) did not improve the farmers' willingness to perform the activities asked of them.
- Many farmers did not the exercise as beneficial and did not appreciate the potential usefulness of such an activity for improving their fish production or their household income. Only three or four families in the first group thought that the exercise was useful and would considered continuing recording their fish farming activities.
- The research team found that most farmers, independent of the group the farmers had come from, were eager to receive information relating to husbandry, management and disease prevention for aquaculture.

Only a handful of farmers actually documented sufficient information to be analysed. Many farmers did not view the exercise as useful and did not see the benefit of keeping records on their livelihood activities. Only three or four families (the ones which kept records in the first group) thought that the exercise was of use and considered continuing with this activity for their own benefit. This in turn may be linked to their perceptions of how important fish farming is to their livelihood activities and how much time they spend fish farming.

The research team found that all of the farmers involved in the study were eager to receive information relating to husbandry, management and disease prevention for aquaculture. This may be one reason why they were willing to participate in the study in the first place.

1.4.2 Activity (2)

The relationship between husbandry practice, environmental conditions, and seasonal influences on disease susceptibility of farmed freshwater fish was examined in an attempt to develop indicators of potential disease outbreaks. This was achieved through (1) field based sampling and (2) laboratory-based studies to examine the clinical significance of the bacterial load within macrophages of health fish and (3) pond studies to simulate environmental conditions found at farms.

1.4.2.1 BIOLOGICAL SAMPLING OF FISH FARMED IN SMALL-SCALE FRESHWATER SYSTEMS IN VIETNAM (see Appendix 3)

The presence of bacteria within the cytoplasm of phagocytic cells isolated from the anterior kidney of freshwater farmed fish now been reported in a number of different studies (Inglis and Crumlish 1997, Thompson and Crumlish 1999, and Crumlish *et al* 2000).

High bacterial loading is often reported within the aquatic environments of the fish farms in Thailand and Southern Vietnam, and a statistical relationship has been found between water quality, bacterial load in the pond and the occurrence of bacteria within macrophages (Thompson and Crumlish 1999). Although the levels of bacteria seen within the isolated macrophages were generally low, most macrophage preparations made from fish from these systems contained bacteria.

The clinical significance of the macrophage bacterial load still remains to be determined, although preliminary assessment using laboratory based studies suggested that fish do succumb to opportunistic infections under stress (Crumlish *et al* 2002). The constant interaction between the macrophages and high levels of bacteria from the aquatic environment may immunocompromise the animal in some way so as to make it more susceptible to infection. If this link is established, it is felt that their presence may be used as an indicator to predict potential disease outbreaks in the farming systems in Thailand and Vietnam.

The purpose of this study was to look for changes in the water quality, the bacterial load of the pond, and the occurrence of bacteria within macrophages, in farms where the farmer had reported previous disease episodes or water quality problems. It was intended to sample each of the nine farms at monthly intervals continuously over a six-month period of their production cycle. The results collected were to be used to look for a relationship between the water quality, the bacterial load of the pond, and the occurrence of bacteria within macrophages and to try to understand the significance of the bacterial load present within the macrophage on the immuno-competence of the fish. As well as looking for the presence of bacteria in macrophages isolated from the head kidney of the fish, the function activity of the cell was also examined using the cells ability to phagocytose yeast, and red and white blood cell counts as a measure of immunocompetence of a number of different fish species in different farming systems in Vietnam.

A 6-month study was carried out in the Mekong Delta region of Vietnam in which farms were visited by research staff from AFSI, CanTho University, to collect biological samples at monthly intervals. The farmers selected to take part in the survey farmed a range of different freshwater fish species in a variety of farming systems. During each visit 4 to 6 fish were sampled, samples were taken for water quality and bacterial counts in the water, blood was samples from the caudal vein for blood cell counts and differential cell counts, and head kidney tissue was taken for macrophage isolation. Macrophages isolated were isolated from this tissue and examined for the presence of bacteria in their cytoplasm and their ability to phagocytose yeast particles.

Research Outputs from Biological Sampling

Main Findings of the study

- In total ten farmers participated in the study and their farms were located in different districts of CanTho and An Giang Province. Unfortunately, "sever flood" took place during the sampling period and some of farms could not be reached during this period. Hence it was difficult for the research team to make six consecutive monthly visits to some farms and therefore the sampling protocol was incomplete and the relationship between many of the parameters could not be determined as anticipated.
- A number of technical difficulties are highlighted in Appendix 3, which has meant that, the full potential of the data collection was not achieved.
- Most of the results were analysed for only three farms (the ones visited monthly between September and December). Water quality was assessed using tests for primary production, acidity or alkalinity, temperature and presence of organic and inorganic particulates in the water column. The three farms appeared to have very similar water quality values.
- Bacterial colonies were recovered from the water samples taken from the three farms. The number of viable bacterial colonies recovered on Tryptone soya agar ranged from 10^3 to 10^7 cfu ml^{-1}. There appeared to be a slight decrease in the number of viable bacteria recovered from farms in November compared with samples taken in September.
- Bacterial colonies were recovered from the tissue and macrophage suspensions sampled fish taken from the three farms, however, there were technical problems in speciating these.
- In the fish sampled from all 3 farms there was a relationship between the percentage of macrophages with bacteria and bacterial growth from the tissue direct or the macrophage suspension.
- This study was the first examination of the immune competence of fish at the pond-site combining two simple assays, which could be performed in the field; the isolation of macrophages stained for the presence of bacteria, and the ability of isolated macrophages to phagocytose yeast.
- The results of breakpoint analysis suggests that when 12% of macrophages have bacteria within their cytoplasm, there was an increase in their ability to phagocytosis yeast.

The use of the tests performed here and in other appendices within this report, combined with other simple techniques developed to examine the immune response of fish in the field, may provide a means of examining the immuno-competence of fish within these culture systems. However, further training is required in basic fish health, disease recognition and the importance of the immune response before the AFSI research team would be able to fulfil such a study. Routine screening at strategic points in the production cycle may reduce the over application of chemicals and antibiotic treatments currently applied. This may in turn will reduce the costs of unnecessary or inappropriate treatments by fish farmers and reduce the number of fish lost from stress-related disease outbreaks. Furthermore, it is recognised that such an activity may only be of use to those families that culture their fish for market or in monoculture systems.

1.4.2.2 EXAMINING THE EFFECTS OF *AEROMONAS HYDROPHILA* ON THE IMMUNE FUNCTION OF AFRICAN CATFISH (*CLARIAS* SPP.) HEAD KIDNEY MACROPHAGES *IN VITRO* (see Appendix 6)

In an attempt to understand the clinical significance of the resident bacteria present in the macrophages, the following study was performed to examine what, if any, effects that resident *Aeromonas hydrophila* might have on the immune function of African catfish (*Clarias* spp.) head kidney macrophages. Macrophages were incubated with the bacterium *in vitro* prior to measuring the respiratory burst produced by the cells. The reduction of nitroblue tetrazolium (NBT) was used as an indicator of superoxide anion production by the macrophages. The superoxide anion is produced during the respiratory burst process and is, in part, responsible for microbial killing activity of the macrophage.

The effect of loading macrophages with the bacterium prior to measuring the respiratory burst was examined. The ability of opsonised bacteria, live and dead bacteria and the extracellular products (ECP) of the bacterium to stimulate the respiratory burst of macrophages was examined and the effect of different concentrations of bacteria on respiratory burst of the cells was also examined.

Research Outputs from Study

Main Findings of Study

- The results of this study suggest that the presence of *A. hydrophila* within the macrophages is able to inhibit the respiratory burst activity of the cells.
- The highest amount of superoxide anion produced was found in wells containing macrophages, which had not been previously exposed to bacteria.
- Cells incubated with *A. hydrophila* for 60 min prior to measuring their respiratory burst in the presence of phorbol myristate acetate (PMA) had higher levels of respiratory burst than cells incubated with bacteria and their respiratory burst measured in the absence of PMA. This suggests that cells containing the bacteria are still able to undergoing respiratory burst however, since the addition of PMA can non-specifically trigger the respiratory burst in the macrophages containing bacteria (PMA is a well-known and frequently used immunostimulant to induce superoxide anion production in the marcophages in a number different fish species).
- The respiratory burst in the absence of PMA is slightly increased by *A. hydrophila* at ratios of approximately 10-100 bacterial cells per macrophage cell. However it was not possible to establish the concentration of bacteria, which was inhibitory for respiratory burst, since cells with bacteria not treated with PMA were not included in the relevant experiment.
- There were no significant differences between uninoculated and inoculated macrophages when superoxide anion production was measured over a 24 h time period.
- The amount of superoxide anion produced in the presence of PMA was similar whether the macrophages had been pre-incubated with unopsonised bacteria, bacteria opsonised with serum or with bacteria incubated with heat inactivated serum, although the repiratory burst was significantly higher in cells without bacteria, reiterating the fact that *A. hydrophila* can inhibit the respiratory burst of the macrophage.

- The ability of *A. hydrophila* to inhibit respiratory burst is only present when the cells are alive, suggesting that this inhibition is an active process, possibly related to the virulence of the bacterium, however the ECP of the bacterium did not affect levels of superoxide anion production.

1.4.2.3 Pond studies (see Appendix 7)

A four month pond study was conducted in Thailand in which some of the potential risk factors identified from the data collected during the fish farming survey in Appendix 1 were examined. The variables tested were polyculture *vs.* monoculture and the influence of pond preparation on the outcome variable identified as fish disease occurrence. Samples of water quality, the aqueous bacterial load and the presence of bacteria within macrophages were analysed monthly.

The study was conducted at a commercial freshwater fish farm in (Wan Mat Cha) located in Minburi Province, which was chosen because the study site was large enough to provide replicate ponds of 800 m^2 and was within daily travelling distance of AAHRI. Two fish species, cultured on the farm routinely were chosen: tilapia and puntius. The farmer had prepared three of ponds for the staff prior to stocking using his "regular" method of preparation, while the other three ponds were not prepared in any way, just water and fish were added.

Research Outputs from the Study

The experiment was designed is present and conducted by AAHRI staff, the results of which will contribute to the MSc thesis of Ms Dumrongphol. Only the experimental design is presented in Appendix 7 as the results are still being analysed and will be available when the thesis is completed in September 2003.

1.4.3 Activity *(3)*

Guidelines devised for the control of bacterial disease in rural farming systems, based on the findings of Activities (1) and (2).

1.4.3.1 Results of the risk analysis out of THAI FISH FARMER SURVEY DATA: GENERAL FINDINGS (See Appendix 1)

Having obtained information about fish disease outbreaks from 304 households from around Thailand using the structured questionnaire in Appendix 1, analysis was performed on the data to identify risk factors associated with the disease episodes.

The data collected from the structured survey was entered into the EPI-Info database, and relative risk analysed and associations identified i.e. if the relative risk (RR) value were not greater than 1 using this package. All exposure variable were tested against the same outcome variable which was “fish disease” and statistical significance measured at $P<0.05$. Statistically significant risk associations were found for only ten exposure variables and the outcome variable “fish disease” (Table 1.1).

Table 1.1 Risk factors associated with disease from the Thai Fish Farmers Survey

Exposure Variable	RR value	R	P value

No preparation of fish farm	1.93	1.16<RR<3.19	0.0067
Fish farming as main source of income	1.76	1.33< RR <2.34	0.0004
Experienced water quality problems	1.54	1.16<RR<2.04	0.0073
Keep written records	1.50	1.13<RR<2.00	0.0138
Monoculture system	1.74	1.33<RR<2.27	0.0001
Polyculture system	0.58	0.44<RR<0.76	0.0002
Sell at local market	1.87	1.42<RR<2.45	0.0000
Feed chicken waste	1.79	1.33<RR<2.40	0.0021
Marks on the surface of fish	4.59	3.59<RR<5.88	0.0000
Dead fish	5.19	3.96<RR<6.80	0.0000

RR = relative risk, P<0.05

- Analysis of the data provided ten-exposure variable that had a statistically significant association with the outcome variable, which was fish disease. Only polyculture systems as an exposure variable provided a 'protective' association' (RR = 0.58, 0.44<R<0.76, P=0.0002).
- Polyculture farmers were more than half as likely to prepare their ponds than monoculture farmers (RR=0.84, P=0.0614), while farmers with integrated farming systems were almost one and a half times more likely to prepare their pond(s) (RR=1.43, P=0.0152).
- Only 24% (n= 69) of the sample population in Thailand claimed that fish farming was their main source of income. Of the 69 households who said this, 39 had experienced some sort of disease problem on their farm. If fish farming was the main household activity, the farmers was nearly twice as likely to have had disease related problems in their stock (RR=1.76, P=0.0004). Thirty three of the households visited, who had fish farming as their main occupation, used monoculture systems to culture their fish, and there was statistically significant association between having fish farming as their main occupation and culturing fish in monoculture ponds (RR=2.09, P=0.0006). Fifty-seven families who had semi-intensive systems, the main type of fish farming system in Thailand, claimed aquaculture to be their main source of income, but again no association was found between this link (RR=0.35, P=0.0574).
- Approximately 22%, or 66 households, claimed to keep written records about their farm. A statistically significant association was found where families with fish farming as a main source of income were twice as likely to keep written records about their fish farm compared with those families that had another activity as a main source of income (R=2.08, 1.38<R<3.12, P=0.001). The reasons given for keeping written records was not unexpected; most families recorded information to help calculate their expenses and hence profit gained from fish farming.
- Families that observed dead fish within their farms were five times more likely to have experienced fish disease according to the relative risk data. This may imply that families who look after and observe their fish may recognise problems with fish health more than those farmers who do not look at their fish stocks regularly. As no fish samples were taken at the time of the questionnaire, disease outbreaks cannot be confirmed making interpretation of the relative risk data difficult. However, the risk factors found would indicate that families who observe and look after the fish were more able to observe when there were fish health problems on their farm.

1.4.3.2 Results of the risk analysis out of VIETNAMESE FISH FARMER SURVEY DATA: GENERAL FINDINGS (See Appendix 2)

- Having obtained information about fish disease outbreaks from 200 households in the Mekong Delta of Vietnam using the structured questionnaire in Appendix 2, analysis was performed on the data to identify risk factors associated with the disease episodes.
- Statistically significant risk associations were found for fourteen exposure variables and the outcome variable "fish disease", and these are shown in Table 1.2.

Table 1.2 Risk factors associated with disease from the Vietnamese Fish Farmers Survey

Exposure Variable	RR value	R	P value
Preparation of farm with lime	1.59	1.16<RR<2.18	0.0054
Preparation of farm with pumping water	1.58	1.13<RR<2.21	0.0095
Fish farming as main occupation	2.07	1.63<RR<2.62	0.0000
Market purpose Export	1.91	1.45<RR<2.51	0.0323
Market purpose family	0.55	0.42<RR<0.72	0.0000
Feeding pellets	2.00	1.59<RR<2.52	0.0053
Feeding trash fish	1.85	1.44<RR<2.38	0.0001
Feeding vegetables	1.40	1.06<RR<1.84	0.0351
Experienced water quality problems	2.58	1.83<RR<3.65	0.0000
Identification of water quality problems	4.46	1.79<RR<11.09	0.0000
Keeping written farm records	1.52	1.11<RR<2.08	0.0530
Use of traditional treatments	2.34	1.97<RR<2.77	0.0000
Use of chemical treatments	2.30	1.90<RR<2.79	0.0000
Use of Antibiotics	3.06	2.42<RR<3.88	0.0000

RR= relative risk value, P< 0.05

Further analysis and verification of the data collected in the surveys will be carried out in DFID project "The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119)".

1.4.3.3 ANALYSIS OF BIOLOGICAL SAMPLING OF FISH FARMED IN SMALL-SCALE FRESHWATER SYSTEMS IN VIETNAM (see Appendix 3)

As mentioned in Section 1.4.2.1, a six-month study was carried out in the Mekong Delta region of Vietnam in which farms were visited by research staff from AFSI, CanTho

University, to collect biological samples at monthly intervals. The farmers selected to take part in the survey farmed a range of different freshwater fish species in a variety of farming systems. During each visit 4 to 6 fish were sampled, samples were taken for water quality and bacterial counts in the water, blood was samples from the caudal vein for blood cell counts and differential cell counts, and head kidney tissue was taken for macrophage isolation. Macrophages isolated were isolated from this tissue and examined for the presence of bacteria in their cytoplasm and their ability to phagocytose yeast particles.

Data analysis

- The data was analysed using Statistica package version 6. A multiple regression analysis was conducted on the dependant variable "percentage bacteria" and the independent variables, "percentage phagocytosis, farm, date and species".
- When a backward stepwise module was applied only percentage phagocytosis and farm remained in the module (R^2 = 0.51253875, P= 0.00000, Phagocytosis β = 0.660 and Farm β = -0.38. The data analysis suggested that there was a positive relationship between the percentage macrophages with bacteria and the percentage phagocytosis observed. This implied that as the percentage of macrophage cells with bacteria in their cytoplasm increased, so did the percentage phagocytosis.
- Furthermore, breakpoint non-linear regression was conducted on the data to provide a breakpoint of 12% where R^2 = 73.042%. The results from the breakpoint analysis suggested that when 12% of macrophages have bacteria within their cytoplasm, there was an increase in their ability to phagocytosis yeast.
- The results of the breakpoint analysis were interesting since they suggested that below levels of 12% of macrophages with bacteria, there was a low level phagocytic response, while 12% of macrophages or more which contained bacteria had a heightened ability to phagocytose yeast.

1.4.4 Activity (4)

Disseminate project findings: (1) an internal workshop at the start of the project to co-ordinate research and provide relevant training; (2) an internal workshop was held at the end of the project to interrupt the results of the project; (3) informal training sessions for extension officers and rural farmers; (4) disseminate of project findings through conference presentations, publications in peer reviewed journals and other media

- An internal workshop was conducted at the start of the project for collaborating groups to co-ordinate research and provide relevant training.
- An internal workshop was held at the end of the project to interrupt the results of the project. A social Scientist was present at this meeting to help with their interruptation.
- The project findings were disseminated to extension officers and rural farmers through farm-based aquatic health management workshops, held in Thailand and Vietnam. The teaching session were delivered in the participant’s native language, promoting health awareness in small-scale rural aquaculture systems.

- The results from the survey, laboratory tank trials and the field studies have been disseminated both local and regional farming communities via workshops for farmers, extension officers, leaflets and informal newsletters (in the native language), and to the general scientific community by way of conference presentations and publications in peer reviewed journals.
- A final workshop was suppose to be conducted at the end of the project in Thailand for regional field research scientists to disseminate project findings and promote understanding of mechanisms involved in fish health; as yet has not taken place take.

1.4.4.1 Dissemination material

Peer Reviewed
(Published and In press)

- Crumlish M, Somsiri T, Dung T. Inglis V. & Thompson K.D. (2000) Development of a sampling method for isolation of head kidney macrophages at the pond-side. Journal of Fish Disease 23, pp 289-293.
- Crumlish M, Somsiri T. & Thompson K.D. (2002). The effects of stress on the susceptibility of Hybrid Catfish *(Clarius gariepinus Burchell x Clarias macrocephalus* Gunther) to an artificial *Aeromonas hydrophila* challenge. Submitted to Asian Fisheries Science (In press).
- Somsiri T, Crumlish M, Dumrongphol Y, Panbanpeaw A & Thompson K.D. (2001). Study of bacterial loading in macrophage cells of hybrid catfish (In Press)

(In Preparation)

- Crumlish M, Dung T.T Somsiri T. & Thompson K.D. (2002). Survey on impact of disease on small-scale freshwater aquaculture in Thailand.
- Crumlish M, Dung T.T, Somsiri T & Thompson K.D (2002). Survey on impact of disease on small-scale freshwater aquaculture in Vietnam.
- Crumlish M, Dung T.T, Somsiri T & Thompson K.D. (2002). A study of freshwater farming systems in South Vietnam: the relationship between water quality, bacterial counts in the water and bacterial levels in the head kidney macrophages of farmed fish.

Articles

- Somsiri, T. (2000) Leaflet on disease prevention, and drugs and chemicals used in Aquaculture. This leaflet is for distribution to farmers via the extension officers
- Thompson K.D. and Crumlish M. (2000) Strategies for improved diagnosis and control of bacterial disease in small-scale fresh water aquaculture in South East Asia *Aquaculture News*
- Crumlish M. (2001) DFID research in Vietnam *Aquaculture News March 2001*
- Dung T.T. (2000) An article about the general aims and activities of the project for a Vietnamese Fish Farmers magazine.
- Dung T.T. (2000) Report in Vietnamese on the findings of DFID project R 7054 at a workshop held at CanTho University.
- Temdoung Somsiri, Supranee Somsiri, Yolprapa Dumrongphol, M. Crumlish K. Thompson (2001) Compilation of Small-scale Freshwater Aquaculture in Thailand. The AAHRI Newsletter 10(2) pp6
- Temdoung Somsiri, Supranee Somsiri, Yolprapa Dumrongphol, M. Crumlish K. Thompson (2001) Survey on Aquaculture Activities of extension Officers. The AAHRI Newsletter 10(2) pp6

Conferences

- Crumlish M., Somsiri T., Dung T.T. and Thompson K.D. The relationship between viable bacteria in farm water and the percentage of head kidney macrophages with bacteria sampled from a variety of cultured fish species (Oral) Aquatic Animal Health for Sustainability", in Cebu, Philippines November 22-26 1999.
- Somsiri T., Crumlish M., Dumrongphol Y., Panbanpeaw A. and Thompson K.D. Study of bacterial loading in macrophage cells of hybrid catfish (Poster) Aquatic Animal Health for Sustainability", in Cebu, Philippines November 22-26 1999.
- Thompson K.D, Crumlish M, Somsiri T. & Dung T.T. (2000). Results of a survey examining husbandry practises and disease outbreaks in small-scale freshwater farming systems in Thailand and Vietnam. (Poster) Third World Fisheries Congress, Beijing 31st October-3rd November 2000.
- Dung T.T (2001) Infection by ecto-parasites in farmed Pangasiid catfish fingerlings raised in the Mekong Delta. Abstract for the Larvi 2001 conference.

Extension Materials

- Somsiri (2000) Leaflet on disease prevention, and drugs and chemicals used in aquaculture. For dissemination to farmers via the extension officers.
- Crumlish (2000) Control of bacterial disease in small-scale fresh water aquaculture. Radio Interview for Wren Media.

Workshops

- Internal project workshop held at AAHRI, Thailand on 18-22nd October 1999 for all personnel involved in project R 7463. Manual for the internal workshop produced.
- Internal project workshop held at AAHRI, Thailand on July 2001 for all personnel involved in project R 7463. Manual for the internal workshop produced.
- Somsiri T. (2000). One-day workshop for Extension Officers in Thailand, held at AAHRI, Bangkok by Dr. Temdoung Somsiri on 25th October 2000.

Seminars

- Crumlish (2000) Survey results of small-scale farming systems in Thailand and Vietnam. The Institute of Aquaculture. October 2000.
- Somsiri, T. (2000) Seminar on fish disease, treatment & prevention together with some of the results of the project at Chiang Mai on 26th June 2000. The participants included 60 fishery biologists from the North of Thailand

Training

- Crumlish M & Millar S. supervised a student for 4 weeks on a Nuffield scholarship. Studies included the extent of artificial loading of catfish macrophages with bacteria. A report of her work was produced at the end of her visit and she presented a poster at the Royal Scottish Museum in Edinburgh on 30th.
- Epi-info database used to compile and analyse data from surveys
- Training document (for internal use) for compiling survey sample frames and conducting structured questionnaires
- Standard operating procedures for field sampling

Activities

- Dung T.T. (2000) "Disease problems in *pangasius* species- prevention and treatment". Two 20 min extension programmes for local and national television
- Crumlish M. (2000) Attended DFID FGRP/ARP workshop in Hanoi, Vietnam *November 2000* A brief double-sided A4 handout relating to the project was prepared and distributed at the conference.
- Production of (a) Survey questionnaire to assess disease in small-scale freshwater aquaculture systems in Thailand; (b) Survey questionnaire to assess disease in small-

scale freshwater aquaculture systems in Vietnam; (c) Survey questionnaire for extension officers, whose jurisdiction cover farms surveyed

New project arising for for DFID-project R 7463

- Risk issues and management strategies for Bacillary Necrosis Disease (BNP) and other factors for Pangasius spp. farming in the Mekong Delta, Vietnam (R 8093)
- The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia- R 8119

1.5 Contribution of these Outputs to Developmental Impact

1.5.1 Outcomes of the project contributing to Developmental Impact

The overall object of the project was to develop strategies to reduce disease outbreaks using the project findings, and establish guidelines to help farmers improve their husbandry management, so as to mitigate losses due to disease and increase harvest yields thus contributing to developmental impact. The following outputs help to achieve this.

- Data relating to the number of Aquaculture families in the provinces surveyed in Thailand and in the Mekong dealt of Vietnam established. Old Department of Fisheries lists of fish farmers were out of date and did not reflect current farmers involved in aquaculture.
- Data relating to the frequency of disease related problems within small-scale fish farming systems was collected together with information on their husbandry practices and disease control strategies used by the farmer. The etiological agents of the outbreaks were not identified however, as no biological samples were taken at the time of the interviews or during periods of disease or poor water quality.
- Some relative risks associated with disease were identified but these will be address further in DFID project "The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119)".
- In response to the findings of the surveys and the request of extension officers, a proposal has been submitted to the DOF of Thailand by AAHRI to set up central laboratories in each province to deal specifically with disease diagnosis and control/ treatments of disease.
- An important finding from the extension officers' questionnaire was that 75% of extension officers claimed that they felt that they did not provide an adequate service for the fish farmers. Reasons for this were thought to be due to their lack of technical knowledge, their lack of appropriate equipment for sampling and the lack of people to help conduct sampling work.
- This study was the first examination of the immune competence of fish at the pond-site combining two simple assays, which could be performed in the field; the isolation of macrophages stained for the presence of bacteria, and the ability of isolated macrophages to phagocytose yeast. The use of a combination of other simple field-based tests may provide a means of examining the immuno-competence of fish within these culture systems and provide potential indicators of disease.
- The clinical significance of the bacteria within the macrophages remains to be determined, but from studies in the laboratory, "live" *A. hydrophila* appears to inhibit the respiratory burst of macrophages, suggesting that the bacteria may in fact be immunosuppressive.

- New project have directly resulted DFID-project R 7463 which will further address the issues dealt with in the current project.
    3. Risk issues and management strategies for Bacillary Necrosis Disease (BNP) and other factors for Pangasius spp. farming in the Mekong Delta, Vietnam (R 8093)
    4. The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119)
- This was originally a very technically based project, examining the incidence of bacterial disease in small-scale fresh water systems in Thailand and Vietnam and establishing strategies to control disease within these systems. However from the work so far, it is evident that there is a lack of understanding by the farmers as to what disease is and how it can affect their systems and livelihoods. We need to understand the farmers' perception of disease, verify true disease outbreaks and establish the impact of disease on the livelihoods of the farmers before we are in a position to establish the incidence of disease and develop strategies to alleviate it.

- It was difficult to define small-scale fish farms according to the DOF classification of fish farms i.e. whether the system was extensive, semi-intensive and intensive. In the opinion of the interviewer semi -intensive farming according to the questionnaire classification is large pond or a number of ponds where regular feeding is given. There appears to be two different levels of fish farming, especially in Thailand both of which could be found located in each province visited

    3. Farms where there is little input; fish farming is not the main source of household income and the farmer experiences few/no diseases or water quality problems possibly because the farmers don't have any concept of these and they are not important to them

    4. Farms where fish farming is the main occupation and disease appears to be a problem. In this case maybe the farmers have a better concept of disease.

Questions arising from this project

- How small-scale fish farmers understand 'fish losses' and fish health management within their systems.
- What is the farmers understanding of the systems and where does it fit in with their families livelihood activities
- Determine the impact of fish losses on livelihoods of rural families involved in raising fish in Thailand and Vietnam.
- What is the farmers perception of problems within the systems specifically related to 'fish losses'
- To understand the knowledge and language used by fish farmers to describe their systems and fish losses
- To establish the causal factor for the fish losses.

Some of these questions will be addressed in DFID project "The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119)" and will be put in context using key informant and group interviews in Thailand and Vietnam. Target groups will be identified from the information collected in surveys in Sections 1.4.1.1 and 1.4.1.2.

1.5.3 Direct and Indirect impacts, on both target beneficiaries and partner

Rural farmers

- Overall, the research attempted to visit a total of 3,614 households, while the number successful visited was 304 farms during the "Thai fish farmer survey" in four different regions of Thailand (the North, North East, Central and Southern regions) (**Direct and Indirect impacts**).
- During "the Vietnamese fish farmer survey", 200 farmers were **Direct and Indirect impacts**).
- The 46 farmers farmer involved in "the recording keeping study" (**Direct and Indirect impacts**).
- Staff at the farm chosen for "the pond study" in Wan Mat Cha (**Direct and Indirect impacts**).
- Radio and television broadcasts (**Direct and Indirect impacts** t)
- New projects arising from this study :- (1) Risk issues and management strategies for Bacillary Necrosis Disease (BNP) and other factors for Pangasius spp. farming in the Mekong Delta, Vietnam (R 8093) (2) The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119) (**Direct and Indirect impacts**)

Extension Officers

- Extension officers survey in four different regions of Thailand (the North, North East, Central and Southern regions). The structured questionnaire was sent to the extension services of 26 different provinces in these regions. Replies to the questionnaire were received from extension officers in only 18 of the 26 provinces where the questionnaire had been sent, with a total of 84 extension officers replying (**Direct and Indirect impacts**).
- A similar survey was conducted in the Mekong Delta of Vietnam- (**Direct and Indirect impacts**).
- One-day workshop for Extension Officers in Thailand, held at AAHRI, Bangkok by Dr. Temdoung Somsiri on 25th October 2000. (**Direct and Indirect impacts**).
- Leaflet on disease prevention, and drugs and chemicals used in aquaculture. For dissemination to farmers via the extension officers. (**Direct and Indirect impacts**)
- Radio and television broadcasts (**Direct and Indirect impacts**)
- New projects arising from this study:- (1) Risk issues and management strategies for Bacillary Necrosis Disease (BNP) and other factors for Pangasius spp. farming in the Mekong Delta, Vietnam (R 8093) (2) The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia (R 8119) (**Indirect impacts**)

Staff at "target institutions"

- Internal project workshops (11 participants) (**Direct and Indirect impacts** t)
- Seminar on fish disease, treatment & prevention together with some of the results of the project at Chiang Mai on 26th June 2000. The participants included 60 fishery biologists from the North of Thailand (**Direct and Indirect impacts)**
- Training of staff in Epi-info database used to compile and analyse data from surveys, compiling survey sample frames and conducting structured questionnaires, biological sampling in the field, situation appraisals (**Direct and Indirect impacts**)

Scientific community (**Direct and Indirect impacts**)

- Delegates at conferences
- Readers of journal and articles
- Seminars

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Appendix 1

This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID

THAI FISH FARMER SURVEY DATA: GENERAL FINDINGS

Dr Kim D. Thompson and Dr Mags Crumlish,

Institute of Aquaculture, University of Stirling, Stirling Scotland, UK

Dr. Supranee Chinabut, Dr. Temdoung Somsiri and Ms. Yolprapa Dumrongphol

Aquatic Animal Health Research Institute (AAHRI), Dept. of Fisheries, Kasetsart University Campus Bangkok 10900 Thailand

BACKGROUND

A small-scale freshwater fish farm survey was conducted in 2000 by staff at AAHRI, to assess problems associated with husbandry and fish disease and to try to establish the frequency of disease related problems within small-scale fish farming systems. The households selected represented farms in the North, North East, Central and Southern regions of Thailand and were selected because they carried out freshwater aquaculture as one of their household activities. In total, 304 households in 24 different provinces were surveyed. The target population of the survey was 'rural poor fish farmers'. However, after pilot testing the questionnaire around Bangkok in Central Thailand and in the Northeast region of Thailand it was found to be more difficult than expected to reach the 'rural poor fish farmers '. This was because they lived in remote areas, which were difficult to reach, especially in the Northeast region of Thailand. Hence, the survey sample frame was extended to include other fish farming groups that had extensive, semi-intensive and intensive fish farm practises.

MATERIALS AND METHODS

A structured questionnaire was designed in English using Microsoft Word and then translated into Thai. This was checked for any differences in the meaning of the questions that may have arose during the translation. Most of the questions used were closed questions, however some open-ended questions were included and the questionnaire was carried out as personal interviews. The design was kept simple with tick boxes or dichotomous replies. It consisted of sections on farm background, production, husbandry and fish diseases and treatments. The farms were randomly selected using a devised survey sample frame, which was designed to include only families currently involved in small-scale freshwater fish farming. Maps of Thailand were used to identify the provinces with freshwater fish farms. A list of the fish farming households was supplied from the Department of Fisheries (DOF) and 50% of the farms identified from province, district, amphoe or hamlet and finally village was randomly selected. An extension officer from the DOF for each district was contacted to assist with either producing the list of fish farmers in their district or by actually arranging the visit by the research staff to the fish farmer on the list.

Information was compiled and analysed using the EPI-INFO version 6 (DOS-based) programme (http://www.cdc.gov/epo/epi/software.htm) to provide frequency data. Relative risk analysis was performed on the dichotomous values where a significant relationship was interpreted as an R-value that was greater than or less than (and did not span) 1. An English version of the survey is presented at the end of the appendix.

RESULTS

It was not always possible to use this information or to follow the survey sample frame for selecting the farms. The actual number of fish farming households successfully interviewed during the survey was 304, which were distributed throughout the various regions of Thailand. The research team initially tried to contact a much larger number of fish farming families (Table 1), contacting some fish farming families was not an easy task even when there were full lists of people available (Table 1). Some farmers had stopped farming fish or fish farming was regarded by the family as a hobby and so they did not want to be included or regarded as a 'farm'.

Table 1: Number of farms visited and reason given for not using the farms randomly selected

Province	**Natural farm**	**Stopped culture**	**Farmer absent**	**Never do aqua-culture**	**Could not locate farm**	**Hobby only**	**Farm too far to visit**	**Farmer dead**	***No of farms visited**
Ayuthaya									**29**
Chaingmai	64	22	2	4	6	0	78	0	**16**
Chaiyapom									**21**
Chaingrai	133	45	40	15	44	3	86	0	**19**
Kalasin	286	8	3	6	9	0	0	0	**5**
Khonkhen	105	53	3	58	8	0	53	4	**12**
Lampang	109	10	0	9	10	0	47	0	**16**
Lopburi	0	12	4	1	7	0	21	2	**11**
Loei	148	9	1	1	10	0	121	0	**10**
Mukdaharn	146	0	0	0	0	0	0	0	**7**
Nakhonnayuk									**3**

Nakornpathom	0	88	8	2	14	0	2	4	**8**
Nongboualompoo	51	2	3	0	19	0	76	0	**4**
Nongkai	42	20	4	16	7	0	66	5	**7**
Pijit	154	25	3	7	0	1	8	0	**19**
Pisanuloke	74	1	0	0	0	0	23	0	**6**
Pattalung	49	3	0	0	0	0	0	0	**18**
Singburi	3	11	4	4	4	0	4	3	**16**
Saraburi	6	13	0	5	5	0	8	2	**7**
Supanburi	0	26	0	13	26	0	0	6	**27**
Surathani	125	15	1	7	26	0	90	0	**11**
Trang	280	8	8	2	5	1	8	0	**20**
Uthaithani	27	5	3	3	17	0	0	2	**3**
Ubonratchathani	0	0	0	0	0	0	0	0	**9**
Total	**1996**	**379**	**99**	**181**	**220**	**5**	**691**	**43**	**304**

Farm Background

Polyculture farming systems were more commonly found compared with any other type of aquaculture system (Table 2). Only one household included in the survey was involved in large cage culture, and this was a newly established farm developed in 1999 in Kokthong district of Loei Province. Some families had two or more types of systems within their farm, with combinations of monoculture and polyculture systems more common than with any other combination of farming system (Table 2).

Table 2: Farming Systems and Number of Households

Farming System	Number of Households
Monoculture only	54
Monoculture + polyculture	21
Monoculture + small cages	10
Monoculture + integrated	6
Monoculture + hatchery	2
Polyculture only	182
Polyculture + small cages	2
Polyculture + integrated	10
Polyculture + hatchery	2
Large cages	1
Small cages	2

Integrated only Integrated + hatchery	7 1
Hatchery only	3
Total	**303**

A greater number of fish farms were established between 1989 to 1999 compared with any other time, with three new farms being set up at the beginning of 2000 (the year of the study). The oldest fish farm in the survey was established in 1941 by a family located in Chaingrai Province. There was a large increase in the number of fish farms in 1995 and again in 1999, with 41 and 45 farms establish during these two year respectively. However, no information was provided on why there was an increase in the number of fish farms established at this time compared with any other time. Of the ones set up in 1995, eighty percent were polyculture based, while in 1999 fifty-five percent of the new farms were monoculture based and forty-four percent polyculture farming systems. There appeared to be no particular pattern in location of the new farms, which appeared scattered throughout the all the provinces surveyed.

Only 42 farmers (57%, n=74) interviewed reported to have had previous fish farming experience and these were located in 11 provinces distributed throughout Thailand (Table 3).

Table 3: Distribution of Households with previous Fish Farming Experience

Province	Number of households	Percentage of households
Ayuthaya	7	17
Chaing Rai	2	5
Lampang	1	2
Pattalung	7	17
Pijit	4	10
Pisanuloke	1	2
Saraburi	1	2
Singburi	1	2
Syurathani	6	15
Trang	9	21
U-bonratchathani	2	5
Total	**42**	**100**

Only 24% (n= 69) of the sample population in Thailand claimed that fish farming was their main source of income. Of the 69 households who said this, 39 had experienced some sort of disease problem on their farm. If fish farming was the main household activity, the farmers was nearly twice as likely to have had disease related problems in their stock (RR=1.76, P=0.0004). Thirty three of the households visited, who had fish farming as their main occupation, used monoculture systems to culture their fish, and there was statistically significant association between having fish farming as their main occupation and culturing fish in monoculture ponds (RR=2.09, P=0.0006). Fifty-seven families who had semi-intensive systems, the main type of fish farming system in Thailand, claimed aquaculture to be their main source of income, but again no association was found between this link (RR=0.35, P=0.0574).

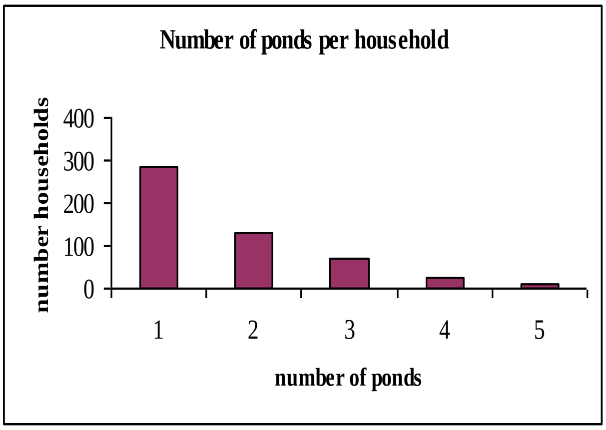
Production

Farm preparation before every crop was practised by 67% (n=201) farming households throughout Thailand (Table 4). Only 5% (n=16) families prepared their farm at the start and did not prepare the farm at every crop (Table 4). Using lime and leaving the pond to dry was the most commonly used form of farm preparation.

Table 4: Number and Percentage of Households that Prepare Fish farm

Farm Preparation	Number of households	Percentage of households
No	58	19
First time only	16	5
Sometimes	24	8
Yes always	201	67
Don't know	1	1
Total	300	100

In total 95% (n= 288) of the households interviewed cultured fish in ponds. Most of the farms visited had one or two ponds (Figure 1), with a higher number of families using 1 pond irrespective of the actual number of ponds available (Figure 2). Some families had two or three ponds, which they claimed to use at the same time (Figure 2).



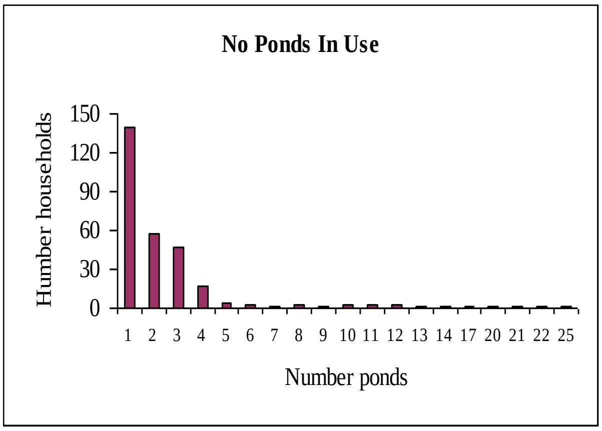


Figure 1: Number of ponds per household Figure 2: Number of ponds in use per household

As the target population of the survey was 'the rural poor' it was thought unlikely that poor farmers would have had more than five ponds. However, when the survey sample frame was extended to include extensive, semi-intensive and intensive fish farm systems, it was found that some households had more than 5 ponds. In fact one family had 25 ponds all of which were in use. The design of the questionnaire meant that production information was only obtained for farmers, which had a maximum of five ponds. This was not really considered a problem, since the majority of fish farming households had five ponds or less (Figures 2). The dimensions of the ponds were found to vary considerably between households (Table 5).

Table 5: Number of Ponds per Households and Range of Pond Dimensions

Number of ponds per Household	Range of Pond Size (m^2)
1	1 to 32,00
2	2.3 to 20,800
3	2.3 to 32,000
4	6 to 4,800
5	6 to 1,600

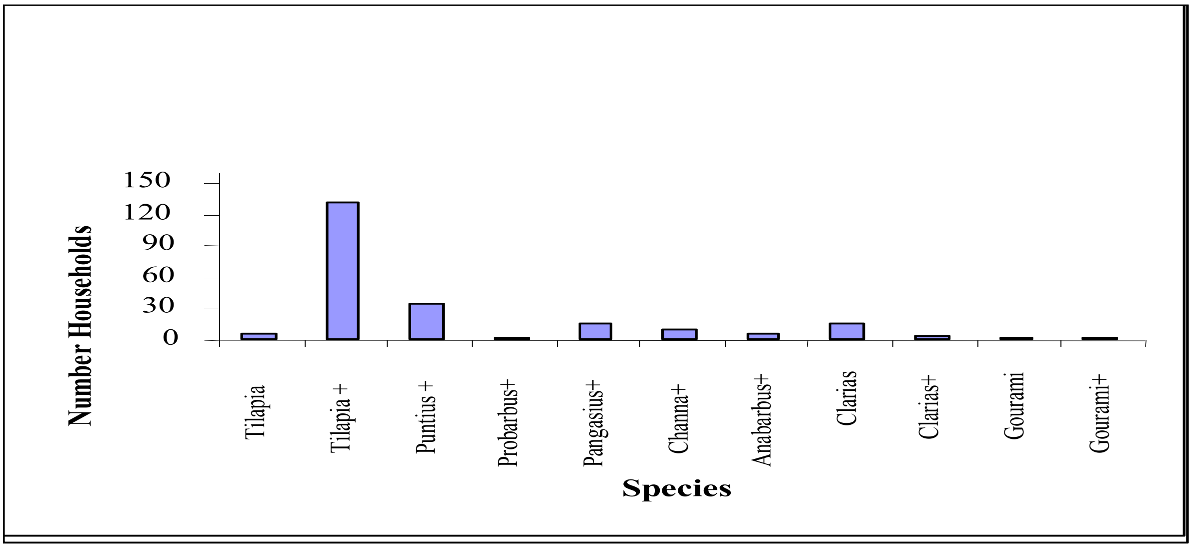
The most common pond size was around 400 m^2, although some farmers had ponds measuring between 800 and 1600 m^2. Where a household had three ponds, the most common size of pond was around 1600 m^2, while the pond size at farms with between 4 and 5 ponds were found to vary greatly (Table 5).

Few families interviewed used cages, and this may have reflected the focus of the survey and the randomisation of the households selected. Of the families that practised cage culture, most had only one cage and only 5 households interviewed owned five cages. A range of different cage sizes were reported (Table 6), but the most common size was found to be 9 m^2, and only 1 family used cages measuring 400 m^2.

Table 6: Number of Cages per Households and Range of Cage Dimensions

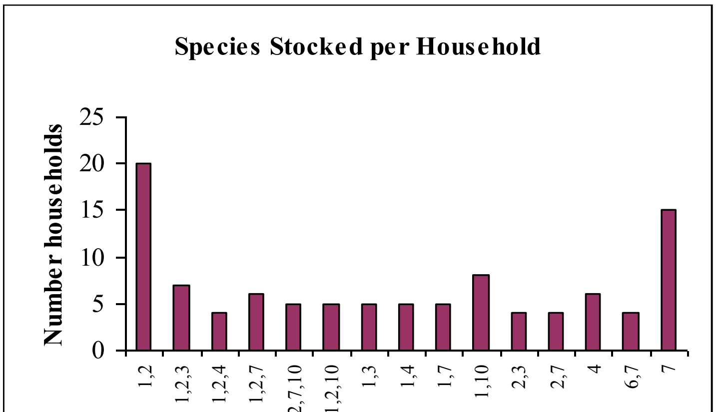
Number of cages per household	Cage Size Range (m^2)
1	6 to 400
2	6 to 25
3	6 to 18
4	8 to18
5	9 to18

An extensive range of fish species was found to be cultured in the ponds (Figure 3a). More households cultured tilapia compared with any other species (Figure 3a). They were usually cultured in polyculture systems with more than one other species. However a large number of families also cultured *Puntius* sp., again in polyculture systems (Figure 3b). It was the opinion of one of the Thai researchers involved in the survey that tilapia appeared to be the most commonly cultured fish species possible due to their promotion by the Thai Government at the time carrying out the survey. In monoculture *Clarias* sp. was the most popular choice of species cultured (Figure 4).



+ = In combination with other

Figure 3a: Fish Species Cultured in Thailand and Number Households



- 1,2=tilapia and puntius
- 1,2,3=tilapia, puntius and probarbus
- 1,2,4=tilapia, puntius and pangasius
- 1,2,7,10=tilapia, puntius, clarias and cyprinus
- 1,2,10=tilapai, puntius and

Figure 3b: Combinations of Fish Species Cultured in Households in Thailand

Eighty-three percent (n= 252) of farmers interviewed, claimed to always farm the same species of fish. Many of the families (48%, n=131) did not to know what would entice them to change the fish species they cultured, but 24% (n=61) suggested that increased income and 12% (n=29) suggested media advice may influence them to change species they normally cultured. It was found that 79 % (n= 239) of families knew the stocking density of their ponds and these varied between households from 0.08 to 1,000-fingerling m^2. The most common stocking density was between 1 to 10 fingerling m^2 (Table 7) and was probably reflective of the size of the pond available and the purpose of farming fish.).

Of the households interviewed only 37% (n=111) added wild fish to their ponds, but only 35 of these claimed to have had disease problems.

Table 7: Number of Households and Range of Stocking Densities

Range Stocking Density (fingerling m^2)	**Number of households**
<1	9
1-10	130

11-20	31
21-30	12
31-40	5
41-50	6
51-60	1
61-70	7
71-80	7
81-90	6
91-100	4
>100	21
Total	**239**

It appeared from the data collected in the survey that farmers stocked their farm throughout the year, but a higher number of families stocked their ponds in May compared with any other month (Table 8). This probably reflected the availability of fingerlings/fry. More households harvested their fish in April compared with any other month (Table 8).

Table 8: Stocking and Harvest Months and Number of Households

Month	Number of households stocking	Number of households harvesting
January	11	10
February	15	8
March	15	13
April	13	**27**
May	**35**	10
June	17	9
July	7	3
August	13	10
September	6	5
October	8	4
November	8	6
December	7	6

Total	155	111

Fewer families knew the month in which they harvested their fish compared to the month they stocked (Table 8), and this may be related to the purpose of their fish farm. If fish farming was the main source of income for the household, the farmer was more likely to be aware of the production within the fish farm compared to families where fish farming was one of many household activities. Forty-two percent (n=127) of the households interviewed knew the total weight of product at harvest and these varied from 20 to 48,000 kg. However, 77% (n=236) families provided information on the individual weight of fish at harvest compared with the total weight of their harvest. Again this was probably a reflection of the types of systems used and the purpose of the fish produced. If the fish were sold at the local market when the family required money, the farmer was more likely to know the weight of individual fish compared to families where fish farming was their main source of income or who used monoculture systems. In this case the market tended to dictate the size/weight of the fish for harvest. The weight of individual fish harvested by the farmers ranged from 0.08 kg and 2 kg. A greater number of families tended to harvest smaller fish, with most families harvesting their fish between 0.11 and 0.3 kg in size. The majority of these households used the harvest for the family and for local market purposes.

The farmers mainly purchased their fry from hatcheries (69%, n=211) compared with any other source, but 16% (n=50) of families did use a fry/fingerling trader as their main source of fry. Most of the households interviewed which practised polyculture obtained their fish seed from various sources throughout the year depending on the purpose of the fish farm and the availability fish. Generally farmers reported low level of fry mortalities upon stocking with only 20% (n= 39) of families experiencing mortalities, with mortalities experienced ranging from 0.001% to 50% of stock. This may be related to the experience of the farmer or the relevance of fish farming to the livelihood activities of the household.

Most farmers produced fish for the local market and family consumption (Table 9). However, a high number of the households had a variety of outlets for their fish, which was usually sold at the local and regional markets (Table 9, "mixed"). None of the families interviewed produced fish for export or claimed to sell to processing plants, and only 4 families farmed their fish for "other" purposes as opposed to selling or eating them (Table 9). Other usually meant that the fish were kept as a hobby or were cultivated and used in the household's restaurant.

Table 9: Market Purpose of Fish produced and Number of Households

Market Purpose	Number of households
Local	32
Regional	8
Export	0

Family	29
Other	4
Local + Family	130
Mixed	101
Total	**304**

Husbandry

In total 74% (n=226) of families feed their fish with commercial pellets, but these were usually combined with other feed types such as agricultural by-products, animal manure, trash fish, rice bran etc. The type of feed used by the farmer depended on the fish species farmed and the purpose of the fish produced. Only 3% (n=9) families did not feed their fish. Additional information gathered at the time of the interview found that many families started their fish on commercial pellets and changed to another feed type during the production cycle. The majority of families used their own experience to know how much to feed. Families either fed their fish once or twice a day, but again this depended on the fish species, stage in the production cycle and intended purpose of the fish produced.

It was found that 23% (n=70) of the households interviewed had experienced water quality problems at some point on their farm. Water quality problems were found on farms in most of the provinces visited, however no households in Kalasin, Lopburi, Surathani or U-Thaitani provinces reported having any previous water quality problems, while the highest percentage of households with problems were found in Mudaharn, Nakornpathom, Nongboulumpoo and U-Bonratchathani province (Figure 4).

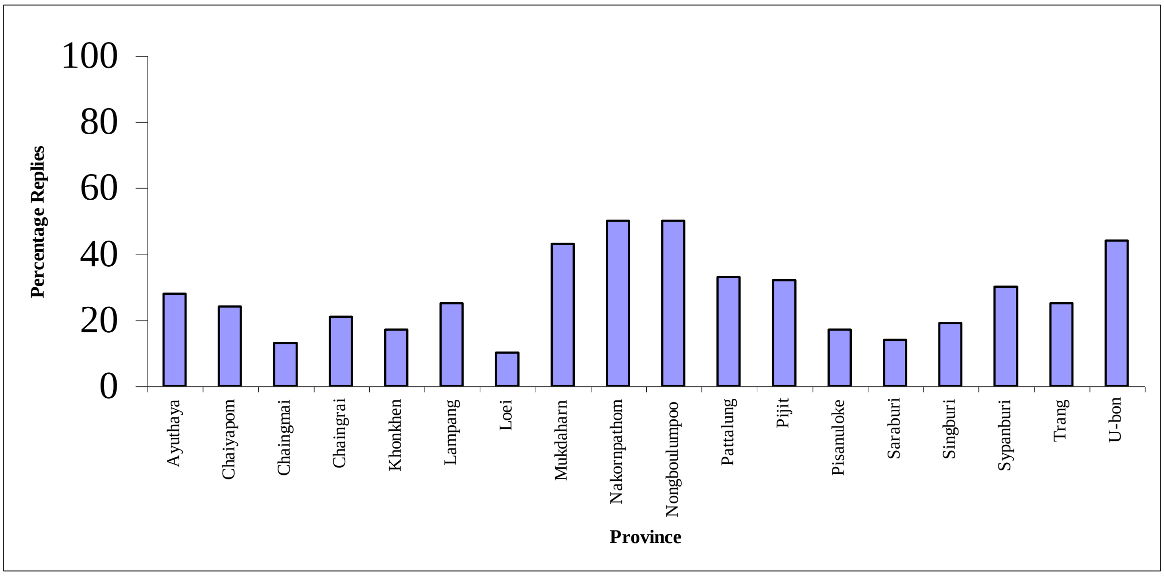


Figure 4: Number of Households per province that had experienced water quality problems

Seventy-four percent (n=52) of farmers claimed to have experienced water quality problems at some point. They used a variety of descriptions to describe the appearance of the water, with the highest number of farmers commenting on water turbidity or as smelling bad (Table 10). No samples were taken during the survey for water quality analysis.

Table 10: Descriptions of Water Quality problems Encountered by Households

Descriptions	**Number of households**
Polluted water	11
Gas at pond bottom	1
Chemicals/toxins in water	11
Turbid water	21
Low oxygen	4
Low temperature	1
Bad smell	15
Change in colour	5
Other	4
Total	**73**

Few households were able to state when water quality problems occurred, because they were unable to recall this information. Most of the farmers claiming to have experienced poor water quality used chemicals/treatment to improve the quality of the water in their ponds. Some families exchanged water or added oxygen depending on the type of problem encountered, while some families claimed to be unable to treat the problem due to lack of knowledge and understanding. The duration of water quality problems identified tended to vary from days to weeks to months. During this time, a high number of families exchange water, add salt or used other chemicals such as lime. Only a few farmers claimed to stop feeding their fish during this time. The majority of households, who replied, relied on their own experience or self-knowledge to apply what they felt were appropriate treatments.

Water exchange was performed on some farms as part of the normal husbandry practise with the greater number of households exchanging 50% or 100% of water.

Approximately 22%, or 66 households, claimed to keep written records about their farm. A statistically significant association was found where families with fish farming as a main source of income were twice as likely to keep written records about their fish farm compared with those families that had another activity as a main source of income ($R=2.08$, $1.38<R<3.12$, $P=0.001$). The reasons given for keeping written records was not unexpected; most families recorded information to help calculate their expenses, and hence profit gained from fish farming.

Fish Disease and Treatment

No biological samples were taken at the time of the survey to verify disease outbreaks, so this section reflects the farmers' interpretation of fish disease within their ponds. It was found that 39% (n= 118) of households, had encountered disease problems on their fish farms at some point since establishing their farm. Poor fish health was reported predominantly in December and in the winter season compared to any other time (Table 11).

Table 11: Months in which Farmers Experience Poor Fish

Time	Number of households
January	8
February	5
March	5
April	0
May	5
June	2
July	0
August	0
September	1
October	3
November	7
December	20
Winter	17
Rainy season	5
Summer	2
Juvenile stage of fish growth	7
Don't know/cannot remember	9
Other[1]	10

Total	106

1= "other" = specific dates were provided and years.

All of the farmers who claimed to have experience poor fish health used the presence of dead fish combined with other signs such as change in colour, reduced feeding and change in shape as the main signs to recognise that their fish stocks were not healthy. The majority of households thought that their fish were most susceptible to disease-associated problems during November to March and again in May. The main explanation for disease occurring during this time was the affect of season. Winter or the cold session occurs from November to March in Thailand, while April/May is the start of the hot wet season with increased rainfall in May.

Only 29% (n=87) of households interviewed had received training on health management. Most of the families interviewed claimed to rely on their own experiences and knowledge to recognise and diagnose poor fish health. Most families had received general aquaculture training from extension officers compared with any other source (Table 12).

Table 12: Sources from whom Fish Farmers receive Aquaculture training

Source	Number of households
Extension Officer	68
Company	5
Fisheries Station	6
NGO	2
Other farmer	3
College/University	3
Total	**87**

NGO = Non-Government Organisation

No more than 40% of farmers, or 111 households, had had contact with an extension officer at some stage during the time they had been fish framing. A higher number of farmers in Nongkai, Pattalung and U-Bonratchathani Provinces had contacted the extension officer at some point compared with those in the other provinces.

Fish losses reported by farmers during the survey were low (between 2-10%) with most families relying on their previous experience or knowledge to diagnose and treat health problems in their stock. Most of the treatments applied by the fish farmer tended to be chemical rather than any other type of treatment (Table 13), however, a high number of households claimed not to treat their fish with anything (Table 13).

Table 13:Types of Treatments used by Farmers

Treatment	Number of households
Exchange water	3
Apply antibiotic in feed	18
Apply antibiotics and chemicals	10
Apply chemicals only	35
Ask advice	3
Combined treatment	10
Remove dead fish only	11
Nothing	25
Total	**115**

DISCUSSION

The most common type of small-scale freshwater system used in inland Thailand was polyculture ponds. This system allows fish farming families to sell their fish at local markets, providing them with both a source of income and a supply of food. Many of the families interviewed owned the land on which their farm was based, and tended to use only one pond, even if they had access to additional ponds. The size of their harvest appeared to be related to the species that they farmed, the size of the pond used and the market purpose of their harvest. Many of the families interviewed both in rural and peri-urban areas of Thailand had a diverse portfolio of livelihood activities and very few carried out only fish farming as their main source of income. Although some of the families interviewed had been involved with fish farming for many generations, there appeared to be an increasing number of people who were starting to practice aquaculture.

The primarily purpose of the survey was to gather information to assess the problems associated with husbandry and fish disease and to try to establish the frequency of disease related problems within small-scale fish farming systems. Further work is required to establish factors, which influence families to make the decisions they do about their fish farming livelihoods. The species cultured by farmers were locally found species, and a higher number of families interviewed produced fish for local market and for family consumption. Therefore, it may be market influences, which dictate the species of fish cultured and the size of fish harvested for sale at the market. Generally, households involved in small-scale fish farming stocked and harvested their fish ponds throughout the year. Fish could be harvested as and when required depending on whether the fish were to be used to feed the family or sold at the market. Although many families regarded it as source of income, aquaculture was not regarded as the main source of income, rather as a resource to be used when the family required additional money.

The information generated from this survey suggested that families involved with fish farming were aware of the importance of good pond preparation, high quality nutrition at strategic points and also the importance of good water quality. It appeared that most of the families understood the importance of providing good quality/high protein diets at the juvenile/fingerling stage, since most families fed pelleted diets at this time. At other times throughout the production cycle most households used household/animal waste and agricultural by-products such as rice bran. Further work is needed to evaluate the farmers understanding of poor water quality, as relatively few families reported ever having had experienced poor water quality. This may reflect the farmers' awareness of water quality, since those who reported having poor water quality provided detailed lists of the problem and the time the problem occurred. Since no water samples were taken at the time of the survey, there is no substantiation as to whether farmers had in fact experienced water quality problems or not.

The questionnaire was pilot tested and adapted as required for Thailand as recommended by the Thai research team. However, it was later found that the word for "disease" in English means "health" in Thai so when the research team asked questions about "fish disease" they were actually asking about "fish health". Just under half of the families involved in the survey experienced and described diseased fish from a range of indicators provided. Interestingly, the majority of households thought that their fish were most susceptible to disease problems during the winter months and also again when most of the families were stocking their ponds. Daily water temperature fluctuate during Thai winters, and this in turn can lead to immunosuppression in fish, which has severe implications for the health status of the animal. Stocking is a critical period in the production cycle for which farmers need an adequate knowledge of fish husbandry to ensure a suitable environment for the culture of the young fish.

The results of this work highlighted that most of the fish farming families relied on other farmers or their own experiences for diagnosing and treating their stocks. This was emphasised by the fact that many of the families obtained their own chemicals and drugs to treat the fish without asking advice and guidance for either extension services or research/diagnostic laboratories.

It was difficult to define small-scale fish farms according to the DOF classification of fish farms i.e. whether the system was extensive, semi-intensive and intensive. In the opinion of the interviewer semi -intensive farming according to the questionnaire classification is large pond or a number of ponds where regular feeding is given. There appears to be two different levels of fish farming in Thailand, both of which could be found located in each of the provinces visited. (1) Farms where there is little input; fish farming is not the main source of household income and the farmer experiences few/no diseases or water quality problems possibly because the farmers don't have any concept of these and they are not important to him (2) Farms where fish farming is the main occupation and disease appears to be a problem. In this case maybe the farmer has a better concept of disease.

Further Study

- Ascertain the level of fish health knowledge by families involved with fish production at all levels in various systems. This is particularly important if families are producing fish for consumption and/or are relying of fish production to provide an income for their household.

- Validate indicators provided by fish farming families on poor water quality and poor fish health with actual biological samples. This will confirm actual water quality problems and disease outbreaks.
- Evaluate the efficacy and application regime of treatments applied by households to enhance the benefit derived from fish farming by rural communities.
- Investigate the current access of rural fish farming households to information on aquatic animal health and application of the information.

SURVEY.1 DRAFT 6 Enter Farmer Code ☐

Enter Interviewer Code ☐

1. BACKGROUND

4 1.1 What Year Did You Establish Fish Farm?:_____________________________

1.2a Do You Have Previous Farming Experience: **YES** [1] ☐ **NO** [2] ☐

1.2b Was Your Previous Farming Experience in Fish Farming? **YES** [1] ☐ **NO** [2] ☐

1.2c If Your Farming Experience Was NOT in Fish Farming,

What Type of Farming Did You Do? ______________________

1.2d When Did You Start This Type of Farming? ____________

1.2e How Many Years Experience Do You Have in Fish Farming? _____________Years

1.3a Please tick all that apply:

1.3a Type of Current Farming System	
Monoculture [1]	
Polyculture [2]	
Large Cage [3]	
Small Cage [4]	
Integrated [5]	
Hatchery/Nursery [6]	
Other [7]	
VAC [8]	

1.3b If Other, What Is It? __________________________

1.4a Please tick one box only:

1.4a Do You Prepare the Farm Before Every Crop?	
Yes [1]	
No [2]	
Don't know [3]	
Cannot remember [4]	
Sometimes [5]	
No, only first time [6]	

1.4b Please tick all that apply:

1.4b If Yes, Do You Do Any of the Following to Prepare before Each Crop?	YES [a]	NO [b]	Sometimes [c]
Remove sediment [1]			
Lime [2]			
Leave to Dry [3]			
Other [4]			

1.4c If Other, What is it? ____

1.4d What is the Total Cost	Baht	1.4e How much Time in Total Does it Take?
Remove sediment[1]		**hours/days/weeks**
Lime [2]		**hours/days/weeks**
Leave to dry [3]		**hours/days/weeks/months**
Other [4]		**hours/days/weeks**

1.4f During the Preparation Time Do You Have Other Work?

YES [1] ☐ **NO** [2] ☐ **SOMETIMES** [3] ☐

1.5a From Stocking to Harvest, What is the Total Input Cost of Farm? __________Baht

1.5b What Month Do You Stock? ____________________________

1.5c What Month Do You Normally Harvest? ______________________________

1.5d Do You Ever Emergency Harvest Your Fish?

YES [1] ☐ **NO** [2] ☐ **Sometimes** [3] ☐

1.5e ***If answer is YES or sometimes please ask the farmer when and why?***

____

1.6aWhat is the Expected Income from Fish Farming (per crop): _______________Baht

1.6b Actual Income from Fish Farming (per crop):_________________________Baht

1.6c Farmer Did Not Know Income ☐

1.7a Is Fish Farming Your Main Source of Income? YES [1] ☐ **NO** [2] ☐

1.7 b Please tick all that apply:

1.7b Do You Also Do Any of the Following?	
Grow Vegetables [1]	
Grow Fruit [2]	
Grow Rice [3[	
Grow Plants [4]	
Grow Other Animals [5]	
Sell Fry or Fingerlings to other Farmers [6]	
Work Elsewhere [7]	

Give details: _____________________________

Give details: ____________________________

1.7c Please insert order of importance with 1 = least and 5 = most

1.7c What is the Most Important Source of Income to You?	

Fish Farming [1]	
Grow Vegetables [2]	
Grow Fruit [3]	
Grow Rice [4[	
Grow Plants [5]	
Grow Other Animals (e.g pigs) [6]	
Sell Fry or Fingerlings to other Farmers [7]	
Work Elsewhere [8]	

1.8a Please tick all that apply:

1.8a Who works on the Fish Farm?	
Husband [1]	
Wife [2]	
Children [3]	
All Immediate Family [4]	
Friends [5]	
Other Family Members (e.g brother-in-law) [6]	
Others [7]	

1.8b Please tick either daily or sometimes only

1.8b How Often Do They Work on the Fish Farm?	**Daily** [a]	**Sometimes** [b]
Husband [1]		
Wife [2]		
Children [3]		
Friends [4]		
Other Family Members [5]		
Others [6]		
Don't Know [7]		

2. Production

2.1 Please tick all that apply and write exact number in box

2.1 What is The TOTAL Number of Cages/Pens/Ponds on Your Fish Farm?	**Cages**	**Pens**	**Ponds**
1 [1]			
2-3 [2]			

4-5 [3]			
More that 5 [4]			

2.2a Please tick all that apply and write exact number in box

2.2a What is The Number of Cage/Pens/Ponds in Use for Fish Farming at Present?	**Cages** [1]	**Pens** [2]	**Ponds** [3]
1 [1]			
2-3 [2]			
4-5 [3]			
More that 5 [4]			

2.2b Please insert species and tick whether they are in cages/pens/ponds

2.2b What are The Fish Species in Cage/Pens/Ponds in Use for Fish farming at Present?	**Cages** [1]	**Pens** [2]	**Ponds** [3]

2.3 Please insert size in box, and tick whether this is the cage/pen or pond

2.3 What is Size of Each Cage/Pen/Pond in Use for Fish Farming? (m^2)	**Cage**	**Pen**	**Ponds**

2.4 Stocking Density for (2.4a)Fingerling: m^2 (2.4b) Adult: m^2

2.5a What is the Total Weight of Fish you Harvest (kg):
2.5b What is the Weight Per Fish you Harvest (kg):

2.6a Please tick all that apply:

2.6a Origin of Fry	
Wild Stocks [1]	
Hatchery [2]	
Neighbouring Farmer [3]	
Self [4]	
Fish Trader [5]	
Other [6]	

2.7 Do You Add Wild Fish? **YES** [1] ☐ **NO** [2] ☐

2.8a Please tick all that apply

2.8a Market Purpose	
Local [1]	
Region [2]	
Export [3]	
Family [4]	
Other [5]	
Fish processing Plant [6]	

2.8b If Other, What Is It?

____

3.Husbandry Regime

3.1a Do You Add Feed to the Fish? **YES** [1] ☐ **NO** [2] ☐

3.1b Please tick all that apply:

3.1b What Do You Use as Feed	
Pellets [1]	
Chicken Waste [2]	
Animal Manure [3]	
Trash Fish [4]	
Agriculture By-Products [5]	
Vegetables [6]	
Other [7]	

Give details:

Give details:

3.2 Please tick one box only:

3.2 How Often Do You Feed Fish?	
Once Daily [1]	
Twice Daily [2]	
Once Weekly [3]	
More Than Once Weekly [4]	
Other [5]	
Don't Know [6]	

3.3 At Each Feeding Time, How Much Feed do you Give the Fish? ______________

3.4 How Much in Total Does Feed Cost (per crop)? _________________Baht

3.5 How Do You Know How Much Feed to Give? ______________________________

3.6a Do You Ever Stop Feeding the Fish? YES [1] ☐ NO [2] ☐

3.6b If YES, Why? ______________________________

5 3.7 Please tick only 1 box for each concern:

3.7 Are You Concerned About the Following?	YES [a]	NO [b]	ALWAYS [c]	DON'T KNOW [d]
Flooding [1]				
High Temperatures [2]				
Low Temperatures [3]				

Too Little Water Available [4]				
Thieves [5]				
Predation [6]				
Water Quality [7]				
Diet [8]				
Water Exchange [9]				
Husbandry Techniques [10]				
Diseases [11]				
Production Rates [12]				
Treatments Cost [13]				
Stocking Density [14]				
Help & Advice [15]				
N^{o} Fish produced Per Crop				

3.8 Please Number (from 1 to 5) With 1 Being the Least and 5 Being the Most Factors That Concern You About Your Farm

Water Quality [1]	
Adequate Food Supply [2]	
Adequate Water Exchange [3]	
Husbandry Techniques [4]	
Disease **[5]**	
Cost of treatment (US $) [6]	
Correct Stocking Density [7]	
Help & Advice [8]	

3.9a Do You Experience Any Water Quality Problems? **YES** [1] ☐ **NO** [2] ☐

<u>3.9b If YES:</u>

Can You Identify Them? [a]	**YES** [1] ☐ **NO** [2] ☐ **DON'T KNOW** [3] ☐
What are they? [b]	
What Months Do They Occur? [c]	
How Long Does This Problem Last? [d]	
What Do You Do? [e] (e.g exchange water/stop feeding)	

Where Do You Get this Information from? [f]	
Does It Affect the Fish? [g]	**YES** [1] ☐ **NO** [2] ☐ **DON'T KNOW** [3] ☐
How Does it Affect the Fish [h] (e.g changes in behaviour/appearance, etc)	
Does it Cost More Money [i]	**YES** [1] ☐ **NO** [2] ☐ **DON'T KNOW** [3] ☐
How Much Money Does it Cost? [j] Ask farmer for a rough estimate	

3.10a Do You Exchange Water ? **YES** [1] ☐ **NO** [2] ☐

3.10bDo You Know What the Water Exchange Rates Are? **YES** [1] ☐ **NO**[2] ☐

3.10c If YES, What are the Water Exchange Rates? ________________

3.11 How Do You Exchange Water? ___________________________

3.12 Do You Always Farm the Same Fish Species? **YES** [1] ☐ **NO** [2] ☐

3.13a Do You Ever Change Fish Species **YES** [1] ☐ **NO** [2] ☐

3.13b If YES, Why? ____

3.14 Please tick all that apply:

3.14 What Factors Would Cause You to Change Fish Species?	
Increased Income[1]	
Advice from Neighbour [2]	
Advice from Other Farmer [3]	
Advice from Extension Officer [4]	
Advice from Research Officer [5]	
Disease Outbreak [6]	
Don't Know [7]	
Media Advice (Television or Newspaper or Radio) [8]	
Would NOT Change Species [9]	

3.15a Do You Keep Written Records From Your Farm? **YES** [1] ☐ **NO** [2] ☐

3.15 b If YES, What Do You Record [1] _____________________________

and Why [2]**?** ______________________________

4. Disease

4.1a Have You Ever Had Any Disease Problems? **YES** [1] ☐ **NO** [2] ☐

4.1b If YES, When? : (insert date if possible)________________________________

4.1c Please Describe Them?[1]

____

____

[1]This does not have to be scientific names, just describe what the farmer can see.

4.1d Were There Any Clinical Signs of Disease? **YES** [1] ☐ **NO** [2] ☐

4.1e If YES, Please Describe Them?[1] ____

4.2a Which Months Are Your Fish Susceptible to Disease Problems?

(**Please tick all that apply**)

Jan [1]	**Feb** [2]	**Mar** [3]	**Apr** [4]	**May** [5]	**June** [6]	**July** [7]	**Aug** [8]	**Sept** [9]	**Oct** [10]	**Nov** [11]	**Dec** [12]

4.2b Please Detail Why?

Jan [1]	**Feb** [2]	**Mar** [3]	**Apr** [4]	**May** [5]	**June** [6]	**July** [7]	**Aug** [8]	**Sept** [9]	**Oct** [10]	**Nov** [11]	**Dec** [12]

4.3 Please tick all that apply:

4.3 How Do You Recognise Diseases?	
Fish Stop Feeding [1]	
Fish Change Colour [2]	
Fish Change Behaviour [3]	
Fish Change Shape or Size [4]	
Marks (lesions/ulcers/spots) on the Fish Surface [5]	
Clinical Signs Of Disease [6]	
Damage to the External Surface Including Fins [7]	
Fish Die [8]	
Don't Know [9]	

4.4a Have You Had Any Training on Health Management? YES [1] ☐ **NO** [2] ☐

4.4b If YES, Please Give Details: (e.g Who was it with and who organised it?)

____

____

4.5 Have you Ever Contacted the Extension Officer? YES [1] ☐ **NO** [2] ☐

4.6a Please tick one box only

4.6a Where Do You Obtain Information on Diseases From?	
Self [1]	
Neighbouring Farmer [2]	
Extension Officer [3]	
Family [4]	
Research Staff [5]	
Media (Television, Newspapers, Radio) [6]	
Other [7]	

4.6b If Other, What Is It? ___________________________________

4.7a Please tick one box only:

4.7a Who Diagnoses A Disease Problem?	
Self [1]	
Research Staff [2]	
Extension Officer [3]	
Other [4]	

4.7b If Other, Who Is It? ___________________________________

4.8 Please Tick One Box Only

4.8 During A Disease Outbreak, What Percentage of Fish Were Lost?	
None [1]	
Less Than 10 [2]	
11-50 [3]	
More Than 50 [4]	
More Than 100 [5]	
All [6]	
Don't Know [7]	
No Response [8]	

4.9 What was the cost (Baht) of additional inputs during disease? _______________Baht

4.10 If Disease Occurs, What Do You Do? ___________________________________

4.11a Do You Ever Treat Before A Disease Occurs YES [1] ☐ **NO**[2] ☐

4.11b If YES Please Give Details? ___________________________________

4.12 Do You Treat When The Disease Occurs [1] ☐ **Or Leave It Until Fish Die?** [2] ☐

4.13 How Often Do The Fish Die Before You Would Treat?	
None [1]	
Daily [2]	
2-6 Days [3]	
Weekly [4]	
Don’t Know [5]	
No Response [6]	

4.14 Please tick all that apply and give details in space if possible:

4.14 What Are The Main Type of Treatments You Use?	
Traditional [1]	
Chemical [2]	
Antibiotics [3]	

4.15 Please tick one box only:

4.15 Where Are These Obtained From?	
Self [1]	
Extension officer [2]	
Shop [3]	
Pharmacy [4]	
Other Farmer [5]	
Other [6]	

4.16 How Do You Know When the Fish are Better? ______________________________

4.17 Does Treatment Increase [1] ☐

or Decrease [2] ☐ **Money Gained from Fish Farming?**

4.18a Please tick all boxes applicable:

4.18a What Happens to Diseased or Dead Fish?	
Discarded [1]	

Used as Fish Feed [2]	
Family Eat Them [3]	
Sell Them [4]	
Other [5]	

4.19Do You Think That Bacterial Disease Is a Problem on Your Farm?

YES [1] ☐ **NO** [2] ☐ **DON'T KNOW** [3] ☐ **Sometimes** [4] ☐

4.20 Please tick all boxes applicable:

4.20 Who Gives You Advice on Diseases and Treatments?	
Self [1]	
Other Farmers [2]	
Relatives [3]	
Extension Officers [4]	
Research Staff [5]	
Other [6]	

4.21 Address and Name of Farmer

NAME:

____

Give each farmer a unique code

ADDRESS:

____

____

Each farm will be coded according to Province

4.22 Data Collected by:____________________date: ____________time: ___________

Each collector will be given a code.

Thank you very much for your time. The information supplied will be used as part of a large study investigating the incidence and impact of bacterial disease in small-scale freshwater farming systems in Asia and will NOT be used for any other purpose.

INTERVIEWERS OPINION OF FARM:

Insert Farmer Code Number: ☐

Insert Interviewers Code Number: ☐

1 Please Explain How Farms were Chosen? ________________________

2. Please Give Opinion of Type of farming System:

Extensive[1] ☐ Semi-Intensive[2] ☐ Intensive[3] ☐

3. Please Give General Opinion of Farm:

Good[1] ☐ Intermediate[2] ☐ Poor[3] ☐

4. Was This Farmer Successful in Aquaculture?

YES [1] ☐ NO[2] ☐ Don't Know [3] ☐

Appendix 2

This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID'

VIETNAMESE FISH FARMER SURVEY DATA: GENERAL FINDINGS

Dr Kim D. Thompson and Dr Mags Crumlish,

Institute of Aquaculture, University of Stirling, Stirling Scotland, UK

Ms. T.T Dung and Ms N.T.N. Ngoc Aquaculture and Fisheries Science Institute (AFSI) College of Agriculture, Cantho University Vietnam

INTRODUCTION

A survey was conducted to assess the problems associated with husbandry and fish disease and to try to establish the frequency of disease related problems encountered by families involved in freshwater aquaculture. The survey was conducted within 7 provinces of the Mekong Delta by staff from AFSI in 2000. The survey included households located within Cantho, Vinh Long, An Giang Dong Thap, Tien Giang, Long An and HoChiMinh provinces (Table 1). In total, 201 families were personally interviewed using a structured questionnaire. The target population of the survey was 'rural poor fish farmers', which were located throughout the 7 provinces (Table 1).

Table 1 Number of Households and Percentage Distribution per Province

Province	N^0 of households	Percentage of households per Province
An Giang	51	25
CanTho	35	18
Dong Thap	14	7
HCM	24	12
Long An	35	17
Tien Giang	27	13
Vinh Long	15	8

Total	201	100

HCM = Ho Chi Minh,

A structured questionnaire was designed in English using Microsoft Word and then translated into Vietnamese. This was checked for misinterpretation in the meaning of questions that may have arose during translation into Vietnamese. The questionnaire was carried out as personal interviews with most of the questions being in the form of closed questions, although some open-ended questions were also included. The design was simple with tick boxes or dichotomous replies. The questionnaire consisted of four main sections: farm background, production, husbandry regime and fish disease. This was pilot tested and adapted for use in Vietnam as recommended the Vietnamese research team. Detailed lists of the fish farming households within districts of each province was supplied from the department of fisheries (DOF) and provincial extension services. From these lists, 50% of the families were randomly selected and visits arranged to ask the household to participate in the study and prearrange suitable interview times. An English version of the survey is presented at the end of the appendix.

METHODS AND PROBLEMS

Initially 50 % of fish farming households were randomly selected from the lists provided by the district and provincial extension services. However, the number of farms selected was too large to be visited within the time frame of the project, and so the number selected was reduced to 25 %. Many of the extension officers were unsure which families under their jurisdiction farmed fish. This reflects the large areas covered by extension officers and the constantly changing numbers of families who participating in fish farming. Contact with farmers to ensure their willingness to participate in the survey and to arrange visits was made either directly by the research team or indirectly through the extension officer. Transport to the farms was sometimes difficult due to their rural located or problems with flooding during the rainy season.

Information was complied and analysed using the EPI-Info version 6 (DOS-based) programme (http://www.cdc.gov/epo/epi/software.htm).

RESULTS

Farm Background

Farms had generally been established from between 1945 and 1999, with 3 main periods of development identified [1990 (n=21), 1995 (n=21) and 1998 (n=24)]. The new farms were distributed throughout all 7 provinces visited in the survey. Of the households interviewed, 83% (n=166) had previous farming experience and of these families 71% (n=141) had previous experience in fish farming. The other types of farming practised on the farms visited included growing rice, fruit vegetables and animals. More families used polyculture systems to culture their fish, than any other type of farming system (Table 2). A high number of farmers used more than one type of culture system on their farm and this was often a mixture of either mono and polyculture ponds, or poly and integrated ponds.

Table 2 Types of Fish farming Systems

System	Number of households
Monoculture only	54
Polyculture only	82
Integrated only	16
Mixed	42
Other	4
Total	**198**

Only 14% (n=25) of the families interviewed had fish farming as their main source of income. The other 86% (n=149) households had a wide range of other livelihood activities. These varied from growing rice and other agricultural crops to farming livestock and working elsewhere.

In the majority of cases, the husband, wife and children, as well as other immediate family members, all worked on the fish farm. Not all of the farms included in the survey were small-scale, with some farmers employing workers to help farm their fish. Sometimes workers were employed at strategic times within the production cycle, such as at the time of pond preparation, stocking ponds or harvesting fish. Workers tended to be employed on a more regular basis if fish farming was the farmers' main source of income.

Nearly all of the families interviewed cultured fish in ponds rather than cages. A high number of families owned between 1 or 2 ponds (Table 3). Even if more ponds were owned by the farmer, usually only 1 or 2 ponds were used (Table 3). This appeared to be related to costs associated with producing the fish, and may reflect the farmers use of the fish. For example, if a family had a range of livelihood activities they may only be only able to cope or afford to look after one pond of fish.

Table 3 Number of ponds owned and the number in use by fish farming households

Number Ponds	Total N^0 Ponds per household	Actual N^0 Ponds in use per household
1	107	129
2	57	51
3	14	7
4	8	3
5	3	2
6	3	1
7	1	0
8	1	1

10	1	0
12	1	1

The size of the fishponds varied from 12.5 to 14,000 m^2 (n=197), however, the most common size was 100, 200 and 500 m^2. The size of the ponds varied depending on the number of ponds on the farm, and also varied in size within individual farms.

Production

The length of the production cycle varied between individual households with farms being stocked and harvested throughout the year (Table 4). A higher number of families claimed to stock their fish farm during April, May and June compared with any other time (Table 4). The main months in which fish were harvested tended to be March, April and December (Table 4). However, some families claimed to harvest their fish as required (i.e. for food or for money).

Table 4 Number of households stocking and harvest fish each month

Month	**N^0 households stocking fish**	**N^0 households harvest fish**
January	2	16
February	6	9
March	18	**17**
April	**47**	**19**
May	**22**	14
June	**28**	6
July	9	2
August	16	8
September	10	10
October	9	8
November	6	5
December	8	**22**
Other	11	3
1-year		3
2-year		1
More than 2 years		2
Anytime		11
Don't know		8

The stocking and harvest times are independent and are not linked to individual fish farms.

Fish Species

Farmers in the Mekong Delta cultured a wide range of fish species (Table 5). More than half of the households interviewed (n=134) cultured *Pangasius hypophthalmus* either in monoculture or polyculture systems. The six most popular species cultured were *P. hypophthalmus*, tilapia, silver barb, common carp, kissing gourami and giant gourami (Table 5).

Table 5 Range of Fish species cultured in the Mekong Delta

Common Name	Scientific Name
Freshwater catfish	***P. hypophthalmus***
Tilapia	***Oreochromis spp.***
Silver carp	Not found
Silver barb	***Puntius spp.***
Common carp	***Cyprinus carpio carpio***
Indian carp	Not found
Kissing gourami	***Helostoma temminckii***
Snake-skin gourami	*Trichogaster pectoralis*
African catfish (hybrid)	*Clarias spp.*
Grass carp	*Ctenopharyhgodon idellus*
Giant gourami	***Osphronemus goramy***
Snakehead	*Channa striata*
Climbing perch	*Anabas testudineus*
Sand goby	Not found
Wild fish	n/a

The most popular species were highlighted in bold font, n/a= not applicable

Families obtained their fry/fingerlings from a variety of different sources including fry from the wild, produced by hatcheries, neighbours or self (i.e. the farmer owned the hatchery or used self-propagating species), from middlemen or other (e.g commercial links). However, the most common source of fish seed was from middlemen/traders or from hatcheries.

In total, 52% (n = 98) of families observed fry mortalities on arrival whereas the remaining 48% (n=91) farmer did not report any fry mortalities on arrival. Although some families claimed to stock their farms with wild fish seed, only 28% (n=55) of families reported actually adding wild fry when asked by the interviewer.

The stocking density of fingerlings ranged from 0.5 fish to 82 fish m^3, (n=155), however most families stocked at either 2, 4, or between 10-20 fish m^3. More families knew the individual weight per fish at harvest time rather than the total weight at harvest, and this probably reflected the type of fish farming practised and the market purpose of the fish. In total, 148 families provided information on the weight per fish at harvest and this ranged from 0.1 to 8 kg, however, most families said the weight per fish was either 0.3, 0.5 or 1 kg. Eighty-eight households provided the total weight of the fish at harvest and this ranged from 1.6 kg to 150,000 kg (Table 6).

Table 6 Number of Households and the Total Weight of Fish at Harvest

Weight (kg)	N^0 households
<10	2
11-100	20
101-200	8
201-300	12
301-400	3
401-500	1
501-600	2
601-700	4
701-800	0
801-900	2
901-1000	3
1001-5000	17
5001-10,000	6
> 10,000	7

A large number of households used their fish for more than one purpose [Table 7-(mixed)]. These tended to be either for household consumption or destine for local market. A high number of families only produced fish for family consumption. Only 4% (n=7) of households produced fish for production companies and none of the families interviewed actually produced fish for export (Table 7), although some of the fish processing companies may have exported the fish as a processed commodity.

Table 7 Production Purpose and Number of Households

Production Purpose	N^0 households	% households
Local market only	25	12
Regional Market only	6	3

Export only	0	0
Family only	60	30
Other	8	4
Processing Plant only	7	4
Mixed	95	47
Total	**201**	**100%**

Husbandry Regime

In total, 84% (n=156) families performed some form of pond preparation before stocking with each crop. This ranged from removing sediment and mud to adding lime and leaving the pond to dry (Table 8). Although most families did one or more of these preparative procedures, some families did not do any preparation and are not included in the results of Table 8.

Table 8 Number of Families Performing Types of Farm Preparation

Preparation	N^0 households	
	YES	NO
Remove sediment/mud	145	17
Add lime	73	94
Leave to dry	80	80
Use Derris Root	7	158
Pump Water	82	82
Other	30	93

This was a multi-answer question

In total 88% (n=160) families interviewed fed their fish, with only 12% of households (n=21) not feeding their fish at any time. Most families fed their fish both once or twice a day with a range of different feeds. The amount given was dependent on the fish species, the type of feed being provided and the stage of the production cycle. The types of feed used by the farmer tended to vary at different stages of the production cycle. Some farmers provides high quality fish pellets in the early stages of production to promote fry growth. Nearer harvest time, fish would be fed less frequently and would usually be fed on a maintenance diet. Most families interviewed used agricultural by-products (produced from other farming activities or from household waste). Trash fish was also popular but this was species-dependent.

Half of the families interviewed did not experience any water quality problems. Further work is required to assess the farmers' awareness of water quality problems and their perceptions on the impact of water quality on fish health. From the data gathered it would appear that half of the families interviewed did not have any water quality problems, but the remaining 50% claimed to have encountered poor water quality on their fish farm at one time or another. Of the families that did experience poor water quality, most of them were able to describe the types of problems encountered (Table 9). They tended to describe changes in the colour of the pond water rather than describe other changes experienced in the system (Table 9). Twelve percent of families (n=12) describe "other" changes which occurred in their ponds e.g. as a results of run-off from insecticide use in the paddy fields. Eleven percent of households (n=11) provided a combination of descriptions ("mixed"), which often included gas bubbles at the surface of the water, a bad smell and a change in water colour.

Table 9 Descriptions of Water Quality Problems Encountered by Households

Description	N^0 households	% households
Change in Water Colour	41	41
Change in Water Smell	5	5
Problems with Soil	5	5
Change in Colour + Smell	8	9
High Turbidity	17	17
Mixed Descriptions	11	11
Other	12	12
Total	**99**	**100%**

Water quality problems were found to occur throughout the year (n=75), but a higher number of families reported poor water quality in January, March and April compared with any other time. No verifiable indicators were measured during the survey. When poor water quality occurred on the fish farms, families responded in various ways but a higher number exchanged water compared with any other type of treatment (Table 10).

Table 10. Types of treatments provided by households to treat poor water quality

Description of Treatment	N^0 households	% households

Chemical treatment	9	9
Exchange Water	45	44
Do Not Exchange Water	9	9
Mixed	7	7
Increased Oxygen Supply	27	27
Don't Know/Nothing	4	4
Total	**101**	**100**

In total 70% (n=141) families always practised water exchange on their fish farm irrespective of whether they had a water quality problem. Off these families, only 103 households knew what the water exchange rates were, and these varied depending on the type and size of the farm as well as the species cultured. However, a higher number of families exchanged 20 or 50% of the pond water compared with any other percentage quoted.

In total, only 11% households (n=23) kept some form of written records related to their fish farming activities, however, two families did not give a reply as to the use of their records (Table 11). Seventeen families recorded information related to production costs, while the other 4 families recorded information relating to water quality, pond preparation and disease outbreaks (Table 11).

Table 11 Information on the Farming Systems that Kept Written Records About their Fish Farm

Record ID	Reason Record Kept	Province	System	Importance of fish farming	Species	Market
2	Cost	Dong Thap	Mono	high	Sand goby	Regional
8	Cost	Dong Thap	Poly	middle	Catfish, CC, KG, Ph	Local
10	Water	Dong	Poly	Middle	SB,SSG,K	Mixed

	quality	Thap			G,IC,CC	
11	Water quality	Dong Thap	Mono	Most	Catfish	Local
12	Pond preparation	Dong Thap	Poly	High	CC,SC,T, SB,Ph	Family
53	Cost	Long An	Integrated	High	GC,KG,CC	Local
99	Cost	HCM	Poly	High	Ph, T, CC	Mixed
105	Cost	CanTho	Poly	Not	SB,CC,Ph,T	Mixed
108	Cost	CanTho	Poly	Not	SB,Ph,CC,SC	Mixed
138	Cost	CanTho	Poly	Not	Prawn	Mixed
139	Cost	An Giang	Mono	Not	Ph	Mixed
140	Cost	An Giang	Mono	Not	Ph	Mixed
141	Cost	An Giang	Mono	Not	Snakehead	Other
142	Cost	An Giang	Mono	Not	Ph	Mixed
143	Cost	An Giang	Mono	Not	Ph	Other
151	Cost	An Giang	Poly	Not	Ph,T,SB	Mixed
153	Cost	An Giang	Poly	Not	Ph	Mixed
154	Cost	An Giang	Poly	Not	SC,Catfish, Ph, T, KG	Mixed
162	No reply	An Giang	Poly	Most	Ph, CC, SB, KG	Mixed
168	Cost	An Giang	Poly	High	KG, SC,SB	Mixed
177	Fish disease	An Giang	Mono	Not	Pb, Ph	Processing P
187	Cost	An Giang	Poly	High	Ph, SB, T, KG	Mixed
194	No reply	Vinh Long	Mono	Low	Ph, IC	Family
Total						

HCM = HoChiMinh, Poly= polyculture, mono= monoculture, Ph = *Pangasius hypophthalmus*, Pb= *Pangasius bocourti*, SSG = snake skin gourami, KG = kissing gourami, SC = silver carp, CC = common carp. SB = silver barb, T = tilapia, IC= indian carp, GC = grass carp

Fish Disease and Treatments

In total 49% (n=97) of fish farming families interviewed had experienced fish disease on their farms at some point during fish farming. Diseases tended to occur throughout the year. A high number of families also reported diseases in November and January (Table 12), which is the cool season in Vietnam. A high number of families reported fish disease outbreaks spanning 2-3 months.

Table 12 Number of households reporting fish disease outbreaks each month

Month	N^0 households	% households
January	8	9
February	1	1
March	3	3
April	7	8
May	2	2
June	3	3
July	5	5
August	5	5
September	7	8
October	9	10
November	10	11
December	3	3
Don't Know/Cannot Remember	5	5
Other	24	26
Total	**92**	**100**

Farmers gave descriptions of diseased fish, with many using changes in colour and shape of the fish as indicators of disease (Table 13). Only changes in colour were also frequently used and these included red fins, white body and white/red spots. Some families gave a variety of descriptions refereed to as a "mixed" response (Table 13). A wide range of descriptions such as gasping for air and epizootic ulcerative syndrome (EUS) were also given and labelled as " other" descriptions (Table 13). No clinical diagnosis was made since no biological samples were taken during disease episodes or at the time of the survey. Therefore no conclusions could be drawn about the aetiological agent involved.

Table 13 Farmers' descriptions of diseased fish

Description	N^0 households	% households
Dead fish	9	9
Change in shape only	4	4
Change in colour only	17	18
Change in shape and colour	22	23
Ulcers/lesions	14	14
Don't know	1	1
Mixed	12	12
Other	18	19
Total	**97**	**100**

Disease outbreaks in ponds appeared to occur throughout the year, but a higher number of families reported disease in their stocks from October to January compared with any other time of year. In total, 6% families (n=11) claimed to have received some form of training in fish health. This training was mostly from the extension officers of district or regional offices, with one family receiving training from a fish-farming club. Few farmers claimed to contact the extension officers with only 10% (n=18) of households interviewed having actually contacted an extension officer. Mostly families relied on their own experience and advice from neighbouring farmers to diagnose fish disease. Treatment given ranged from providing antibiotics mixed with feed, to treating with antibiotics together with chemicals such as lime, malachite green and salt (Table 14). Twenty-nine percent families (n=26) did not treat their fish as they were unsure of what treatment to use and how to apply it (Table 14). Some families gave treatments other than antibiotics or chemicals, included in the category "other", which entailed removing dead fish or applying oxygen. Diseased or dead fish were often discarded by the farmer or used to feed the family. Only a minority of households claimed to sell the fish that had died in their ponds.

Table 14 Range of treatments provided by households

Treatment	N^0 households	% households

Antibiotics alone	26	29
Chemicals alone	5	6
Nothing	26	29
Antibiotics + Chemicals	21	24
Exchange water	5	6
Other	5	6
Total	**88**	**100**

Most fish farmers interviewed purchased their own treatments from pharmacies located provincially. Information and advice on diseases, and the use of treatment were mostly as a result of the families' own experience of disease or form other farmers.

DISCUSSION

Many of the families included in the survey had had previous farming experience, which is not surprising since many of the households in the Mekong delta are active in agri and aquaculture activities. Many of the households were rurally located and most of the farmers owned the land on which the pond was located. Fish farming appeared to be only one of many livelihood activities that the households were involved in. A higher number of households had polyculture systems compared with monoculture or other types of systems, and this probably reflected the market purpose of the fish being produced. Most of the families interviewed not only sold their fish locally, but used the fish to feed their family, and so having a diverse range of fish species in their farming system ensured that they always had food available or something to sell.

The most common species cultured was the indigenous Asian freshwater catfish (*P. hypophthalmus*), which has been farmed in Mekong delta for a number of decades. However, a wide range of fish species were cultured and again this was probably influenced by the types of farming systems used and the market purpose of the fish produced. Smaller sized fish were more popular with the local consumers, and indigenous species are more frequently purchased, because they are recognised by the local market-goers.

Half of the farmers interviewed had experienced water quality problems and disease problems at some point, although not always on the same farm. No biological samples were taken at the time of the survey to confirm the farmer's interpretation of these problems. However, it was interesting to observe from the frequency data that fish farmers appeared to rely on their own experience and the knowledge of other farmers for advice on the management of water quality problems and disease outbreaks. The fish farmer commonly applied treatments when diseases were recognised, and again this was usually after self-diagnosis of the problem. It was often the opinion of the AFSI research team that many households did not report problems on their fish farms because they lacked understanding of the concept of disease and water quality problems. Households were frequently unaware that fish could have disease problems or that poor water quality could affect fish and result in fish mortalities. This was reflected in the fact that few families had received any form of training on fish health and disease management. It is intended to determine relative risk factors relating to this data during DFID project R

8119 (*The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia*).

SURVEY.1 **DRAFT 7** **Enter Farmer Code** ☐

Enter Interviewer Code ☐

1. BACKGROUND

6 1.1 What Year Did You Establish Fish Farm?:______________________

1.2a Do You Have Previous Farming Experience: **YES** [1] ☐ **NO** [2] ☐

1.2b Was Your Previous Farming Experience in Fish Farming?

YES [1] ☐

NO [2] ☐

1.2c If Your Farming Experience Was NOT in Fish Farming,

What Type of Farming Did You Do? ______________________

1.2d When Did You Start This Type of Farming? ____________

1.2e How Many Years Experience Do You Have in Fish Farming? _____________**Years**

1.3a Please tick all that apply:

1.3a Type of Current Farming System	
Monoculture [1]	
Polyculture [2]	
Large Cage [3]	
Small Cage [4]	
Hatchery/Nursery [5]	
Other [6]	
VAC [7]	

1.3b If Other, What Is It? ___________________________

1.4a Please tick one box only:

1.4a Do You Prepare the Farm Before Every Crop?	
Yes [1]	
No [2]	
Sometimes [3]	

1.4b Please tick all that apply:

1.4b If Yes, Do You Do Any of the Following to Prepare before Each Crop?	**YES** [a]	**NO** [b]	**Sometimes** [c]
Remove sediment/mud [1]			
Lime [2]			
Leave to Dry [3]			
Clean Cage [4]			
Derris Root [5]			
Pump Water [6]			
Other [7]			

1.4c If Other, What is it? ____

1.4d What is the Total Cost (Vn Dong)	**100 m^2**	**Q1.41d** Insert calculation for Size of actual farm
Remove sediment/mud[1]		
Lime [2]		
Leave to dry [3]		
Clean Cage [4]		
Derris Root [5]		
Pump Water [6]		
Other [7]		

1.4e What is the Total Time for Preparation? ____________________days

1.4f During the Preparation Time Do You Have Other Work?

YES [1] ☐ **NO** [2] ☐ **SOMETIMES** [3] ☐

1.5a From Stocking to Harvest, What is the Total Input Cost of Fish Farm? ______VnD

1.5b What Month Do You Stock Fish Farm? ______________________________

1.5c What Month Do You Normally Harvest Fish Farm? ___________________________

1.5d Do You Ever Emergency Harvest Your Fish?

YES [1] ☐ **NO** [2] ☐ **Sometimes** [3] ☐

1.5e ***If answer is YES or sometimes please ask the farmer when and why?***

____

1.6aWhat is the Expected Income from Fish Farming (per crop): _______________VnD

1.6b Actual Income from Fish Farming (per crop):_________________________VnD

1.6c Did Farmer Know Income YES/NO

1.7a Is Fish Farming Your Main Source of Income? **YES** [1] ☐ **NO** [2] ☐

1.7 b Please tick all that apply:

1.7b Do You Also Do Any of the Following?	
Grow Vegetables [1]	
Grow Fruit [2]	
Grow Rice [3[	
Grow Seed Plants [4]	
Grow Other Animals [5]	
Sell Fry, Fingerlings or Juveniles to other Farmers [6]	
Work Elsewhere [7]	

1.7c Please insert order of importance with 1 = most and 5 = least

1.7c What is the Most Important Source of Income to You?	
Fish Farming [1]	
Grow Vegetables [2]	

Grow Fruit [3]	
Grow Rice [4[	
Grow Plants [5]	
Grow Other Animals (e.g pigs) [6]	
Sell Fry or Fingerlings to other Farmers [7]	
Work Elsewhere [8]	

1.8a Please tick all that apply:

1.8a Who works on the Fish Farm?	
Husband [1]	
Wife [2]	
Children [3]	
All Immediate Family [4]	
Friends [5]	
Other Family Members (e.g brother-in-law) [6]	
Others [7]	

1.8b Please insert number of hours per day

1.8b How Often Do They Work on the Fish Farm?	**Hrs**
Husband [1]	
Wife [2]	
Children [3]	
Friends [4]	
Other Family Members [5]	
Others [6]	
Don't Know [7]	

2. Production

2.1 Please tick all that apply and write exact number in box

2.1 What is The TOTAL Number of Cages/Pens/Ponds on Your Fish Farm?	**Cages**	**Pens**	**Ponds**
1 [1]			
2-3 [2]			
4-5 [3]			
More that 5 [4]			

2.2a Please tick all that apply and write exact number in box

2.2a What is The Number of Cage/Pens/Ponds in Use for Fish Farming at Present?	Cages [a]	Pens [b]	Ponds [c]
1 [1]			
2-3 [2]			
4-5 [3]			
More that 5 [4]			

2.2b Please insert species and tick whether they are in cages/pens/ponds

2.2b What are The Fish Species in Cage/Pens/Ponds in Use for Fish farming at Present?	Cages [a]	Pens [b]	Ponds [c]

2.3 Please insert size in box, and tick whether this is the cage/pen or pond

2.3 What is Size of Each Cage/Pen/Pond in Use for Fish Farming? (m^2)	Cage	Pen	Ponds

2.4 What is the Stocking Density:

2.4a Fingerling m^2

2.4b Juvenile m^2

2.4c Adult m^2

2.5a What is the Total Weight of Fish you Harvest (kg):

2.5b What is the Weight Per Fish you Harvest (kg):

2.6a Please tick all that apply:

2.6a What is the Origin of Fry/Juvenile Fish	
Wild Stocks [1]	
Hatchery [2]	
Neighbouring Farmer [3]	
Self [4]	
Middleman [5]	
Other [6]	

2.6b When Fry/Juvenile Arrive, Do You See Mortalities?

YES [1] ☐ **NO**[2] ☐ **DON'T KNOW**[3] ☐

2.6c If Yes, What Are The Percentage Losses? ____________________

2.7 Do You Add Wild Fish? **YES** [1] ☐ **NO** [2] ☐

2.8a Please tick all that apply

2.8a Market Purpose	
Local [1]	
Region [2]	
Export [3]	
Family [4]	
Other [5]	
Fish processing Plant [6]	

2.8b If Other, What Is It?

____

3.Husbandry Regime

3.1a Do You Add Feed to the Fish? **YES** [1] ☐ **NO** [2] ☐ **Sometimes** [3] ☐

3.1b Please tick all that apply:

3.1b What Do You Use as Feed	
Pellets [1]	
Chicken Waste [2]	
Animal Manure [3]	
Trash Fish [4]	
Agriculture By-Products [5]	
Vegetables [6]	
Other [7]	

3.2 Please tick all that apply:

3.2 How Often Do You Feed Fish?	
Once Daily [1]	
Twice Daily [2]	
Other [3]	
Don't Know [4]	

3.3 At Each Feeding Time, How Much Feed do you Give the Fish? ______________

3.4Where Do You Get Feeding Information From?_____________________________

3.5 How Much in Total Does Feed Cost (per crop)? _________________VnD

3.6a Do You Ever Stop Feeding the Fish? YES [1] ☐ NO [2] ☐

3.6b If YES, Why? ______________________________

7 **3.7a Please tick only 1 box for each concern.**

8 **3.7b Please insert number (with 1 = most and 5 = least) what concerns farmer about HIS farm.**

3.7a Are You Concerned About the Following?	YES [a]	NO [b]	ALWAYS [c]	DON'T KNOW [d]	3.7b Farmers Opinion [e]
Flooding [1]					
High Temperatures [2]					
Low Temperatures [3]					
Too Little Water Available [4]					
Thieves [5]					

Predation [6]					
Water Quality [7]					
Diet [8]					
Water Exchange [9]					
Husbandry Techniques [10]					
Diseases [11]					
Production Cycle [12]					
Treatments Cost [13]					
Stocking Density [14]					
Help & Advice [15]					
N^{o} Fish produced Per Crop [16]					

3.8a Do You Experience Any Water Quality Problems? **YES** [1] ☐ **NO** [2] ☐

If YES:

3.8b Can You Identify Them? [a]	**YES** [1] ☐ **NO** [2] ☐ **DON'T KNOW** [3] ☐
3.8c What are they? [b]	
3.8d What Months Do They Occur? [c]	
3.8e How Long Does This Problem Last? [d]	
3.8f What Do You Do? [e] (e.g exchange water/stop feeding)	
3.8g Where Do You Get this Information from? [f]	
3.8h Does It Affect the Fish? [g]	**YES** [1] ☐ **NO** [2] ☐ **DON'T KNOW** [3] ☐
3.8i How Does it Affect the Fish [h] (e.g changes in behaviour/appearance, etc)	
3.8j Does it Cost More Money [i]	**YES** [1] ☐ **NO** [2] ☐ **DON'T KNOW** [3] ☐
3.8k How Much Money Does it Cost? [j] Ask farmer for a rough estimate	

3.9a Do You Exchange Water ? **YES** [1] ☐ **NO** [2] ☐

3.9bDo You Know What the Water Exchange Rates Are? **YES** [1] ☐ **NO**[2] ☐

3.9c If YES, What are the Water Exchange Rates? _________________

3.10 How Do You Exchange Water? ____________________________

3.11 Do You Always Farm the Same Fish Species? **YES** [1] ☐ **NO** [2] ☐

3.12 Do You Ever Change Fish Species **YES** [1] ☐ **NO** [2] ☐ **Sometimes** [3] ☐

3.13 Please tick all that apply:

3.13 What Factors Would Cause You to Change Fish Species?	
Increased Income[1]	
Advice from Neighbour [2]	
Advice from Other Farmer [3]	
Advice from Extension Officer **[4]**	
Advice from Research Officer [5]	
Disease Outbreak [6]	
Other [7]	
Media Advice (Television or Newspaper or Radio) [8]	
Would NOT Change Species [9]	

3.14a Do You Keep Written Records From Your Farm? **YES** [1] ☐ **NO** [2] ☐

3.14 b If YES, What Do You Record [1] ______________________________

and Why [2]**?** ______________________________

4. Disease

4.1a Have You Ever Had Any Disease Problems? **YES** [1] ☐ **NO** ☐

4.1b If YES, When? : (insert date if possible)________________________________

4.1c Please Describe Them?[1]

____

____

[1]This does not have to be scientific names, just describe what the farmer can see or common names.

4.1d What are the Most Common Diseases[1] That Occur on Your Farm?

____

____

4.2a Which Months Are Your Fish Susceptible to Disease Problems?

(**Please tick all that apply**)

Jan [1]	Feb [2]	Mar [3]	Apr [4]	May [5]	June [6]	July [7]	Aug [8]	Sept [9]	Oct [10]	Nov [11]	Dec [12]

4.2b Please Detail Why?

Jan [1]	Feb [2]	Mar [3]	Apr [4]	May [5]	June [6]	July [7]	Aug [8]	Sept [9]	Oct [10]	Nov [11]	Dec [12]

4.3 Please tick all that apply:

4.3 How Do You Recognise Diseases?	
Fish Stop Feeding [1]	
Fish Change Colour [2]	
Fish Change Behaviour [3]	
Fish Change Shape or Size [4]	
Marks (lesions/ulcers/spots) on the Fish Surface [5]	
Clinical Signs Of Disease [6]	
Damage to the External Surface Including Fins [7]	
Fish Die [8]	
Don't Know [9]	

4.4a Have You Had Any Training on Diseases? YES [1] ☐ **NO** [2] ☐

4.4b If YES, Please Give Details: (e.g Who was it with and who organised it?)

____

____

4.5 Have you Ever Contacted the Extension Officer? **YES** [1] ☐ **NO** [2] ☐

4.6 Please tick all that apply

4.6 Where Do You Obtain Information on Diseases From?	
Self [1]	
Neighbouring Farmer [2]	
Extension Officer [3]	
Family [4]	
Research Staff [5]	
Media (Television, Newspapers, Radio) [6]	
Other [7]	

4.7 Please tick all that apply:

4.7 Who Diagnoses A Disease Problem?	
Self [1]	
Research Staff [2]	
Extension Officer [3]	
Other [4]	

4.8 Please Tick One Box Only

4.8 During A Disease Outbreak, What Percentage of Fish Were Lost?	
None [1]	
Less Than 10 [2]	
11-50 [3]	
More Than 50 [4]	
All [5]	
Cannot Remember [6]	
No Response [7]	

4.9 What was The Total Cost of Additional Inputs during Disease? ______________VnD

4.10 If Disease Occurs, What Do You Do? ___________________________________

4.11 Do You Ever Treat Before A Disease Occurs? YES [1] ☐ **NO**[2] ☐ **Sometimes** [3] ☐

4.12 Please tick one box only:

4.12 How Often Do The Fish Die Before You Would Treat?	
None [1]	
Daily [2]	
2-6 Days [3]	
Weekly [4]	
Don't Know [5]	
No Response [6]	

4.13 Please tick all that apply and give details in space if possible:

4.13 What Are The Main Type of Treatments You Use?	
Traditional [1]	
Chemical [2]	
Antibiotics [3]	
Other [4]	

4.14 Please tick all that apply:

4.14 Where Are These Obtained From?	
Self [1]	
Extension officer [2]	
Pharmacy [3]	
Other Farmer [4]	
Other [5]	

4.15 How Do You Know When the Fish are Better? ____________________________

4.16 In Your Opinion, Do Treatments Increase or Decrease the Money Gained from Fish Farming?

4.16a Increase [1] ☐

4.16b Decrease [2] ☐

☐

4.16c Don't Know [3]

4.17 Please tick all that apply:

4.17 What Happens to Diseased or Dead Fish?	
Discarded [1]	
Used as Fish Feed [2]	
Family Eat Them [3]	
Sell Them [4]	
Other [5]	

4.18 Please tick all that apply:

4.18 Who Gives You Advice on Diseases and Treatments?	
Self [1]	
Other Farmers [2]	
Relatives [3]	
Extension Officers [4]	
Research Staff [5]	
Other [6]	

4.19 Address and Name of Farmer

NAME:

____

ADDRESS:

____

____

4.20 Data Collected by:____________________date: ___________time: __________

Thank you very much for your time. The information supplied will be used as part of a large study investigating the incidence and impact of bacterial disease in small-scale freshwater farming systems in Asia and will NOT be used for any other purpose.

5. INTERVIEWERS OPINION OF FARM:

5.1 Insert Farmer Code Number: [ ]

5.2 Insert Interviewers Code Number: [ ]

5.3 Please Give Opinion of Type of Farming System:

Extensive**[1]** ☐ Semi-Intensive**[2]** ☐ Intensive**[3]** ☐

5.4 Please Give General Opinion of Farm:

Good**[1]** ☐ Intermediate**[2]** ☐ Poor**[3]** ☐

5.5 Was This Farmer Successful in Aquaculture?

YES **[1]** ☐ NO**[2]** ☐ Don't Know **[3]** ☐

Appendix 3

This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID'

BIOLOGICAL SAMPLING OF FISH FARMED IN SMALL-SCALE FRESHWATER SYSTEMS IN VIETNAM

Dr Kim Thompson and Dr Mags Crumlish,

Institute of Aquaculture. University of Stirling, Stirling UK

Ms. T.T Dung and Ms N.G.N. Ngoc Aquaculture and Fisheries research Institute (AFSI), College of Agriculture, Cantho University. CanTho. Vietnam

BACKGROUND

The presence of bacteria within the cytoplasm of phagocytic cells isolated from the anterior kidney of freshwater farmed fish has now been reported in a number of different studies (Crumlish 1999, Thompson and Crumlish 1999, and Crumlish, Somsiri, Dung, Inglis and Thompson 2000). This phenomenon has been seen in a range of freshwater fish cultured in a variety of different farming systems examined in Thailand and Vietnam (Thompson and Crumlish 1999). High bacterial loading is often reported within the aquatic environments of the fish farms in Thailand and Southern Vietnam, and a statistical significant relationship was found between high bacterial load in the pond water and the occurrence of bacteria within macrophages from clinically healthy farmed fish (Thompson and Crumlish 1999). Although the levels of bacteria seen within the isolated macrophages were generally low, most macrophage preparations made from fish from these systems contained bacteria.

The dominant bacterial species recovered from the macrophages isolated from the fish were identified as Aeromonads and Pseudomonads (Crumlish 1999, Thompson and Crumlish 1999, and Crumlish *et al* 2000): potential opportunistic pathogens. The clinical significance of the bacterial load in the macrophage still remains to be determined, although preliminary assessment using laboratory based studies suggested that fish do succumb to opportunistic infections under stress (Crumlish *et al* 2002). Constant interactions between macrophages and high levels of bacteria from the aquatic environment may result in immunocompromised animals, hence increased susceptible to infection. This relationship if proven, could be used as an indicator to predict potential disease outbreaks in the farming systems in Thailand and Vietnam.

Many different methods are available to examine the cellular immune response of fish in the laboratory. However, there are few studies whereby the cellular immune response of farmed fish has been measured at the pond-side. A crude but reliable method was developed to isolate macrophage cells from the anterior kidney of farmed fish on site (Crumlish *et al* 2000). In a previous DFID project R7054, macrophages from healthy fish with no overt clinical signs of disease were examined for the presence of bacteria in their cytoplasm. The functional activity of these macrophages was not examined in that particular study however.

A study was designed to examine water quality parameters, the bacterial load of the pond, and the occurrence of bacteria within macrophages, in farms where the farmer had reported previous disease episodes or water quality problems. Farms were chosen where sampling could be performed for six continuous months during the production cycle. A number of different fish species in different farming systems were examined. As well as looking for the presence of bacteria in macrophages isolated from the head kidney of the fish, the functional activity of the cell was also examined using the cells ability to phagocytose yeast as a measure of immunocompetence of the cell. White and blood cell counts were also used as an indicator of the health status of the animal.

MATERIALS AND METHODS

The study design

A 6-month study was carried out in the Mekong Delta region of Vietnam in which farms were visited by research staff from AFSI, CanTho University, to collect biological samples at monthly intervals. The farmers selected to take part in the survey farmed a range of different freshwater fish species in a variety of farming systems. During each visit 4 to 6 fish were sampled. Samples of water were taken for analysis and used to determine bacterial counts in the water. Blood samples from the caudal vein were taken for blood cell counts and differential white cell counts, and macrophage cells isolated from anterior kidney and examined for the presence of bacteria in their cytoplasm and their ability to phagocytose yeast particles. This work was performed at the farm site.

Farmer Selection

The selection criteria for participating farmers were that their farms had to be within easy travelling distance from AFSI, and they were farmers from the questionnaire in Appendix 2 who claimed to have experienced poor water quality and/or fish disease. The farms also had to be actively involved in aquaculture at the time of the survey and finally that they would have completed a full production cycle within the time frame of the six month study. The farmers also had to be willing to participate in the record keeping study in Appendix 4.

Water samples

(a) Water quality analysis

At each visit the pH and temperature of the farm water was measured on site. Dissolved oxygen was measured using a probe which was often faulty and associated data was therefore unreliable and removed. The total ammonia (nitrates/nitrites) and total phosphate levels were measured on site using a commercially available kit purchased from Thailand. A sample of water was taken back to the AFSI water chemistry laboratory in a sterilised container where it was assessed for total suspended solids (organic and inorganic).

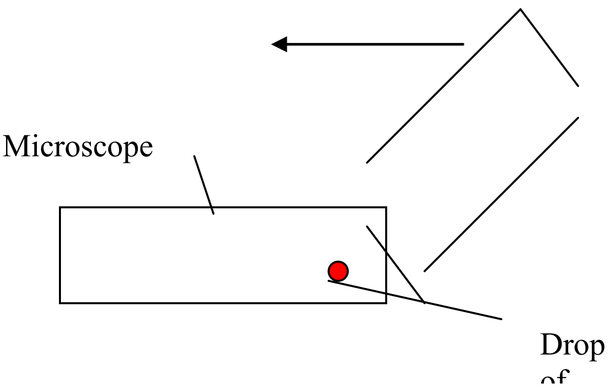
(b) Bacterial counts in the water and their identification

The spread plate method described by Heritage *et al* (1996) was used to determine the number of viable bacteria present in samples of farm water. Dilutions of samples were dropped and spread onto tryptone soya agar (TSA) and Aeromonas selective media plates. The plates were then sealed and incubated at 28^0C at the AFSI laboratory, for 24 to 48 h after which time the number of colony forming units (cfu) was determined. The dominant bacterial colonies from the TSA and Aeromonas selective media were subcultured so as to obtain pure colonies of the dominant bacteria and these were identified to genus level using primary identification tests (Millar and Frerichs 1996).

<u>Blood samples</u>

(a) Differential white blood cell counts

The fish were bled from the caudal vessel and a sample of blood was prepared for differential white blood cell counts using the method of Rowley (1990). Briefly, 2 blood smears were prepared per fish (Figure 1). These were allowed to air dry, then fixed in alcohol for 30 sec and stained using eosin and giemsa based dyes of the DipQuick or Rapi-Diff II kits for 1 min in each stain. The slides were air dried, taken back to the AFSI laboratory and mounted. The slides were examined under oil immersion (x100 magnification) and the percentage of lymphocytes, monocytes, neutrophils, basophils and where appropriate eosinophils determined for a total count of 200 cells.



- Place clean microscope slide on flat surface.
- Put small drop of blood onto slide
- Take another clean slide and place edge against drop of blood

Figure 1 Blood smear preparation.

(b) Total white blood cell counts

Total white blood cell counts were performed at the AFSI laboratory following the protocol of Siwicki and Anderson (1993). At the farm a small volume of blood (200 µl) from each fish was transferred into a sterile eppendorff tube containing 10 µl of heparin. The eppendorff was inverted slowly to mix the blood and heparin and taken back to AFSI where the cell count was made. A 1:20 dilution (100µl of blood in 1.9ml of phosphate buffered saline) was prepared and the cells then counted using a haemacytometer. All cell counts were performed at the laboratory on the same day as the sampling.

(c) Serum collection

The remainder of the blood collected was placed into another eppendorff tube without heparin, transported back to the laboratory and left overnight at 4°C. The next day the blood was centrifuged at 13,000 rpm for 5 min in a microcentrifuge and serum removed. This was placed into an eppendorff tube and kept at -20°C until required (it was the intention to use these to measure serum lysozyme levels, but these samples remain to be analysed).

<u>Recovery of bacteria from the fish and their identification</u>

A small section of anterior kidney was sampled from each fish and streaked onto both TSA and Aeromonas selective agar. Suspensions of the macerated kidney suspension were also streaked onto TSA and Aeromonas selective agar. The plates were sealed, incubated at 28^0C and checked for presence of bacterial growth 48 h later. Any growth was recorded (number, shape and colour of colonies) before selecting the dominant colony and producing a pure culture by subculturing, which was identified to genus level using primary tests (Millar and Frerichs 1996).

<u>Recovery and Isolation of Macrophage Cells</u>

All solutions were prepared the day before sampling, kept at 4°C overnight and checked for contamination by streaking on to TSA. Macrophages were isolated at the pond-side following the method described by Crumlish *et al.* (1999). Briefly, after streaking the anterior kidney from each fish onto TSA and Aeromonas selective media, the remainder of the organ was placed into 2mls of isolation media (total volume: 20mls Leibovitz-15 medium supplemented 20µl foetal calf serum (FCS) or control fish sera, 80µl heparin). This was macerated through a sterile mesh using the end of a sterile syringe to produce a mixed cell suspension. Three circles were then marked onto the surface of cleaned microscope slides using a PAP pen with two slides per fish and these were kept in a sterile container. One slide was labelled macrophage and bacteria and the other slide macrophage and yeast. The cell suspension (100µl) was placed onto each circle of the slide using sterile tips. The slide was incubated in a sterile container for 40 min after which unattached cells were removed gently by washing the slides with sterile saline and tapping each slide gently to remove excess liquid.

<u>Detection of bacteria in macrophages</u>

The slides labelled macrophage and bacteria were fixed in alcohol for 30 sec, and stained using RapiDiff II kit. Slides were washed in normal water until no further colour was removed and then left to air dry. These were transported back to the laboratory and mounted before examining under oil immersion by light microscopy. The number of macrophages containing bacteria were counted and expressed as a percentage of the total cell count. The aim was to count a total of 200 cells but this number was not always possible to obtain as the number of cells recovered was related to the size/age and the species of fish.

Phagocytosis

Bakers yeast was used as an indicator to assess the ability of the macrophages to phagocytose particles. Yeast (5 mg) was placed in a sterile universal and taken to the farm dry. The yeast was resuspended at the farm in 5 mls of L-15 supplemented with either 5% FCS or normal fish serum). The yeast suspension (100 μl circle^{-1}) was placed onto the 3 wax circles on the slides labelled macrophage and yeast, and gently aspirated to ensure contact between the cells and yeast. This was incubated in a plastic container (previously sterilised) for 60 min. After this time the slides were gently removed and washed using sterile physiological saline and tapped to removed excess fluid. The whole slide was immersed in alcohol fixative for 30 sec before staining it with the Rapi-Diff II kit. The slides were washed in water until no further colour was removed and left to dry before taking them back to the laboratory for mounting. The mountant was left to dry for 24 h before examining them by light microscopy under oil immersion.

The number of macrophages containing yeast were counted and expressed as a percentage of the total cell count. The aim was to count a total of 200 cells but again this was not always possible.

RESULTS AND DISCUSSION

Farm visits

In total, ten farmers participated in the study and their farms were located in different districts of CanTho and An Giang Province (Table 1). Unfortunately, "sever flooding" took place during the sampling period and some of farms could not be reached during this period. Hence it was difficult for the research team to make six consecutive monthly visits to some farms. The three farms highlighted in Table 1 were the only farms to be visited for 4 consecutive months in a row. No farms were visited during the month of February, since this month included a two-week National holiday.

Table 1 Farmers Selected, Farm Site and Sampling Times

Farmer Name	Farm Site	Samples collected*	Fish Species Stocked
1. Mr Tri	Long My District, CanTho Province	Sept, Nov. and Jan.	Climbing perch
2. Mr Von	Long My District, CanTho Province	Sept, Nov. and Jan	Snakeskin gourami
3. Mr Dinh	**Phung Hiep District, CanTho Province**	**Sept., Oct., Nov., Dec and March**	**Kissing gourami, silver carp and barb, Indian carp and Tilapia**
4. Mr Do	**Mang Thit District, CanTho Province**	**Sept., Oct., Nov., Dec. and March**	**Tilapia and silver carp**
5. Mr	**Mang Thit**	**Sept., Oct.,**	**Carps (silver, Indian and**

Hai	District, CanTho Province	Nov., Dec. and March	common), and *P. hypophthalmus*
6. Mr Liem	Chau Phu District, An Giang Province	Sept., Nov., Jan. and March	*P. hypophthalmus* and Tilapia
7. Mr Nhat	A Chau Phu District, An Giang Province	Sept., Nov., Jan. and March	Silver barb, tialpia and Indian carp
8. Mrs Hoa	Chau Phu District, An Giang Province	Sept., Nov., Jan. and March	Tilapia, Indian and common carp
9. Mr Tung	Chau Phu District, An Giang Province	Sept., Nov., Jan. and March	*P. hypophthalmus* and kissing gourami
10. Mr Ngoc	Chau Phu District, An Giang Province	Nov., Jan. and March	Silver and common carp and Tilapia

* 2000/2001

Water samples

(a) Water quality samples

Water analysis was performed at each farm visit only. The quality of the water was assessed using tests for primary production, acidity or alkalinity, temperature and presence of organic and inorganic particulates in the water column. The three farms, visited monthly between September and December appeared to have very similar water quality values (Figure 2). The primary production in the farm water was low and similar for all three farms visited as shown by the levels of total nitrates (TN) and total phosphates (TP) (Figure 2). The acidity and alkalinity of the water was more or less uniform in the three farms over the sampling period, but there were sight variations in water temperature during this time. There was a significant decrease in the water transparency in Farmer Do's system on the second sampling, which was probably related to the increase seen in total suspended solids (TSS) (Figure 3). In all farms sampled, the amount of TSS in the farm water column appeared to be mostly from organic rather than inorganic matter as shown in Figure 3.

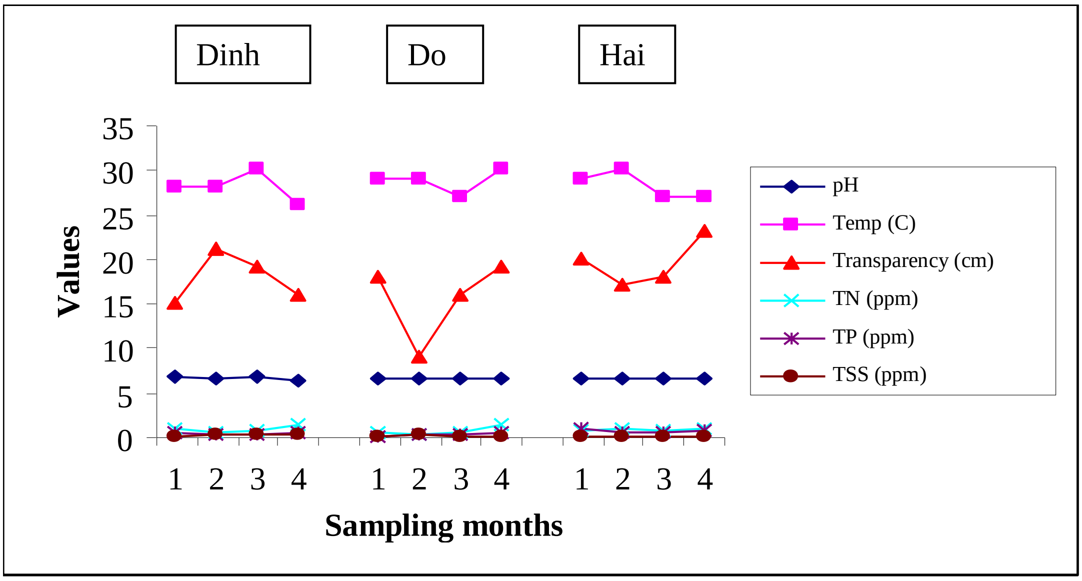


Figure 2 **Water quality values of the three farms sampled monthly between September and December (Farmers Dihn, Do and Hai)**

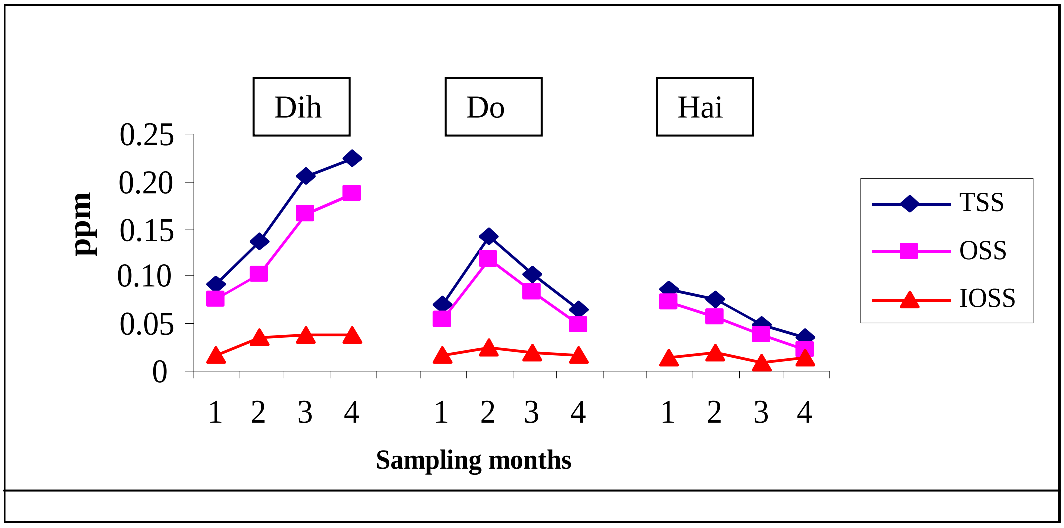


Figure 3 **Amount of suspended solids in the water columns of the three farms sampled monthly between September and December (Farmers Dinh, Do and Hai)**

<u>Bacterial counts in the water and their identification</u>

Bacterial colonies were recovered from the water samples taken from the three farms (Table 2). Water samples were grown on TSA and Aeromonas selective medium, but it was later found that other bacteria also grew on the Aeromonas selective medium. An antibiotic (amoxocyllin) was added to the Aeromonas selective medium against which most aqueous aermonads are resistant. In previous work (Crumlish 1999, Thompson and Crumlish 1999) high numbers of bacteria from the water column in freshwater fish farms were identified as aeromonads hence the application of a selective media for aeromonad bacteria. The principal in using selective agar was to inhibit the growth of non-aeromonad bacteria, however it was found that other Gram negative bacteria also grew on the aeromonad selective agar. This made it difficult to determine the number of *Aeromonas* sp colonies in the water samples. Therefore, only the total colonies counted (cfu ml^{-1}) grown on the TSA are presented. The number of viable bacterial colonies recovered from the sampled water ranged from 10^3 to 10^7 cfu ml^{-1} (Table 2). There appeared to be a slight decrease in the number of viable bacteria recovered from farms in November compared with samples taken in September (Table 2).

Table 3. Total cfu ml^{-1} recovered on TSA from the farm water sampled during the survey

Farmer Name	Sept	Oct	Nov	Dec	Jan	March

Mr Tri	3x10^{5}		4x10^{5}		2x10^{5}	
Mr Von	2x10^{6}		2x10^{4}		7x10^{4}	
Mr Dinh	**7x10^{4}**	**1x10^{4}**	**4x10^{4}**	**9x10^{5}**		**5x10^{5}**
Mr Do	**5x10^{4}**	**1x10^{5}**	**9x10^{3}**	**2x10^{5}**		**3x10^{6}**
Mr Hai	**6x10^{5}**	**1x10^{7}**	**7x10^{4}**	**2x10^{5}**		**2x10^{5}**
Mr Liem	8x10^{4}		9x10^{3}		2x10^{7}	4x10^{4}
Mr Nhat	5x10^{4}		(contam)		0	9x10^{5}
Mrs Hoa	1x10^{5}		1x10^{5}		1x10^{5}	2x10^{5}
Mr Tung	5x10^{5}		4x10^{6}		TNTC	5x10^{3}
Mr Ngoc	N/d		1x10^{5}		TNTC	6x10^{4}

Contam = contaminated plate usually fungus or swarming bacteria present and overgrown everything else.

TNTC = too numerous to count

Bacterial recovery from the fish and their identification

The numbers of bacterial colonies present in samples taken from the fish were recorded in Table 3, and were predominantly mixed cultures of bacteria on the TSA plates. Although dominant colonies were selected from the mixed growth on the agar plates, the research team was unable to identify any of the bacterial species recovered from the fish (or the water). Large numbers of different bacterial species were recovered, subcultured and stored as pure cultures until they could be identified at a convenient time. However, there was a problem storing the bacteria at AFSI due to a lack of reliable equipment and relevant expertise.

Table 3. Number of fish sampled at the different farms on different sampling days that produced bacterial growth on TSA

Farm/ Month	numbers of CFU				[e]Comments
	[a]tissue	[b]suspension	[c]both	[d]No growth	
Dinh					n=24sampled, actual n=23 + 1 sample contaminated
September	3	0	3	0	
October	1	0	2	2	
November	0	0	2	4	
December	4	0	2	0	

Do September	4	0	0	2	n= 24sampled; actual n= 21 + 3 contaminated sample
October	0	0	6	0	
November	3	01	0	2	
December	0	0	1	2	

Hai September	4	0	0	2	n=15 sampled, actual n=14 1 contaminated sample
October	0	1	0	2	
November	0	1	3	1	
December	0	0	0	0	

a: number of fish with bacterial colonies isolated from their kidney

b: number of fish with bacterial colonies isolated from their macrophages (in suspension)

c: number of fish with bacterial colonies isolated from their kidney and their macrophages

d: number of fish with no bacterial colonies isolated from their kidney or their macrophages

e: due to the bad road conditions because of the flooding, some fish samples were contaminated during transport. The results from these samples were therefore excluded from the analysis.

A range of different bacterial colonies was recovered from the tissue and macrophage suspensions, varying in the different fish species, farms and sampling time (Table 4). Overall, the number of colonies recovered was low and predominantly considered as non-specific growth.

Table 4 Mean values and Range of colonies recovered on TSA

Dinh	n	From Tissue (Range)	From Tissue Mean	n	From Suspension (Range)	From Suspension (Mean)
Septembe r	6	2-28	11 ±10	6	0-2	1 ±1
October	6	0-20	6 ±7	6	0-12	6 ±6
Novembe r	6	0-9	2 ±4	6	0-1	0

December	6	1-52	17 ±19	6*	0-7	2 ±3

n= number fish sampled * = 1 sample contaminated

Do	n	Tissue Range	Tissue Mean	n	Suspension Range	Suspension Mean
Septembe r	6	0-27	11 ±12	6	0	0
October	6	1-6	4 ±2	6	1-4	2 ±1
Novembe r	6	0-4	2 ±2	6	0-7	1 ±3
December	6*	0-4	1 ±2	6	0-3	1 ±2

* = 3 bacterial samples were contaminated

Hai	n	Tissue Range	Tissue Mean	n	Suspension Range	Suspension Mean
Septembe r	6	0-27	7 11	6	0	0
October	3	0	0	3	0-2	1 ±1
Novembe r	5	0-57	27 ±27	5	0-15	5 ±6
December	1	Contam.	Contam	1	Contam.	Contam.

Contam. = contaminated samples

<u>Blood cell counts</u>

Both erythrocyte and leukocyte counts are useful indicators of the health status of fish, as they alter during infection or times of stress. However, the levels of these cells are susceptible to external influences such as seasonality, temperature, stress or pollution. Such factors are known to suppress the immune response of fish and poor water quality, "the cold season", transport stress, high stocking densities may all lead to a reduction in the levels of white or red blood cell counts obtained.

There is a lack of baseline data for total and differential white blood cell levels for "normal" fish cultured in freshwater farming systems in Asia (NB a variety of species not preciously reported in the literature). Without baseline levels, it is difficult to measure the effects of different factors on the blood cell counts. Total white and red blood cell counts, and differential white blood cell counts were performed for a variety of fish species farmed at the nine fish farms visited over the six-month sampling period. It was intended to use the data to set up baseline values for each species and try to see if there was any relationship between the cell counts and the other parameters measured during the biological sampling i.e water quality, numbers of macrophages with bacteria, bacterial counts in the water. However the data set was considered to be incomplete due to the inability to take samples during the flood hence no statistical analysis was performed.

Total white and red blood cell (TWBC) counts

The total white blood cells counts varied over time and farm sampled (Figure 4). The number of TWBC per species sampled also varied although there did not appear to be a significant difference in these levels between species (Figure 5). The lowest values were found in *C. mirhigal* and *P. hypophthalmus* (Figure 5).

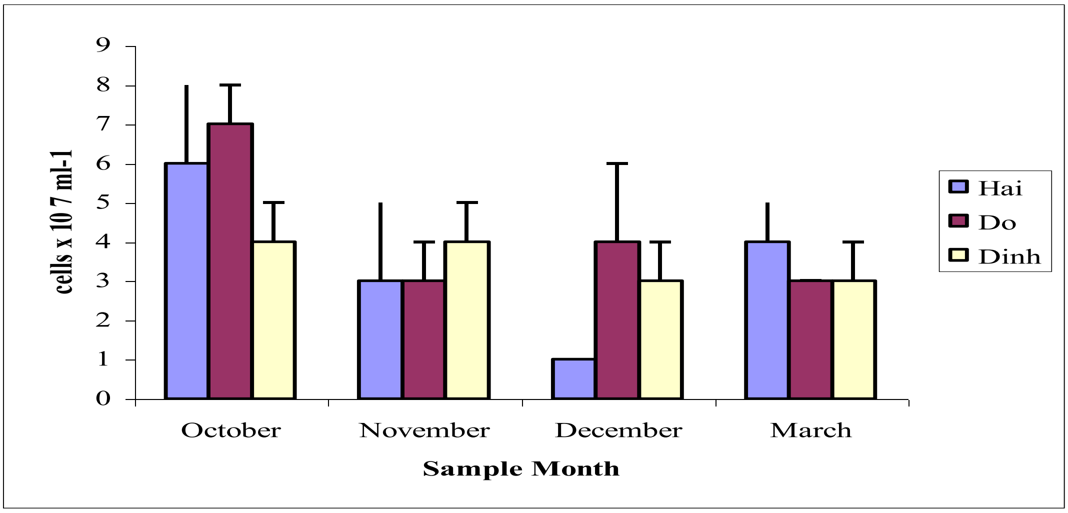


Figure 4. Mean Total White Blood Cell count per Farm at each sampling
NB Different sample numbers were taken at different sampling time for individual farms, mean value ±standard deviation.

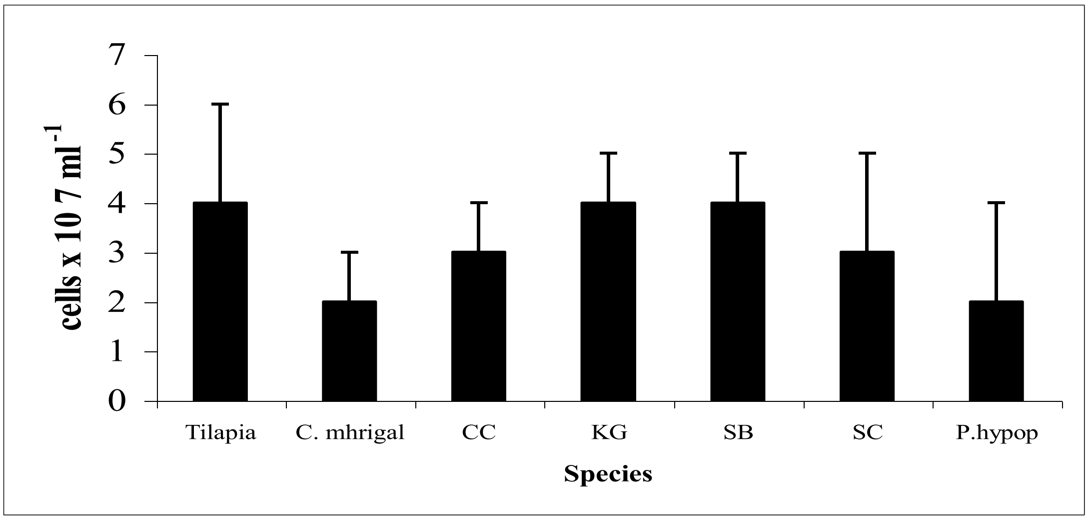


Figure 5 . Mean Total White Blood Cell count per Per Species
tilapia n=18, *C.mirhigal* n=2, CC = common carp (n=2), KG = kissing gourami (n=2), SB = silver barb (n=21), SC = silver carp (n=7), P.ypop = *Pangasius hypophthalmus* (n=3). Mean value ±standard deviation.

Differential white blood cell counts

The number and species of fish sampled and the date of sampling per farm were recorded, however the differential white blood cell counts are not presented here since the level of the monocytes counts (around 20%), were considered to be very high (K.D. Thompson pers.com), and the slides need to be re-evaluated before values are presented.

Isolation of Macrophage Cells from head kidney

(a) Number of macrophages with bacteria observed

The farms of Dinh, Do and Hai were the only sites where samples were collected for four consecutive months, and therefore the results obtained from these farms are the only ones which will be discussed here. A high number of cells were recovered from silver carp in all of the farms sampled with the mean number being more than 100 cells per fish except in November at the Hai Farm where the average number was low (Table 5). Consistently high yields of macrophages were recovered from Tilapia in Do Farm, except in November where the average yield was lower (Table 5). Good yields of macrophages were also obtained from *P. hypophthalmus* from the Hai Farm in Sept, Oct and December, although the number of fish sampled was low (Table 5).

Table 5 Fish species, mean number and range of macrophages with bacteria

Month	Kissing G	Silver carp	Silver barb	Indian carp	Tilapia	P. hypophth.
Farm Dinh						
Sept	n=1, 160	n=5, (136-175) 157 ±19	0	0	0	0
Oct	0	n=6, (70-170) 120 ±39	0	0	0	0
Nov	0	n=4,	n=6,	0	0	0

		(60-150) 100 ±39	(90-120) 105 ±21			
Dec	0	0	n=4, (110-197) 162 ±39	n=1, 87	n=1, 65	0
Farm Do		**Silver carp**	**Silver barb**	**Indian carp**	**Tilapia**	**P. hypophth.**
Sept	0	0	0	0	n=6, (90-200) 150 ±56	0
Oct	0	0	0	0	n=6, (100-140) 120 ±14	0
Nov	0	n=1, 200	0	0	n=5, (16-124) 62 ±47	0
Dec	0	0	0	0	n=6, (50-158) 107 ±44	0
Farm Hai	**Com.carp**	**Silver carp**	**Silver barb**	**Indian carp**	**Tilapia**	**P. hypophth.**
Sept	n=2, (112-120) 116 ±6	n=1, 156		n=1, 98		n=2, (45-169) 107 ±88
Oct		n=2, (112-140) 126 ±20				n=1, 145
Nov		n=5, (2-123) 69 ±43				0
Dec		0				n=1, 160

Numbers in parentheses = range.

A high percentage of macrophages recovered from silver carp from the Dinh Farm had bacteria in their cytoplasm, and the percentage of cells observed with bacteria were similar in Sept, Oct and November (Table 6). A lower percentage of macrophages with bacteria from silver carp was found in the Hai Farm in September and October, but a similar value was found in November compared with those values observed for Farm Dinh. Samples in Sept and October in the Hai Farm were only from 1 or 2 fish. A higher percentage of macrophages sampled from silver barb in Farm Dinh was found in December compared with November (Table 6). The values for tilapia differed between the months sampled on the Do farm (Table 6) as the lowest percentage cells with bacteria were observed in December compared with the highest in November.

Table 6 Percentage Macrophages with Bacteria visible per species

DINH	**Kissing G**	**Silver C**	**Silver B**	**Indian C**	**Tilapia**	**P. hypop.**
Sept	9 (n=1)	13 ±6 (n=5)				
Oct		12 ±4 (n=6)				
Nov		12 ±2 (n=4)	10 ±3 (n=2)			
Dec			24 ±12 (n=4)	3 (n=1)	5 (n=1)	
DO	**Kissing G**	**Silver C**	**Silver B**	**Indian C**	**Tilapia**	**P. hypop.**
Sept					16 ±2 (n=6)	
Oct					12 ±3 (n=5)	
Nov		38 (n=1)			22 ±7 (n=5)	
Dec					8 ±7, (n=6)	
HAI	**Common C**	**Silver C**	**Silver B**	**Indian C**	**Tilapia**	**P. hypop.**
Sept	4 ± 0 (n=2)	5 (n=1)		3 (n=1)		3 ±1 (n=2)
Oct		1 ±0 (n=2)				0 (n=1)
Nov		14 ±12 (n=3)				
Dec						7 (n=1)

P.hypop = *Pangasius hypophthalmus*

In the fish sampled from all three farms, there was a relationship between the percentage of macrophages with bacteria and bacterial growth from the tissue streaked directly onto agar plates or recovered from the suspension of macrophages. Often it is only possible to recover bacteria from the macrophages isolated from clinically healthy animals using a resuscitation step in liquid medium (Crumlish 1999). The need for a resuscitation step suggested that the bacteria inside the macrophages may have been 'damaged' in some way, and was thus unable to grow directly onto solid agar and required a liquid media for growth.

The numbers of bacterial colonies recovered directly from the tissue or from the macrophage suspension was usually low. Identification of the bacterial species present in the cultures recovered was unsuccessful. It is unknown if the bacteria recovered are the same bacteria as seen in the macrophages. Further work is needed to establish if the bacteria inside the macrophages are dead or damaged, or whether they were being phagocytosed as some sort of bacterial clearance mechanism by the immune response of the animal. A commercially live/dead kit was used in the previous DFID project (R 7054) which was able to identify live and dead bacteria in the cells, However, a fluorescent microscope was required for this which was not available at the laboratory in CanTho.

Ability to macrophages to phagocytosis yeast

It would appear from the data present in Table 7, that the macrophages were able to phagocytosis yeast particles when they had bacteria present within their cytoplasm. Only one fish sampled (Table 7, November Fish 1 silver carp Farm Hai) had no bacteria present in its macrophages and its macrophages did not phagocytose any yeast particles. Another two fish sampled from the 3 farms did not have bacteria present in their macrophages, but their macrophages were able to phagocytose yeast particles.

Table 7 Percentage macrophages with Bacteria and Percentage Phagocytosis

Farm	Month Sampled											
	Sept			Oct			Nov			Dec		
	Fish	(%) +B	(%) +Y	Fish	(%)+B	(%) +Y	Fish	(%)+B	(%)+Y	Fish	(%) +B	(%)+Y
Dinh												
	1KG	9	6	1 SC	7	6	1 SC	10	6	1IC	3	8
	2SC	7	8	2SC	11	6	2	13	9	2 T	5	0
	3SC	11	8	3SC	20	12	3	14	8	3 SB	33	30
	4 SC	7	10	4SC	12	7	4	12	9	4 SB	6	46
	5 SC	21	8	5SC	12	8	5SB	12	16	5 SB	30	0
	6 SC	18	10	6SC	11	7	6SB	8	12	6 SB	28	30
DO												
	1T	12	13	1 T	12	8	1SC	38	83	1T	3	3
	2T	15	12	2T	10	10	2T	28	57	2 T	4	4
	3T	17	17	3T	8	8	3T	16	30	3 T	10	10
	4T	16	15	4T	14	5	4T	25	60	4 T	21	21

	5T	19	11	5T	14	10	5T	14	41	5 T	8	0
	6T	15	8	T		10	6T	28	67	6 T	0	8
Hai												
	1 SC	5	21	1 SC	1	6	1 SC	0	0	1Pan	7	5
	2 IC	3	20	2SC	1	9	2 SC	19	33			
	3 CC	4	17	3 Pan	0	7	3 SC	23	42			
	4 CC	4	16				4 CC	9	12			
	5Pan	2	17				5 CC	7	10			
	6Pan	4	19									

T = tilapia; Pan = pangasius; SC = silver carp; SB = silver barb; IC = Indian carp; KG = Kissing gourami; CC = common carp.

(%) +Y – percentage of macrophages with yeast; (%) +B – percentage of macrophages with beacteria

Analysis of the data in Table 7 was performed by Dr James Turnbull, Institute of Aquaculture, Stirling University using Statistica package version 6. A multiple regression analysis was conducted on the dependant variable "**percentage bacteria**" and the independent variables, "**percentage phagocytosis, farm, date and species**". When a backward stepwise module was applied only percentage phagocytosis and farm remained in the module (R^2 = 0.51253875, P= 0.00000, Phagocytosis β = 0.660 and Farm β = -0.38) the analysis suggested that there was a positive relationship between the percentage macrophages with bacteria and the percentage phagocytosis observed. This implied that as the percentage of macrophage cells with bacteria in their cytoplasm increased, so did the percentage phagocytosis. Furthermore, breakpoint non-linear regression was conducted on the data to provide a breakpoint of 12% where R^2 = 73.042%. The results from the breakpoint analysis suggested that when 12% of macrophages have bacteria within their cytoplasm, there was an increase in their ability to phagocytosis yeast.

The results of the breakpoint analysis suggest that below levels of 12% of macrophages with bacteria, there was a low level phagocytic response, while 12% of macrophages or more which contained bacteria had a heightened ability to phagocytose yeast. From the resulsts it would appear that the phagocytic ability of the fishes cellular immune response was performing well, and was able to engulf bacteria and other organic material (yeast cells). No analysis were performed to measure the killing potential of the macrophages with bacteria (using the tests described in Apendix 6) in relation to their ability to phagocytosis yeast. This would have helped determine the ability of the macrophages to clear and kill bacteria from its host. Further field and laboratory work are needed to test this hypothesis.

CONCLUSIONS

The purpose of this study was to look for changes in the water quality, the bacterial load of the pond, and the occurrence of bacteria within macrophages, in farms where the farmer had reported previous disease episodes or water quality problems. The results collected were to be used to look firstly for a relationship between the water quality, the bacterial load of the pond, and the occurrence of bacteria within macrophages and secondly to try to understand the significance of the bacterial load present within the macrophage on the immuno-competence of the fish. As well as looking for the presence of bacteria in macrophages isolated from the head kidney of the fish, the function activity of the cell was also examined using the cells ability to phagocytose yeast, and red and white blood cell counts as a measure of immunocompetence of a number of different fish species in different farming systems in Vietnam.

It was intended to sample each of the nine farms at monthly intervals continuously over a six-month period of their production cycle. Due to sever flooding experienced in Vietnam over the course of the study, the sampling protocol was incomplete and the relationship between many of the parameters could not be determined as anticipated. There were a number of technical difficulties highlighted above, which have meant that full potential of the data collection was not achieved. However, this study was the first examination of the immune competence of fish at the pond-site combining two simple assays, which could be performed in the field. The analysis should be repeated at a time in the farms' production cycle when there might be heightened organic loads in the water and hence increased bacterial load in the water.

The use of the tests performed here and in other appendices within this report, combined with other simple techniques developed to examine the immune response of fish in the field, may provide a means of examining the immuno-competence of fish within these culture systems. However, further training is required in basic fish health, disease recognition and the importance of the immune response before the AFSI research team would be able to fulfil such a study. Routine screening at strategic points in the production cycle may reduce the over application of chemicals and antibiotic treatments currently applied. This may in turn will reduce the costs of unnecessary or inappropriate treatments by fish farmers and reduce the number of fish lost from stress-related disease outbreaks. Furthermore, it is recognised that such an activity may only be of use to those families that culture their fish for market or in monoculture systems.

Appendix 4

This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID

FARMER RECORDING KEEPING STUDY

Dr Kim Thompson and Dr Mags Crumlish,

Institute of Aquaculture. University of Stirling, Stirling UK

Ms. T.T Dung, Aquaculture and Fisheries Research Institute (AFSI), College of Agriculture, Cantho University, CanTho. Vietnam.

BACKGROUND

It was evident from the results of a fish farmers' survey carried out in the Mekong Delta, Vietnam (Appendix 2), that many small-scale farmers were unable to recall information related to the fish farming practices that they carried out either daily or in general. For example many rural families did not known the number of fish in their pond or were unable to calculate the productivity of their farm, since they did not have the relevant information to do so. Such details were more often available for more intensive monoculture farms, where high value species often were produced. It was found from the fish farmers survey in Vietnam (Appendix 2), that 11% (n=22) of farmers interviewed kept some form of written record about their fish farming activities. These farmers were located in 6 provinces distributed throughout the Mekong Delta (Table 1).

Table 1 Number and Percentage of Fish Farmers Who Kept Written Records

Province	Total number of farmers interviewed	Number of farmers who kept written records	(%) kept written records in each province
An Giang	51	12	**23**
Can Tho	35	3	**9**
Dong Thap	14	5	**36**
HoChiMinh	24	1	**4**
Long An	35	1	**3**
Tien Giang	27	0	**0**
Vinh Long	15	1	**7**

204 households interviewed in total, covering 7 provinces

The information recorded was related to costs associated with fish production. Only one farmer recorded information relating to disease outbreaks in his pond. Information relating to fish losses due to disease is necessary to help establish the economic impact of disease outbreaks on the livelihoods of the household. This information can be determined directly thorough loss of income or indirectly through the cost of purchasing supplemented/medicated feeds and treatments. A record keeping study was initiated to establish if keeping written records was compatible with farmer's lifestyles, how useful these were to rural small-scale farmers producing fish for the local market and whether they thought that such an exercise would help increase their overall productivity. The study was also used to investigate the influence of previous or regular contact with the research staff on the farmer's willingness to participate in the study and their commitment to perform the activities asked of them. As an incentive to participate farmers were invited to contact the research team if they experienced any health-related problems within their stock over the course of the study. It was agreed that the research team would try to provide them with a field diagnosis of their problems (only a field diagnosis could be provided for logistical reasons).

METHODS

Two groups of fish farming families located in the Mekong Delta were invited to participate in the study. In the first group, the research team visited each farmer at the beginning of the study, to explain the type of information required and how to record data in the notebooks supplied (Figure 1). These farmers were then visited at the end of the study and their notebooks collected. Half of the farmers in this group were chosen from the list of farmers who had participated in the fish farming survey and who had reported having problems with disease in their stock (Appendix 2). The remaining half of the group was unknown to the research team, and they were randomly selected from lists supplied by the Department of Fisheries and the provincial extension services. In total 40 families were approached and 37 agreed to participate in the study.

Figure 1. The research team visiting with farmers at the start of the project

The smaller second group of farmers (n=9), were visited by the research team monthly throughout the study period. These farmers were selected from the previous fish farmer survey in Appendix 2, and had previously reported having had fish disease problems on their farms. These farmers were again visited by the research team at the beginning of the study and shown the type of information required and how to record it. The research team then visited each farm in this group at monthly internals to take biological samples (see Appendix 3) and to see how the farmers were coping with the records keeping activity.

Participating farmers were supplied with notebooks and pens with which to record the information over the six-month study period. The research team had prepared each notebook before hand indicating where the relevant information should be recorded. The researcher ensured that someone in the family was able to read and write. The team explained information should be record daily or at the very least on a weekly basis. Initially the farmer provided background information related to each farm. The farmers were then asked to record information about the feeding regime of the fish, i.e. the type and the amount of feed given and when they actually fed their fish. They were asked to remark on the general weather conditions (i.e. if it was sunny, rainy, dry or cold) and on the water quality of their ponds. They were also asked to comment on periods when they experienced poor water quality, whether they exchanged exchange water, and if they did so what were the exchange rates and quantities of water they used. Finally, they were asked to record any disease problems they had in their stock i.e. a description of the problem, the types of species affected, the number of mortalities they experienced, and the course of action they took when their believed their fish to be abnormal (diseased). All of the descriptions and opinions recorded were those of the fish farmers involved in the study.

At the end of the study period all farmers were visited and the notebooks collected. During this time farmers were interviewed briefly to ask them what they thought about the record keeping study.

RESULTS AND DISCUSSION

At the onset of the study, 97% of farmers from both groups claimed to be willing to participate in the study irrespective of whether the farmers had had previous contact with the research team. However, this was not reflected in their ability or commitment to maintain the records throughout the six-month study period. All of the farmers in both groups produced fish for the local market, however fish farming was not their only source of income. They tended to be involved in many other livelihood activities, which contributed towards the total household income. The species produced by the farmers were species commonly cultured in Vietnam for sale at local markets.

Of the 40 farmers asked to keep records in Group 1, only three families did not want to participate in the study. The reason given by these farmers for not participating was that their farm was too small, they thought that they had no information to record or they regarded themselves as too old and could not write clearly, if at all.

At the end of the six-month period, only three farmers had kept detailed records for the duration of the study (Table 2a-c). Twelve families had kept good records for the first month of the study, after which they forgot to record the information or were too busy to do so, and ten farmers had lost their notebooks, of which eight families had lost them during the sever floods of October/November 2000. The three farmers who kept the records all belonged to the group that had had no previous contact with the research team.

Five farmers recorded the information themselves, while the remaining ten farmers asking other people in their family to write the information down for them. The research team felt that this was not because farmers were unable to record the information themselves, but rather wanted the information to appear neat and legible. Sometimes the farmers would quickly record information on table tops, scrap paper, walls etc., and then ask others to write it into their books at a later date. Two farmers in this group, both from Vinh Long Province, believed that they had disease problems in their stock during the study. They contacted the research team, one directly and the other through the extension officer, who provided a field diagnosis with the limited information provided. The research team was unable to take biological samples from these sites.

All nine farmers invited to participate in the record keeping study, together with the biological sampling study in Appendix 3, were willing to participate. However, these farmers actually recorded very little information apart for one family (Table 3). The farmers in this group claimed that they did not know what information to record since they felt they had been asked to record too much detailed information.

CONCLUSIONS

The results of the record keeping study showed that previous contact with the research team had no bearing on the Vietnamese fish farmers' willingness to participate in the study. Furthermore, regular contact with the research team (through monthly visits) did not improve the farmer's ability to perform the activities asked of them. Only a handful of farmers actually documented sufficient information to be analysed. Many farmers did not view the exercise as useful and did not see the benefit of keeping records on their livelihood activities. Only three or four families (the ones which kept records in the first group) thought that the exercise was of use and considered continuing with this activity for their own benefit. This in turn may be linked to their perceptions of how important fish farming is to their livelihood activities and how much time they spend fish farming.

The research team found that all of the farmers involved in the study were eager to receive information relating to husbandry, management and disease prevention for aquaculture. This may be one reason why they were willing to participate in the study in the first place.

GROUP-1 FARMERS

Table 2a: **Farmer:** NGUYEN TAN LOI

Address: **Phung Hiep - Can tho**

Species of fish culture: Snakeskin gourami (*Trichogaster pectoralis*)

Stocking density: 2 fishes/m^2

Stocking date: 02/07/2000

Total number pond: 01 (1500 m^2)

Month	Notes
08/2000 August	**Feed:** rice bran at 0.5 kg/day **Weather:** sunshine Water quality**: water colour = sometimes green or clear water** **Comments:** Changed water daily using tidal exchange
09/2000 September	**Feed:** duckweed at 15 kg/day **Weather:** sunshine, sometimes rainy **Water quality**: water colour = green or clear **Comments:** sometimes dirty water, need to be changed by pipe[1]
10/2000 October	**Feed:** tiny shrimp (collect from canals or rivers) at 1-5 kg/day **Weather:** nothing recorded **Water quality:** nothing recorded
11/2000 November	**Feed:** tiny shrimp (collect from canals or rivers) at 1 kg/day **Weather:** recorded nothing **Water quality**: nothing recorded **Comments:** Natural food was reduced daily as it was difficult to enough tiny shrimp. The farmer relied on fish finding natural food in the pond by themselves from now until to harvest (March 2001)

[1] = this was not clear why water was changed by a 'pipe' at this stage.

Table 2b: Farmer: NGUYEN VAN BAY

Address: **Phung Hiep - Can tho**

Species of fish culture: Snakeskin gourami (*Trichogaster pectoralis*)

Stocking density: 0.38 fish/m^2

Stocking date: 02/12/1999

Total number pond: 01 (5200 m^2)

Month	Notes
08/2000 **August**	**Feed:** rice bran at 1-2 kg/day and sometimes adding 10kg of duckweed for the first 10 days after stocking **Weather:** sunshine Water quality: water colour = green or clear, sometimes dirty **Comments:** daily water exchange by tide. Ten days post stocking fish fed rice bran with alternating 'fish' milk[2] and bran corn (bran corn = waste from corn cake)
09/2000 **September**	**Feed:** rice bran at 2.5-4 kg/day, sometimes duckweed at 2-3 kg/day added. Weather: **sunshine or very hot, sometimes rainy** **Water quality:** water colour = green. **Comments:** Dirty water occurred at the end of this month. Water change by tide[3]
10/2000 **October**	**Feed:** rice bran at 4kg/day Weather: **rainy, cold and sometimes very hot** **Water quality**: water colour = green
11/2000 **November**	**Feed:** rice bran at 3-4 kg/day, sometimes duckweed added. **Weather**: rainy, cold and sometimes very hot **Water quality:** water colour = green water
12/2000 **December**	**Feed:** rice bran at 3-4 kg/day, sometimes duckweed added. **Weather**: rainy, cold and sometimes very hot **Water quality:** water colour = green water
01/2001 **January**	**Feed:** rice bran at 3-4 kg/day sometimes duckweed added. **Weather:** rainy, cold and sometimes very hot **Water Quality:** water colour = green. **Comments:** Dirty water occurred at the end of this month. Practised tidal exchange.

[2] = fish milk = dried trash fish ground into a powder and then added to the feed

[3] = freshwater exchange but under influence of tide

Table 2c: Farmer: VO THANH PHONG

Address: Chau Phu District - An Giang Province

Species cultured: *Pangasius hypophthalmus,* kissing gourami (*Helostoma temminckii*)

Stocking density: 3.75 fishes/m2

Stocking date: 29/08/2000

Expect harvest date: 03 - 04/2001

Total number pond: 02 ponds

Number of ponds in use = 01 pond for fish culture

Month	Notes
08/2000 **August**	- **Feed**: rice bran (5 kg) + broken rice (2 kg) + water spinach (5 kg) /day. (**Unless otherwise stated all the materials were cooked before fed to fish)** - **Weather:** rainy and cold - **Pond preparing**: lime (50 kg/800m2) applied - **Comments:** Thirteen pangasius and two kissing gourami died due to transportation stress, as determined by farmer.
09/2000 **Septembe r**	- **Feed:** rice bran (5 kg) + broken rice (2 kg) + water spinach (5 kg) /day. - **Weather**: sunshine -**Water quality**: water colour = clear and green
2.9.00	- Five *Pangasius* and seven kissing gourami died
3.9.00	- Eighteen *Pangasius* and nine kissing gourami died Again the farmer thought that fish died because of transportation stress
4.9.00	+ **Feed:** rice bran (5 kg) + broken rice (2 kg) + water spinach (4 kg) /day. + **Weather**: rainy + **Water quality:** water colour = clear or green **Comments:** Two pangasius and one kissing gourami died
7.9.00	+ **Feed**: rice bran (7 kg) + broken rice (2 kg) + water spinach (5 kg) /day. + **Weather**: it was sunshine and rainy at all day but cold on night + **Water quality**: water colour = clear or green
9.9.00	+ **Feed**: rice bran (10 kg) + broken rice (2 kg) + water spinach (2 kg) /day. + **Weather:** sunshine but cold at night + **Water quality**: water colour = green
11.9.00	+ **Feed**: rice bran (10 kg) + broken rice (2 kg) + water spinach (2 kg) + yellow edible snail (2 kg)/day.

	+ **Weather**: sunshine but cold at night + **Water quality**: water colour = green **Comments:** Fish eat normal but had one pangasius died
15.9.00	+ **Feed**: rice bran (10 kg) + broken rice (3 kg) + water spinach (3 kg) + yellow edible snail (4 kg)/day. + **Weather**: good + **Water quality**: water colour = greenish **Comments:** Fish ate normal
20.9.00	+ **Feed**: rice bran (12 kg) + broken rice (3 kg) + water spinach (2 kg) + yellow edible snail (3 kg)/day. + **Weather**: a little rainy and cool + **Water quality**: water colour = greenish **Comments:** All the materials were cooked then put for fish fed, 5 kg in morning and 15 kg in afternoon. Farmer observed that fish reduced feeding at this time.
24.9.00	+ **Feed:** rice bran (18 kg) + broken rice (4 kg) + water spinach (10 kg) + yellow edible snail (8 kg)/day. + **Weather:** it was sunshine on morning and rainy on night + **Water quality**: water colour = green water
29.9.00	+ **Feed:** rice bran (20 kg) + broken rice (5 kg) + water spinach (12 kg) + yellow edible snail (13 kg)/day. + **Weather:** it was sunshine and dry on morning + **Water quality**: water colour = green
10/2000 **October**	
1/10/2000	**+ Feed:** rice bran (25 kg) + broken rice (5 kg) + water spinach (10 kg) + yellow edible snail (10 kg)/day. + **Weather:** sunshine on morning, rainy afternoon and cool at night + **Water quality**: water colour = green **Comments:** Fishes came to water surface at night, which farmer thought may be due to low oxygen content in water.
5.10.00	**+ Feed:** rice bran (35 kg) + broken rice (5 kg) + water spinach (15 kg) + yellow edible snail (5 kg)/day. **+ Weather:** sunshine on morning, rainy afternoon and cool at night + **Water quality**: colour = green **Comments:** Fishes came to water surface at night, which farmer thought may be due to low oxygen content in water. **+ Feed:** rice bran (30 kg) + broken rice (4 kg) + water spinach (6 kg) /day.

10.10.00	+ **Weather:** rainy at morning and afternoon but cold at night + **Water quality**: colour = green **Comments:** *Pangasius* came to water surface at night, which farmer thought may be due to low oxygen content in water.
20.10.00	**+ Feed:** rice bran (40 kg) + broken rice (10 kg) + water spinach (10 kg) /day + **Weather**: very rainy and cold + **Water quality**: green water so water pump more 4 cm **Comments:** one fish died
25.10.00	**+ Feed:** rice bran (50 kg) + broken rice (7 kg) + water spinach (13 kg) /day + **Weather:** very rainy and cold + **Water quality**: colour = green **Comments:** Fish still coming to surface which may be due to low oxygen and dirty water so farmer treatment by pumping water and adding salt and lime
29.10.00	**+ Feed:** rice bran (60 kg) + broken rice (10 kg) /day. All the materials were cooked then put for fish fed **+ Weather:** sunshine and rainy + **Water quality**: water colour = green **Comments:** Fishes activate and normal
11/2000 **November** **5/11/2000**	**+ Feed:** rice bran (60 kg) + broken rice (10 kg) + water spinach (15 kg) /day. + **Weather:** sometimes sunshine sometimes rainy + **Water quality:** water colour = green **Comments:** Fishes activate and normal
8.11.00	**+ Feed:** rice bran (60 kg) + broken rice (10 kg) + water spinach (10 kg) /day. + **Weather:** sunshine at morning, cold at night + **Water quality**: colour = green **Comments:** Fishes ate normal
10.11.00 / 15.11.00	**+ Feed:** rice bran (60 kg) + broken rice (10 kg) + water spinach (15 kg) /day. + **Weather:** sunshine in morning and rainy in afternoon + **Water quality**: colour = very green +**Comments:** Fishes ate normally

	+ Feed: rice bran (65 kg) + broken rice (10 kg) + water spinach (15 kg) /day. **+ Weather:** sunshine in morning and rainy in evening + **Water quality**: colour = green + **Comments:** Fish reduced feeding compared with previous days
16.11.00	**+ Feed:** rice bran (65 kg) + broken rice (10 kg) + water spinach (15 kg) /day. + **Weather:** sunshine + **Water quality**: colour = green + **Comments:** Fishes reduced feeding so farmer changed water using a pump and added lime
19.11.00	**+ Feed:** rice bran (55 kg) + broken rice (10 kg) + water spinach (20 kg) /day. + **Weather:** sunshine and cool **+ Water quality**: colour = clear + **Comments:** Fish eat normally
22.11.00	**+ Feed:** rice bran (55 kg) + broken rice (10 kg) + water spinach (15 kg) /day. + **Weather**: very rainy + **Water quality**: colour = clear + **Comments:** Fishes ate normally
25.11.00	+ **Feed:** rice bran (65 kg) + broken rice (10 kg) + water spinach (15 kg) /day. + **Weather:** rainy and cold + **Water quality:** colour = clear +**Comments:** Fishes ate normally
28.11.00	**+ Feed:** rice bran (60 kg) + broken rice (10 kg) + water spinach (15 kg) /day. + **Weather:** sunshine and rainy + **Water quality**: colour = green +**Comments:** Fishes ate normally
30.11.00	+ **Feed:** rice bran (65 kg) + broken rice (10 kg) + water spinach (20 kg) /day. + **Weather:** sunshine and rainy **+ Water quality**: colour = green + **Comments:** Fishes ate normally
12/2000	

December 4/12/2000	+ **Feed:** rice bran (70 kg) + broken rice (10 kg) + water spinach (20 kg) /day. + **Weather:** sunshine + **Water quality:** colour = greenish **+ Comments** : Fish reduced feeding
10.12.00	+ **Feed:** rice bran (70 kg) + broken rice (10 kg) + water spinach (20 kg) /day. + **Weather:** sunshine all day and rainy in evening + **Water quality**: colour = greenish + **Comments:** Fishes ate normally
14.12.00	+ **Feed:** rice bran (70 kg) + broken rice (10 kg) + water spinach (20 kg) /day. + **Weather:** sunshine + **Water quality**: colour = greenish **+Comments**: Fishes ate normally
17.12.00	+ **Feed**: rice bran (60 kg) + broken rice (10 kg) + water spinach (20 kg) and fresh trash fish (20 kg)/day. + **Weather:** sunshine + **Water quality**: colour = greenish + **Comments:** Fishes ate well (strongly)
20.12.00	+ **Feed**: rice bran (60 kg) + broken rice (10 kg) + water spinach (20 kg) and fresh trash fish (20 kg)/day. + **Weather:** sunshine and rainy + **Water quality**: colour = green +**Comments:** Fishes ate well (strongly)
22.12.00	+ **Feed**: rice bran (50 kg) + broken rice (10 kg) + water spinach (20 kg) and fresh trash fish (30 kg)/day. + **Weather**: rainy + **Water quality**: colour = green + **Comments:** Fishes ate well (strongly)
25.12.00	+ **Feed**: rice bran (60 kg) + broken rice (10 kg) + water spinach (20 kg) and fresh trash fish (20 kg)/day. + **Weather:** sunshine **+ Water quality**: colour = very green + **Comments:** Fish reduced feeding
27.12.00	+ Feed: rice bran (50 kg) + broken rice (10 kg) + water spinach (20 kg) and

	fresh trash fish (30 kg)/day. + **Weather:** rainy and sunshine + **Water quality**: colour = very green + **Comments:** Fish reduced feeding
28.12.00	**+ Feed:** rice bran (50 kg) + broken rice (10 kg) + water spinach (10 kg) and fresh trash fish (30 kg)/day. **+ Weather**: sunshine + **Water quality**: colour = very green + **Comments:** Fishes ate well (strong). Farmer exchanged water as a 'treatment'.
30.12.00	+ **Feed:** rice bran (50 kg) + broken rice (10 kg) + water spinach (10 kg) and fresh trash fish (30 kg)/day. **+ Weather:** rainy and sunshine + **Water quality**: water colour = clear + **Comments:** Fishes reduced feeding and farmer added lime
01/2001 **January**	
02/01/2001	+ **Feed:** rice bran (60 kg) + broken rice (10 kg) + water spinach (20 kg) and fresh trash fish (10 kg)/day. + **Weather:** rainy and sunshine + **Water quality**: colour = clear + **Comments:** Fish reduced feeding and swimming near the side and around the pond. A treatment of lime and salt was applied with gasoline. *Some of farmers usually treated by lime and salt but seldom use gasoline because it was special treatment for crustaceans.*
4.1.01	+ **Feed:** rice bran (60 kg) + broken rice (10 kg) + water spinach (20 kg) and fresh trash fish (10 kg)/day. + **Weather**: rainy and sunshine + **Water quality**: colour = green +**Comments:** Fish ate normally
7.1.01	+ **Feed:** rice bran (70 kg) + broken rice (10 kg) + water spinach (20 kg) and fresh trash fish (20 kg)/day. + **Weather:** rainy and sunshine + **Water quality**: colour = clear + **Comments:** Fish ate normally. Two dead fish observed. + **Feed:** rice bran (70 kg) + broken rice (10 kg) + water spinach (25 kg) and fresh trash fish (15 kg)/day.

10.01.01	+ **Weather:** sunshine + **Water quality**: colour = green + **Comments:** Fish ate normally + **Feed:** rice bran (70 kg) + broken rice (10 kg) + water spinach (20 kg) and fresh trash fish (20 kg)/day.
15.01.01	+ **Weather**: sunshine + **Water quality**: colour = green + **Comments:** Fish ate normally + **Feed:** rice bran (80 kg) + broken rice (10 kg) + water spinach (20 kg)/day.
18.01.01	+ **Weather:** rainy and sunshine + **Water quality**: colour = green + **Comments:** Fish ate normally + **Feed:** rice bran (80 kg) + broken rice (10 kg) + water spinach (20 kg)/day.
20.01.01	+ **Weather**: rainy and sunshine + **Water quality**: colour = green + **Comments:** Fish ate normally **+ Feed:** rice bran (80 kg) + broken rice (10 kg) + water spinach (20 kg) and product from alcohol (30 kg)/day[4].
22.01.01	+ **Weather**: sunshine + **Water quality**: colour = green + **Comments:** Fish ate well (strongly) + **Feed:** rice bran (80 kg) + broken rice (10 kg) + water spinach (20 kg) and product from alcohol (30 kg)/day.
25.01.01	+ **Weather:** sunshine + **Water quality**: colour = green + **Comments:** Fish ate well but one fish died + **Feed:** rice bran (80 kg) + broken rice (10 kg) + water spinach (20 kg) and product from alcohol (30 kg)/day.
28.01.01	+ **Weather**: sunshine and rainy + **Water quality**: colour = green + **Comments:** Fish ate well (strongly)
02/2001 **February** **01/02/2001**	+ **Feed:** rice bran (80 kg) + broken rice (15 kg) + water spinach (20 kg)

	and waste from producing alcohol (20 kg)/day. + **Weather**: sunshine and rainy + **Water quality:** colour = greenish + **Comments:** Fish ate well (strong)
04/02/2001	+ **Feed:** rice bran (80 kg) + broken rice (15 kg) + water spinach (15 kg) and waste from producing alcohol (30 kg)/day. + **Weather**: It was sunshine and rainy + **Water quality**: colour = greenish + **Comment:** Fish ate well (strongly)
10/02/2001	+ **Feed:** rice bran (80 kg) + broken rice (15 kg) + water spinach (20 kg) and waste from producing alcohol (20 kg)/day. + **Weather**: sunshine + **Water quality**: colour = greenish + **Comment:** Fish ate well (strongly)
15/02/2001	+ **Feed**: rice bran (80 kg) + broken rice (15 kg) + water spinach (15 kg) and waste from producing alcohol (30 kg)/day. + **Weather**: sunshine and rainy + **Water quality**: colour = greenish + **Comment:** Fish ate normally
18/02/2001	+ **Feed:** rice bran (80 kg) + broken rice (15 kg) + water spinach (20 kg) and waste from producing alcohol (30 kg)/day. + **Weather**: sunshine and rainy + **Water quality**: colour = green + **Comment:** Fish ate normally
20/02/2001	+ **Feed:** rice bran (80 kg) + broken rice (15 kg) + water spinach (20 kg) and waste from producing alcohol (30 kg)/day. + **Weather:** sunshine + **Water quality**: colour = green + **Comment:** Fish ate normally
27/02/2001	+ **Feed:** rice bran (80 kg) + broken rice (15 kg) + water spinach (20 kg) and waste from producing alcohol (30 kg)/day. + **Weather**: cool + **Water quality**: colour = green + **Comment:** Fish ate normally

[4] = this is residual product after rice alcohol has been produced. This was provided at end of January and throughout February because the amount of natural food available was reduced and this is a cheap substitute. It is commonly used for fish and livestock in Vietnam.

Group-2 Farmer

Table 3: **Farmer:** LY MINH NHUT

Address: **Chau Phu District - An Giang Province**

Species of fish culture: Tilapia (*Oreochromis* sp.), silver barb (*Puntius* sp.)

Stocking density: 12 fish/m^2

Stocking date: 01/05/2000

Expect harvest date: 02/2001

Total number pond: 01

Month	Notes
08/2000 **August**	- **Feed:** rice bran (65%) + broken rice (35%) = 30 kg/day + water spinach and other vegetables, antibiotics (e.g Tetra) and Vitamin C were mixed with food every four days for the prevention of disease problems during changing seasons **Weather:** Sunshine: fish ate normally Rainy: fishes reduced feeding and sometimes stopped eating **Comments (farm or fish):** -Supply lime around pond before raining - Sometimes use salt for bathing - Lime and $CuSO_4$ were dissolved in water, then solution on the surface were used to supply around pond. *- The mortality rate depended on how dirty the water was as increased mortalities occurred when the farmer noticed bubbles with black decay rising from the pond bottom to the water surface.* - Water exchange every 20 days by both inlet and outlet with 40 - 50 cm of water changed. To increase the water oxygen content the water inlet was combine with a "shower"[5], after which the fish looked normal with no mortalities observed. **- After raining for a few days, fish mortality occurred mostly silver barb died with about 10 fishes/day**
09/2000 **September**	- **Feed**: rice bran (30 kg/day) + pellet (4 kg/day) + small crab (20 kg/day) **-Water quality:** It was difficult to change water during this time and this resulted in very bad water quality, as determined by farmer. Lime and other chemicals such as salt or CuSO4 was applied around the pond and in the water to bathe the fish which improved the water quality (again as determined by fish farmer). **-Comments:** - Silver barb died 2 - 3 fish/day.

	- Pelleted feed was given at an average of 25 kg/day until harvest. - Water quality looked good with no dead fish observed.
10/2000 **October**	- **Feed:** pelleted feed at 20 - 30 kg/day **-**Water quality**: appeared good as no fish died and they grew well**
11/2000 **November**	The same on October
12/2000 **December**	The same on October
01/2001 **January**	The same on October
Additional Informatio n	Farmer described 'sick' fish as those that swam strangely, which occurred predominantly when the environment was polluted, particularly during season changes.

[5] = this was from the farmers bathroom and was used to supply oxygen to the pond water.

Appendix 5

This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID'

SURVEY ON THE AQUACULTURE ACTIVITIES OF EXTENSION OFFICERS IN THAILAND

Dr Kim D. Thompson and Dr Mags Crumlish,

Institute of Aquaculture, University of Stirling, Stirling Scotland, UK

Dr. Supranee Somsiri, Dr. Temdoung Somsiri and Ms. Yolprapa Dumrongphol

Aquatic Animal Health Research Institute, Dept. of Fisheries, Kasetsart

University Campus Bangkok 10900 Thailand

BACKGROUND

Verbal communication between research staff and extension officers highlighted that the extension services were often unable to provide an effective service to the numerous families involved in aquaculture. This was thought to be due mainly to lack of personnel, time and funds. The aim of this study was to examine the relationship between the extension services in Thailand responsible for fish farming communities and their target communities. Extension officers involved directly with fish farming families were interviewed using a structured questionnaire and their opinions of this relationship analysed.

MATERIALS AND METHODS

A survey of aquaculture activities of extension officers in four different regions of Thailand (the North, NorthEast, Central and Southern regions) was conducted by the Aquatic Animal Health Research Institute (AAHRI) in Bangkok. A structured questionnaire was produced as a WORD document at Stirling and sent to AAHRI for translation. This was then pilot tested by the AAHRI staff and changes made before being sent to the extension services of 26 different provinces in these regions. Personal interviews were conducted in provinces more accessible to the researchers from AAHRI, while postal questionnaires were sent to the extension services in provinces further away in Nakornpathom, Ayuthaya, Pattalung, Chaingrai, Trang, Pijit, Nakhonna-yok and U-Bonratchathani. A database was produced using Epi-Info (version 6 DOS based) and the results were then entered into the database over a period of 6-months. These were sent to IOA and frequency analysis performed within Epi-Info.

RESULTS

A total of 84 extension officers replied and these were located in 18 of the 26 provinces (Table 1). Under half of the replies (n = 41) originated from the postal survey and the remainder (n = 43) were collected as personal interviews.

Table 1 Location of Extension Officers

Region/Province	N^0 Officers	% Officers
North		
Chiang rai	13	15
Chiang mai	7	8
Pijit	1	1
Pisanuloke	6	7
Lampung	5	6
NorthEast		
Chiayapum	7	8
Khonkhen	5	6
Loei	6	7
Nongboulumpoo	5	6
Central		
Ayuthaya	1	1
Lopburi	1	1
Nakhonna-yok	4	5
Nakornpathom	1	1
Singburi	4	5
Saraburi	1	1
South		
Pattalung	5	6
Trang	5	6
U-Bonratchathani	7	8
Total	**84**	**100**

Sixty-nine percent (n= 57) of the extension officers believed their main activities, which related to aquaculture, were providing advice to fish farmers and promoting small-scale freshwater aquaculture within their region. The majority of extension officers questioned said their knowledge of fish farming was obtained from "on-job" training and previous experience although a relatively high number of them had also had attended university (Figure 1). Eighty-three percent (n= 57) of extension officers had received some form of

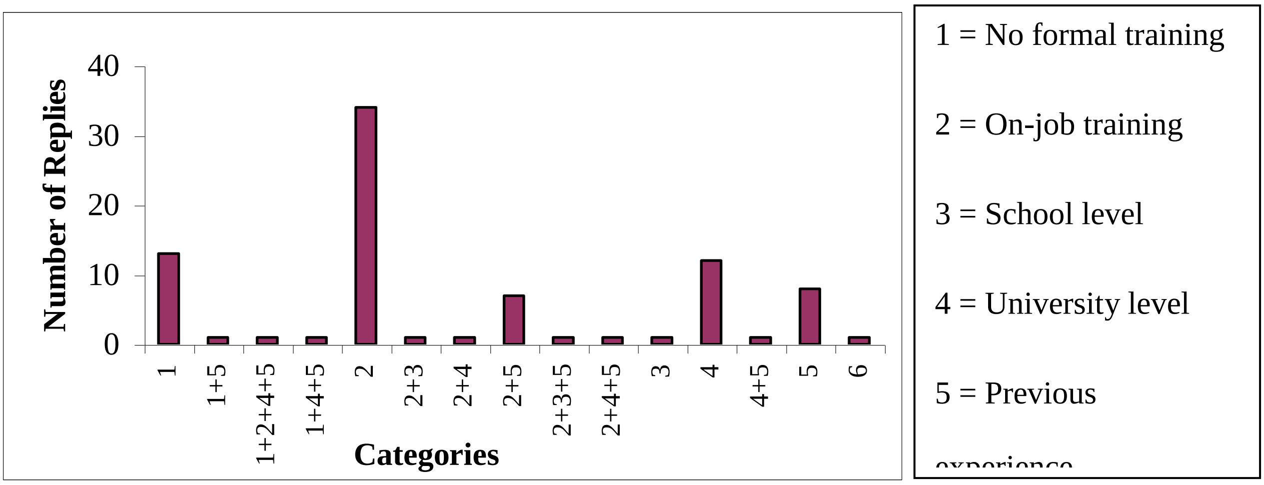


Figure 1 Level of Extension Officers Knowledge about Aquaculture

training from the Department of Fisheries (DOF). No information was obtained about the contents of the training courses.

Of the extension officers who replied to the questionnaire, it was found that they had responsibility for between 34 to 800 villages, and most of the officers were responsible for more than 20 fish farms in total. Sixty two percent of these officers (n=50) claimed to visit fish farms monthly and 15% (n=12) of officers claimed to visit fish farms weekly. Twenty percent (n=16) visited when the farmer contacted them only, 1% (n=1) did not know when they visited and 1% (n=1) did not never visited fish farms.

Extension officers were asked to rank the importance of different issues that the farmers seemed most concerned about (Table 2). A higher number of extension officers thought that anti-theft strategies, water exchange rates and use of lime were the most import questions most frequently asked of them (Table 2). The least important issues as judged by the extension officers were stocking density, treatments, diseases and disease prevention (Table 2).

Table 2 Ranked values of importance on issues Extension Officers most frequently asked

Advice topics	Total replies	(%) Importance				
		1	**2**	**3**	**4**	**5**
Fertiliser	49	29	24	31	10	6
Stocking density	64	14	16	27	17	27

Disease problems	67	7	16	18	21	37
Treatments	65	13	9	18	25	35
Feeding rates	63	22	27	19	17	14
Disease prevention	60	13	13	27	20	27
Liming	60	32	25	27	7	10
Anti-theft strategies	54	76	6	7	2	9
Water quality problems	70	17	13	27	21	21
Water exchange rates	58	33	29	26	9	3
General advice	64	25	6	23	13	33

1= most important and 5 = least important

The data ranked the most importance did not correlate with the advice given, since 90% of officers said that they had given advice on disease prevention and treatment to fish farmers at some point. It was found that a greater number of farmers in Nongkhai, Pattalung and U-bonratchathani had contacted extension officers when they encountered disease problems compared with farmers in other provinces.

Sixty-two percent (n=46) of the extension officers said that they recorded information relating to the fish farms they had visited. Most of the information they recorded was related to production data (18% of extension officers claimed this), advice on disease prevention together with production data (12% of extension officers said they recorded this) and information relating to management strategies (12% of extension officers said that they recorded this). Thirty two percent of extension officers said that they used the information they recorded to help them to provide general advice and guidance to fish farmers generally, while some extension officers (19% of extension officers) said that they used the information for statistical analysis and/or in databases.

Forty-three percent (n=36) of extension officers had received some form of training predominantly from the DOF and 63% (n=20) of these officers had received training in fish health and disease prevention.

The officer's were asked to rank their concerns about the problems which fish farmers encountered in order of most (1) to least (5) importance (Table 3). The biggest concern to extension officers appeared to be the advice that farmers received from other people rather than the extension services, feeding rates used by the farmer and inappropriate methods of treatment. Health management, water quality and seed quality were not ranked as a high importance by the extension officers.

Table 3 Ranking of the Importance of Extension Officers Concerns relating to disease

Concerns of the Extension	**Total replies**	**(%) Importance**

Officers		**1**	**2**	**3**	**4**	**5**
Feed type	67	16	28	27	16	12
Feeding rate	68	22	25	25	13	15
Water quality	76	9	14	24	16	39
Bacterial diseases	68	9	18	24	24	26
Advice given to farmers by others	72	40	15	25	7	13
Inappropriate treatments	68	21	19	25	12	24
Seed quality	75	19	8	16	25	32

Extension officers claimed to use various methods to recognise diseased fish. A greater number of extension officers used a combination of signs to recognise disease including change in the fish's appearance, fish not feeding, farmers' description of abnormal fish, clinical signs and the number of mortalities, which occur in the pond. According to the extension officers, the main cause of health problems in fish farm was related to poor husbandry.

Ninety-nine percent (n=81) of extension officers interviewed claimed that they recommended treatments to fish farms were asked. Most of the officers claimed to use their own experience or previous knowledge to determine which treatment to recommend. Twenty-three percent of officers did claim to contact research staff to ask advice and guidance before recommending treatments.

Ninety-eight percent (n=79) of extension officers interviewed claimed to recommend prophylactic treatments. However, very few (n=3) officers claimed to recommend antibiotics. The most common prophylactic treatment recommended was related to good farm management, such as preparation of ponds, water quality, and guidance on choosing fingerling and stocking density. More that half of the extension officer who replied to the questionnaire said that they returned to the fish farm after the farm had experienced a disease outbreak (Figure 2).

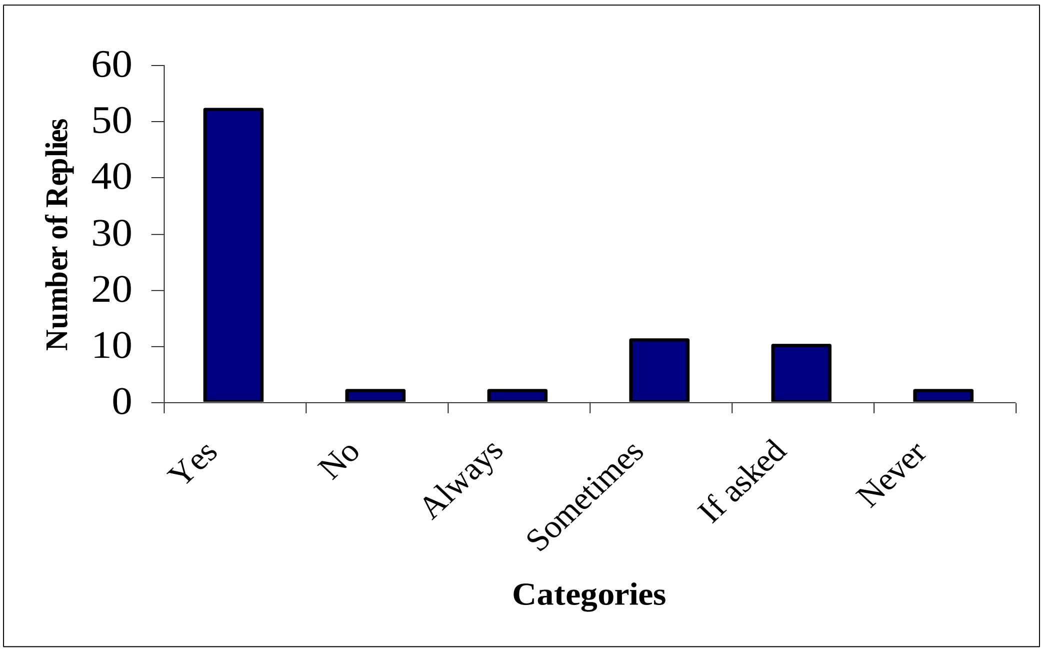


Figure 2 Number of extension officers returning to a farm after a disease outbreak

Nearly all extension officers (87%) said that they would like to have additional training relating to disease issues, and 86% of them also wanted to have additional resources for disease control i.e. training, technical information, documentation and data. Some also wanted central laboratories in each province to deal specifically with disease diagnosis and control/ treatments of disease.

An important finding from the questionnaire was that 75% of extension officers claimed that they felt that they did not provide an adequate service for the fish farmers. Reasons for this were thought to be due to their lack of technical knowledge, their lack of appropriate equipment for sampling and the lack of people to help conduct sampling work.

CONCLUSIONS

One of the main findings of the study was the size of the area and number of fish farms that individual extension officers were responsible for. These tended to be very large and therefore it was difficult for many of the extension officers to visit their designated farms on a regular basis. However, it was apparent that the officers did attend training courses and they did appear to pass on information to the fish farmers. Nevertheless, it was interesting that a high percentage of officers who replied, thought that they did not provide an adequate service to fish farming communities felt that they needed greater support in the form of training and access the literature.

SURVEY.2 **INSERT CODE** ☐

(EXTENSION OFFICERS SURVEY)

1. BACKGROUND

1.1 Can You Describe Your Role in Small Scale Fresh-Water Aquaculture?

____

____

1.2 Please Give Details on the Service You Provide to Fish Farmers?

____

____

1.3 Please tick all that apply:

1.3 What Level of Knowledge Do You Have About Fish Farming?	
No Formal Training [1]	
On-Job Training Only [2]	
School Level Only [3]	
University Only [4]	
Previous Experience Only [5]	
Don't Know [6]	

1.4a Do You Attend Any Training Sessions? **YES** [1] ☐ **NO** [2] ☐

1.4b If YES, Who Organises Them? ____________________________________

1.5a What is the Area (Distance) of Responsibility? __________________________

1.5b Please Give Province: ________________________________

1.5c Please Give Number of Villages Included: ______________________________

1.6 Please tick one box only:

1.6 How Many Farms in Total Are You Responsible For?	
***None**[1]*	
1 [2]	
Less Than 5 [3]	
Less Than 10 [4]	
More Than 10, Less Than 20 [5]	
More Than 20 [6]	
Don't Know [7]	

1.7 Please tick one box only:

1.7 How Many Of These Are Fish Farms?	
1 [1]	
Less Than 5 [2]	
Less Than 10 [3]	
More Than 10, Less Than 20 [4]	
More Than 20 [5]	
All [6]	
None [7]	
Don't Know [8]	

1.9 Please tick one box only:

1.9 How Often Do You Visit These Fish Farms?	
Never [1]	
Daily [2]	
Weekly [3]	
Monthly [4]	
When Farmer Contacts You [5]	
Don't Know [6]	

1.10a Do You Have Regular Times When You Visit Fish Farms?

☐ ☐

YES [1] **NO** [2] **DON'T KNOW** [3] ☐

1.10b If YES, Please Give Details: _________________________daily/weekly/monthly

2. Farmer Knowledge

INSERT FARMER SURVEY CODE: ☐

THIS SECTION WILL ONLY BE USED AT A LATER DATE, PLEASE GO STRAIGHT TO Q 2.9 DURING PILOT/VALIDATION

2.1 Insert Name and Contact Address for Farmer:

____

2.2 Please tick one box only:

2.2 What is Your Relationship with this Farmer?	
Friend [1]	
Colleague [2]	
Neighbour [3]	
No Previous Knowledge [4]	
Limited Previous Knowledge [5]	
None [6]	

2.3 Please tick one box only:

2.3 How Many Times Have You Visited This *Farm In The Last 12 Months?*	
Many (more than 10) [1]	
Often (less than 10 but more than 5) [2]	
Few (less than 5) [3]	
Infrequently (less than 2) [4]	
Never [5]	

2.4 When You Visit This Particular Farm What Is the Purpose of The Visit?

____

2.5 Does This Farm Have Disease Problems?

YES [1] ☐ **NO** [2] ☐ **DON'T KNOW** [3] ☐

2.6 Please tick one box only:

2.6 If There Is A Problem When Will Farmer Contact You?	
Immediately [1]	
Less Than 1 Week [2]	
More Than 1 Week Later [3]	
Don't Know [4]	

2.7 After The Farmer Contacts You What Action Do You Take?

____

2.8 Please tick one box only:

2.8 Does this Farmer Ask your Advice?	
No [2]	
Regularly [3]	
Occasionally [4]	
Only When There is a Serious Problem [4]	

Please Rank These in Order of Importance with 1 = most and 5 = least

2.9 What Are You Most Asked Advice On?	
Fertilisers [1]	
Stocking Density [2]	
Disease problems [3]	
Treatments [4]	
Feeding Rates [5]	
Disease Prevention [6]	
Liming [7]	
Anti-Theft Strategies [8]	
Water Quality Problems [9]	
Water Exchange Rates [10]	
General Fish Farming Advice [11]	

2.10a Do You Record Information on the Farms Visited? YES [1] ☐ NO [2] ☐

2.10b If YES, Please Give Details:
____

2.11 Please Explain What Happens to this Information? ________________________

3. Disease

3.1a Have You Attended Any Formal Training on Disease? YES [1] ☐ **NO** [2] ☐

3.1b If YES Please Give Details:____

Please Rank These in Order of Importance with 1 = most and 5 = least

3.2 How Concerned Are You With the Following?	
Health Management in General [1]	
Type of Feed Given [2]	
Feeding Rate [3]	
Water Quality [4]	

Diseases in General [5]	
Bacterial Disease [6]	
Advice Given to Farmers By Others [7]	
Quality of Fry [8]	
Inappropriate Treatments [9]	

3.4 Tick as Many Boxes as Applicable

3.4 How Do You Recognise Diseases?	
Cannot [1]	
Change in Appearance [2]	
Change in Size [3]	
Fish Not Feeding [4]	
From Farmers Description Only [5]	
Clinical Signs [6]	
Number of Mortalities [7]	
Results from Lab. Diagnosis [8]	
Don't Know [9]	

3.5 Can You Distinguish Bacterial Disease Problems From Other Health Problems?

YES [1] ☐ **NO** [2] ☐ **DON'T KNOW** [3] ☐

3.6 Please tick one box only:

3.6 What is the Main Cause Of Disease Problems in Fish Farms?	
Don't Know [1]	
Bacterial [2]	
Viral [3]	
Parasites [4]	
Fungal [5]	
Poor Husbandry [6]	
Poor Feed Quality [7]	
Poor Environmental Conditions [8]	
Lack of Farmer Understanding [9]	

9 Treatments

☐ ☐

Do You Recommend Treatments to Farmers? **YES** [1] **NO** [2]

4.2 Please tick one box only:

4.2 How Do You Decide Which Treatment To Recommend?	
Previous Experience [1]	
Ask Pharmacy [2]	
Ask Research Scientist [3]	
Ask Friends & Colleagues [4]	
Ask Other Farmers [5]	
Guess [6]	
Don't Know [7]	

4.3a Please tick as many boxes as applicable:

4.3a Who Would You Ask Advice From On Treatments for Diseases?	
Self [1]	
Other Farmers [2]	
Family [3]	
Research Staff [4]	
Friends [5]	
Media (Television or Newspaper) [6]	
Extension Officers [7]	
Guess [8]	
Others [9]	

4.3b If Others, Please Give Details: ____

4.4a Do You Recommend Prophylactic Treatment? **YES** [1] ☐ **NO** [2] ☐

4.4b If YES then:

What Is It? [1]	

5. Additional Information

5.1 Would You Like to Attend Training Sessions in Disease? **YES** [1] ☐ **NO** [2] ☐

5.2a Would You Like to have Additional Resources for Disease Control? **YES** [1] ☐ **NO** [2] ☐

5.2b If YES, Please Describe Why: ___________________________

5.3a Do You Think That You Provide a Service for the Fish Farmers? **YES** [1] ☐ **NO** [2] ☐

5.3b If NO, Why?: ____

5.4 Please tick one box only:

5.4 Do You Go Back To The Farm After A Disease Outbreak?	
Yes [1]	
No [2]	
Always [3]	
Sometimes [4]	
Only If Asked [5]	
Never [6]	
Don't Know [7]	

5.5 Name and Contact Address

____

____

9.5 Data Collected by: ______________Date: ______________Time: ______________

Thank you for your time and effort. The information given will be used as part of a large study investigating the incidence and impact of bacterial disease in small-scale freshwater aquaculture systems in Asia and will NOT be used for any other purpose.

Appendix 6

This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID'

EXAMINING THE EFFECTS OF *AEROMONAS HYDROPHILA* ON THE IMMUNE FUNCTION OF AFRICAN CATFISH (*CLARIAS* SPP.) HEAD KIDNEY MACROPHAGES *IN VITRO*

Dr Kim D. Thompson and Dr Mags Crumlish,

Institute of Aquaculture, University of Stirling, Stirling Scotland, UK

6.1 INTRODUCTION

Bacteria have been observed in macrophages isolated from both the spleens of clinically healthy farmed tropical frogs and the head kidney of a number of different freshwater fish species farmed in Thailand and Vietnam (Inglis and Crumlish 1997, Crumlish 1999, Thompson and Crumlish 1999 and Crumlish, Somsiri, Dung, Inglis and Thompson 2000). Macrophages are phagocytic by nature, engulfing invading organisms once the organism has infected its host. Functionally, these cells recognise, attach, ingest and kill microbes by the production of bactericidal enzymes, and they play an important role both in the non-specific and specific immune responses of the animal.

High bacterial loading is often reported within the aquatic environments of frog and fish farms in Thailand and Southern Vietnam. A statistical relationship has been found between water quality, bacterial load in the pond and the occurrence of bacteria within macrophages (Thompson and Crumlish 1999), and although levels of bacteria within the macrophages are generally low, most macrophage preparations made from fish within these systems contained bacteria.

It was not surprising to find bacteria within the cytoplasm of macrophages isolated from animals cultured in environments of high bacterial loading. As one of the roles of macrophages in animals exposed to high bacterial challenges is to ingest and kill invading bacteria. However, it remained uncertain whether the bacteria observed in the macrophages of clinically healthy farmed fish were simply due to natural bacterial clearance, or whether in fact the presence of the bacteria was due to an inability of the host to clear the microbes. If the latter was true and the host was immuno-compromised then farmed fish may succumb more easily to opportunistic infections from opportunistic bacteria found in the surrounding aqueous environment. The dominant bacterial species recovered the macrophages isolated from both the fish and the frogs were identified as Aeromonad and Pseudomonad spp. (Crumlish 1999, and Crumlish *et al* 2000).

Farmers continually report low level mortalities within their farming systems, even though there are no overt clinical signs of disease within their stock (verbal communication). Previously bacterial isolation was only possible using a resuscitation step in liquid medium from animals that had bacteria within their macrophages (Crumlish 1999, Crumlish *et al.*

2000). This suggested that bacteria were either present in very low levels within the macrophages or the bacteria have been damaged by the macrophages in some way rather than killed, so as to prevent their growth on an agar medium. In an attempt to understand the clinical significance of the resident bacteria present in the macrophages, the following study was performed to examine what, if any, effects that resident *Aeromonas hydrophila* might have on the immune function of African catfish (*Clarias* spp.) head kidney macrophages. Macrophages were incubated with the bacterium *in vitro* prior to measuring the respiratory burst produced by the cells. The reduction of nitroblue tetrazolium (NBT) was used as an indicator of superoxide anion production by the macrophages. The superoxide anion is produced during the respiratory burst process and is, in part, responsible for microbial killing activity of the macrophage. The effect of loading macrophages with the bacterium prior to measuring the respiratory burst was examined. The ability of opsonised bacteria, live and dead bacteria and the extracellular products (ECP) of the bacterium to stimulate the respiratory burst of macrophages was examined and the effect of different concentrations of bacteria on respiratory burst of the cells was also examined.

6.2 MATERIALS AND METHODS

6.2.1 Measurement of superoxide anion production by catfish anterior kidney macrophages *in vitro* pre-incubated with *Aeromonas hydrophila*

Catfish with an average length of 29cm ±4.62 and average weight 178g ±93.7 were obtained from the tropical aquarium, IOA, Stirling. Head kidney macrophages were isolated from the anterior kidney of fish and cell monolayers prepared according to Secombes (1990). The macrophage monolayers were prepared in sterile 96-well microtitre plates. Prior to use the monolayers were washed twice with sterile saline to remove unattached cells and number of viable cells counted using the trypan blue exclusion test (Secombes 1990). The macrophages were exposed to four different treatments: either (1) NBT; (2) NBT and PMA; (3) *A. hydrophila* and NBT; (4) *A. hydrophila* and PMA. The bacteria (*A. hydrophila* isolate T4) obtained from the bacterial collection held at the Institute of Aquaculture, University of Stirling, were cultured in 20 mls of Tryptone Soya Broth (TSB) overnight at 22^0C. The bacteria were harvested by centrifuged at 3,500 rpm for 15 min and the resulting cell pellet was resuspended in a volume of Leibtoz's-15 medium (L-15).

Macrophages (treatment 3 and 4) were incubated with *A.hydrophila* at a ratio of 1 macrophage to 10 bacteria for 60 min at 22^oC prior to adding either NBT or PMA. A solution of filtered sterile NBT (Sigma Chemical Co. Poole, UK) ($1mgml^{-1}$) or NBT and PMA (Sigma) at $1ugml^{-1}$ was added to triplicate wells of the monolayer at 100 µl $well^{-1}$ The cells were incubated with the solutions for 60 min 22^0C ±2^0C, after which time the reaction was stopped by removing the solution from the wells, fixing cells with 100% methanol for 2 min and washing three times with 70% methanol. After air-drying the plates, 120µl of 2M potassium hydroxide (KOH) and 140µl of dimethyl sulphoxide (DMSO), (Sigma) were added to the wells and mixed to dissolve the formazan produced by the reduction of the NBT. The colour reaction, which resulted, was read at 620nm using a scan spectrophotometer. The results were adjusted to represent 1 $x10^5$ cells ml^{-1}.

6.2.2 Measurement of superoxide anion production by catfish anterior kidney macrophages *in vitro,* pre-incubated with opsonised *A. hydrophila*

Macrophage monolayers were prepared from three African catfish as described in Section 6.2.1. Three different samples of *A. hydrophila* were prepared: (1) 2ml of bacteria suspension, 2.5ml sterile saline and 0.5ml normal catfish serum; (2) 2ml of bacteria suspension, 2.5 ml sterile saline and 0.5ml normal catfish serum heated at 55°C for 30min to inactivate complement; (3) 2ml of bacteria suspension and 3ml sterile saline.

Bacteria were incubated with the sera or sterile saline for 30min at 22°C, after which the bacteria were centrifuging at 3000rpm for 15 min, the supernatant discarded and washed once with sterile saline and the pellet resuspended in 2ml sterile saline at a concentration of 5.1 x10^7 CFU ml^{-1}. A fourth group was included in which medium was added in place of bacteria as a negative control. Each bacterial suspension was added to six wells of the monolayers made from different fish. A further six wells were incubated with medium as a negative controls. Plates were incubated for 1 h at 22^{o}C, then three wells per treatment were incubated with NBT and three wells with NBT and PMA for 30min at 1 h at 22^{o}C as described in Section 6.2.1. The reaction was then developed and read as described in Section 6.2.1.

6.2.3. The effect of pre-incubating catfish anterior kidney macrophages with A. *hydrophila in vitro* at different time intervals on the superoxide production of the cells

Macrophage monolayers were prepared from three African catfish as described in Section 6.2.1. The cells were divided between five different microtitre plates. Eight wells were prepared on each plate per fish. Four of the eight wells were incubated with *A. hydrophila* at 5.8 x10^6 CFU $well^{-1}$ (100μl $well^{-1}$), and the other four wells were incubated with medium as a negative control. The five plates were incubated with the bacteria at 22°C for 30min, 1h, 3h, 18h or 24h. Solutions of NBT, or PMA and NBT were then added for 30min and the reaction developed as described in Section 6.2.1. Analysis was performed by Dr. D. Miles at IOA using ANOVA using fish, treatment, time and the presence or absence of PMA as crossed, fixed factors. All possible interactions between treatment, time and presence or absence of PMA were considered. Natural log transformation was applied to the data.

6.2.4 The effects of pre-incubating catfish anterior kidney macrophages with live and dead *A. hydrophila* or its ECP *in vitro* on the superoxide production of the cells

A total of twelve African catfish were sampled over three days, four per day. Macrophages were isolated from the anterior kidney of each fish and macrophage monolayers prepared as described in Section 6.2.1. *A. hydrophila* suspensions were prepared daily and inoculated at concentrations of 8.7 x10^6, 1.4 x10^7 and 5.0 x10^6 CFU ml^{-1} to macrophage monolayers on Days 1, 2 and 3 respectively. Some of each sample was heat killed by incubation in a water bath at 60°C for 1h, although this was not found to kill all bacteria present. The ECP were prepared by passing broth culture medium through a 0.22 μm sterile filter, and 10μl of this was used. The concentration of ECP was not determined.

Six wells were assigned to each of the four treatments: (1) live bacteria; (2) dead *A. hydrophila*; (3) ECP recovered from an *A. hydrophila* culture; (4) Control macrophages incubated with medium as a negative control. Three of the wells from each treatment were stimulated with PMA and three were left untreated. Solutions of NBT, or PMA and NBT were then added for 30min and the reaction developed as described in Section 6.2.1.

6.2.5 The effects of pre-incubating catfish anterior kidney macrophages with different concentrations of *A. hydrophila* or with ECP *in vitro* on the superoxide production of the cells

A total of seven African catfish were sampled over two days including four male and three female fish. Macrophage monolayers were prepared as described in Section 6.2.1. Tenfold dilutions of an *A. hydrophila* suspension ranging from 3.2 x10^7 to 3.2 x10^2 ml $^{-1}$ on Day 1 and 6.8 x10^7 to 6.8 x 10^2 ml^{-1} on Day 2 were prepared in medium and 100 μlwell^{-1} of each dilution was added to four wells of each of the fish macrophage monolayer for 60 min at 22^{o}C. A further four wells were incubated with only medium as a negative control. After this time the respiratory burst assay was performed as described in Section 6.2.1.

6.3 RESULTS

6.3.1 Measurement of superoxide anion production by catfish anterior kidney macrophages *in vitro* pre-incubated with *A. hydrophila*

An increase was observed in the amount of superoxide anion produced by the catfish macrophages when they were incubated with PMA (0.017 ±0.010) compared with cells not incubated with PMA (0.013 ±0.006) (Figure 6.1). The lowest levels of superoxide anion production in any of the four groups was seen with cells incubated with *A. hydrophila* for 60 min prior to measuring their respiratory burst (0.009 ±0.004). In contrast, the highest levels of respiratory burst were seen with macrophages incubated with bacteria and their respiratory burst measured in the presence of PMA (0.023 ±0.014 group).

6.3.2 Measurement of superoxide anion production by catfish anterior kidney macrophages *in vitro,* pre-incubated with opsonised *A. hydrophila*

The amount of superoxide anion produced by the macrophages was greater in wells incubated with PMA for all four groups compared with those incubated without PMA. The highest amount of superoxide anion produced was found in wells containing control macrophages, which had not been previously exposed to bacteria. The amount of superoxide anion produced in the presence of PMA was similar whether the macrophages had been pre-incubated with unopsonised bacteria, bacteria opsonised with serum or with bacteria incubated with heat inactivated serum. The results of analysis by ANOVA using individual, treatment and presence or absence of PMA as crossed, fixed factors are shown in Table 6.1. The differences between the treatments are shown in Table 6.2 and all results are summarised in Figure 6.2.

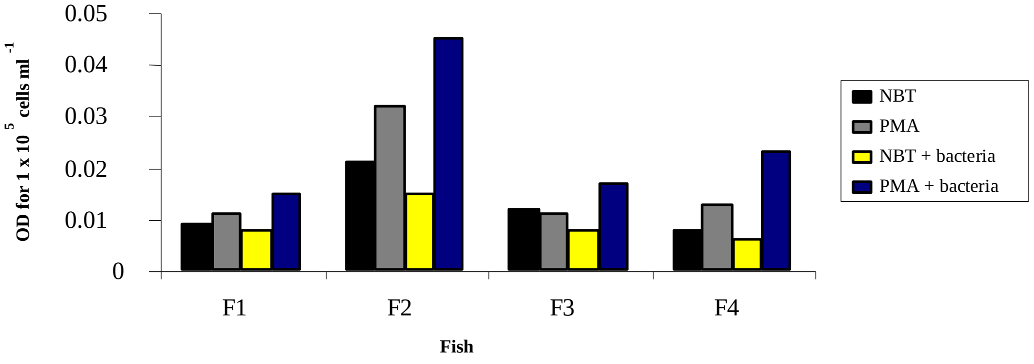


Figure 6.1 Measurement of superoxide anion production by catfish anterior kidney macrophages *in vitro* pre-incubated with *Aeromonas hydrophila*

Table 6.1 Result of ANOVA of individual fish, treatment and presence or absence of PMA

Source	F	Df	p
Individual	136.40	2	<0.0005
Treatment	5.24	3	0.003
Presence or absence of PMA	59.32	1	<0.0005
Interaction between treatment and PMA	1.6	3	0.20

Table 6.2. Results of Tukey multiple comparison of treatments. Expressed as row-column, t-value over p-value

Treatment	Ctrl	Unopsonised	Serum opsonised
Opsonised with inactivated serum	-3.22 0.011	-0.16 1.00	-2.44 0.08
Opsonised with serum	-0.78 0.87	2.28 0.11	
Un-opsonised	-3.06 0.017		

n=3

6.3.3. The effect of pre-incubating catfish anterior kidney macrophages with *A. hydrophila in vitro* at different time intervals on the superoxide production of the cells

Again, the amount of superoxide anion produced by the macrophages was greater in wells containing PMA (Figure 6.3a) compared to wells incubated without PMA (Figure 6.3b) at all sampling times. Generally, when no PMA was present, the amount of superoxide anion produced in the control wells without bacteria was greater than in wells incubated with bacteria except at 3h when the opposite was seen (Figure 6.3a). The amount of superoxide anion produced by the cells was seen to increase in the control wells containing no bacteria with time and was still increasing when the experiment was terminated at 24 h. A similar pattern of activity was observed in wells incubated with PMA as seen in wells incubated without PMA over the experimental period. That is, a greater increase in superoxide anion production by macrophages not pre-incubated with bacteria was seen compared to cells pre-incubated with bacteria (Figure 6.3b). Levels of superoxide anion production in macrophages pre-incubated with bacteria in the presence of PMA was greatest at 30 min, although similar levels of activity were seen in wells with bacteria and PMA at 24h. The inhibition by *A. hydrophila* appeared to be reduced at 18h and 24h in the presence of PMA. The results of the ANOVA are presented in Table 6.3, and differences between times are presented in Table 6.4.

Table 6.3. Results of ANOVA of time series data

Source	F	Df	P
Time	27.81	4	<0.0005
Individual fish	5.69	2	0.005
Treatment	5.72	1	0.019
Stimulation	4.96	1	0.028
Interaction between time and stimulation	1.34	4	0.26
Interaction between treatment and stimulation	8.76	1	0.004
Interaction between time and treatment	2.52	4	0.047
Interaction between time, treatment and	0.52	4	0.72

stimulation			

P = statistical significance, df = degrees of freedom and F = ANOVA value

Table 6.4. Results of Tukey multiple comparisons of respiratory burst after incubation for various time periods. Results expressed as row-column, t-value over p-value

Time	30min	1h	3h	18h
24h	**5.33** **<0.00005**	**5.94** **<0.00005**	**10.47** **<0.00005**	**3.92** **0.0016**
18h	0.73 0.95	1.27 0.71	**5.22** **<0.00005**	
3h	**-5.14** **<0.00005**	**-4.53** **0.0002**		
1h	-0.61 0.97			

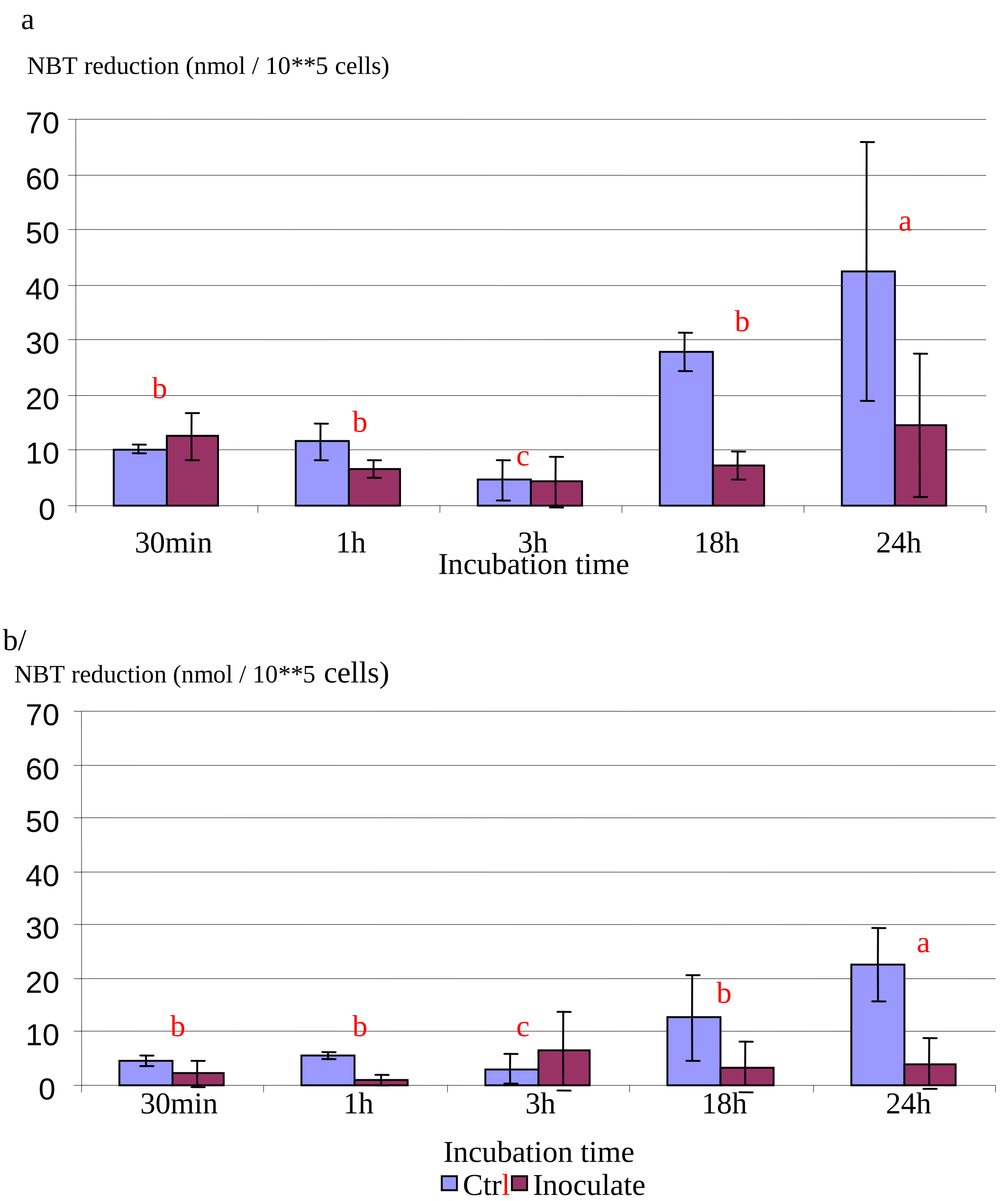


Figure 6.3a and b. Respiratory burst of a/ PMA-stimulated and b/ unstimulated macrophages inoculated with *A. hydrophila*, and unstimulated macrophages after various incubation times. Letters indicate significance groups between times. Bars indicate 95% confidence intervals.

6.3.4 The effects of pre-incubating catfish anterior kidney macrophages with live and dead **A. hydrophila** *or its ECP* **in vitro** *on the superoxide production of the cells*

A greater amount of superoxide anion was produced in the wells containing PMA compared with those without PMA (Figure 6.4). Similar levels of superoxide anion were production by macrophages incubated with live bacteria and without PMA. Levels of superoxide anion produced were similar between the wells containing bacteria, ECP and control macrophages when PMA was not added, although there was no statistical difference between them. On the other hand, PMA stimulated control macrophages to substantially increase their respiratory burst. Live A. hydrophila *inhibited the respiratory burst in the presence of PMA to the extent that it was not different from the respiratory burst without PMA.*

Dead A. hydrophila *or ECP had no effect on the respiratory burst. The treatments were compared by ANOVA, using the different days, treatments and presence or absence of PMA as crossed fixed factors. Individual fish were nested within the days and the interaction between PMA and treatment was considered. A square root transformation was applied. The results of the ANOVA are presented in Table 6.5. The differences between the treatments are given in Table 6.6 and Fig. 6.4. The effect of treatment with PMA is given in Table 6.7.*

Table 6.5 ANOVA of results of Live dead bacteria test

Effect	F	df	P
Day	253.35	2	<0.0005
Individual	3.50	9	0.016
Treatment	201.90	3	<0.0005
Presence of PMA	27.80	9	<0.0005
Interaction between presence of PMA and treatment	10.33	3	<0.0005

Table 6.6. Differences between respiratory burst of control macrophages compared to macrophages inoculated with live or dead *A. hydrophila* or ECP compared by Tukey multiple comparisons.

Overall	Ctrl	Live	Dead
ECP	0.18 1.00	2.43 0.07	0.097 1.00
Dead	0.10 1.00	**2.76** **0.03**	
Live	-2.57 0.050		
PMA-stimulated			
ECP	0.099 1.00	**5.34** **<0.00005**	0.034 1.00
Dead	0.078 1.00	**4.58** **0.0001**	
Live	**-5.10** **<0.00005**		
Unstimulated			
ECP	0.15 1.00	-1.10 0.96	0.11 1.00
Dead	0.06	-1.41	

	1.00	0.85	
Live	1.42 0.85		

Results for the entire trial and results for PMA-stimulated or unstimulated macrophages alone are given. Results are presented as the column subtracted from the row, with t-value over p-value.

Table 6.7. Results of Tukey multiple comparisons between PMA-stimulated and unstimulated macrophages within the four sample groups

	Ctrl	Live *A. hydrophila*	Dead *A. hydrophila*	ECP
t	9.01	2.53	9.90	7.35
p	**<0.00005**	0.18	**<0.00005**	**<0.00005**

6.3.5 The effects of pre-incubating catfish anterior kidney macrophages with different concetntrations of A. hydrophila *or with ECP* in vitro *on the superoxide production of the cells*

Bacterial concentrations of 5 x10^{5} CFU ml^{-1} or below, or approximately 1 bacterial cell per macrophage, had no effect on the respiratory burst (Figure 6.5). Only the highest concentration, approximately 5 x10^{7} CFU ml^{-1} or 100 bacterial cells per macrophage cell, induced significant inhibition of PMA-stimulated macrophages. The respiratory burst of untriggered macrophages was slightly stimulated at bacterial concentrations of 5 x10^{6} CFU ml^{-1}, or 10 bacterial cells per macrophage cell, or above. Results were analysed by ANOVA using sample day, presence or absence of PMA, dilution and fish nested within sample day as fixed, crossed factors. Interaction between the presence of PMA and dilution also considered. Analysis was applied to data after natural log transformation. The power of the analysis is likely to be low due to the small sample sizes. The results of the ANOVA are presented in Table 6.8, the effect of PMA stimulation at each dilution of A. hydrophila *in Table 6.9 and differences between dilutions in Table 6.10. All results are summarised in Fig. 6.5. Further analysis assessed the effect of the bacteria: macrophage ratio on NBT reduction by replacing the fixed factor of dilution with the ratio as a covariate, but no significant effect was found.*

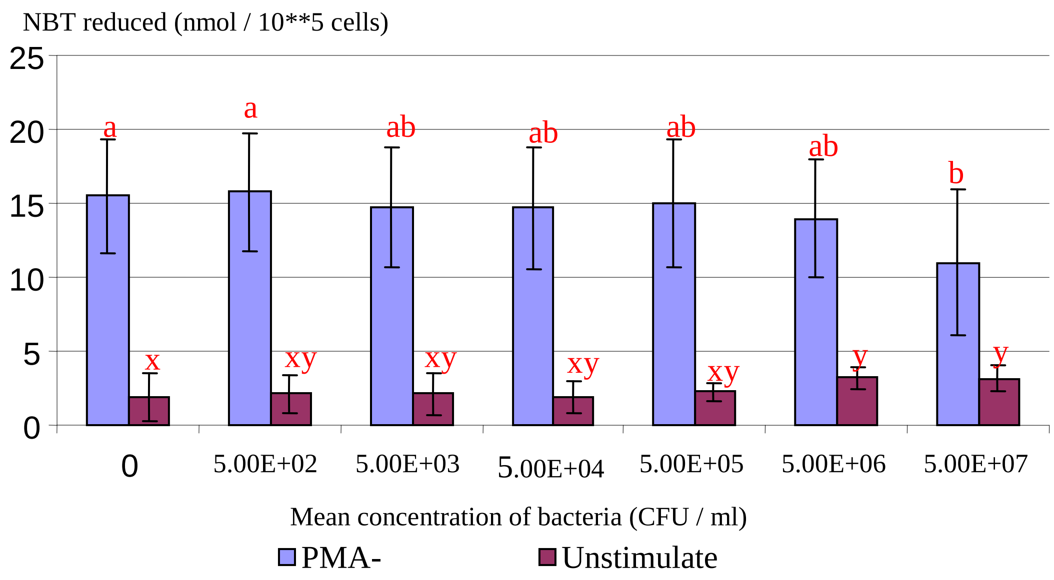


Fig. 6.5. NBT reduction of PMA-stimulated and unstimulated macrophages at different concentrations of *A. hydrophila*. Bars indicate 95% confidence interval. Letters (a,b) indicate significance groups between PMA-stimulated macrophages and (x,y) between unstimulated macrophages.

Table 6.8 ANOVA of effect of different concentrations of *A. hydrophila* on respiratory burst.

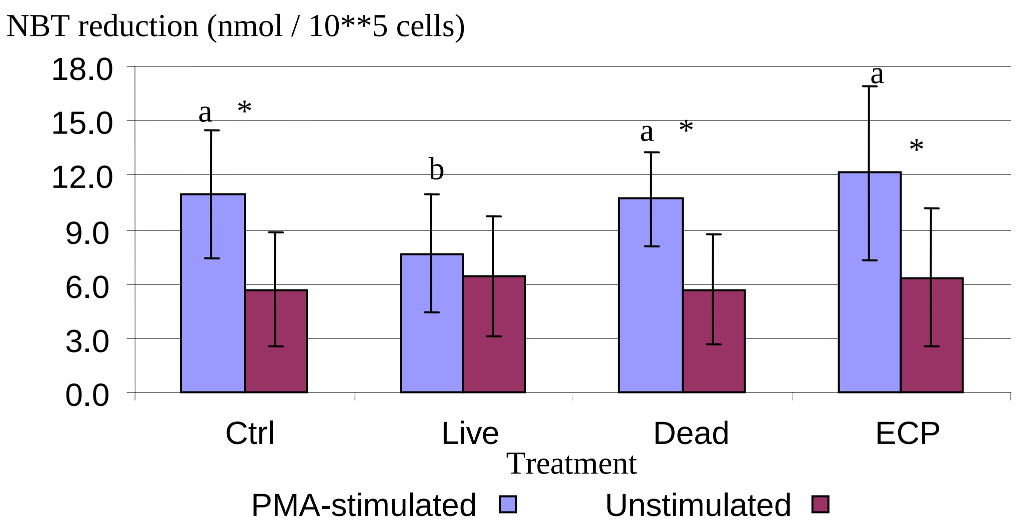


Figure 6.4. Reduction of NBT by PMA-stimulated and unstimulated macrophage cells inoculated with live or dead *A. hydrophila* or ECP. *Indicates significant difference between stimulated and unstimulated. Letters indicate significant differences between PMA-stimulated macrophages.

	F	df	p
Day	22.40	1	<0.0005
Presence of PMA	1197.15	1	<0.0005
Individual	47.12	5	<0.0005

Dilution	1.16	6	0.33
Interaction between PMA and dilution	6.39	6	<0.0005

Table 6.9 Effect of PMA-stimulation at each concentration of *A. hydrophila*.

Dilution	Mean concentration of *A. hydrophila* (CFU ml^{-1})	t	P
Ctrl	0	15.70	<0.00005
1	5.0×10^2	13.98	<0.00005
2	5.0×10^3	13.55	<0.00005
3	5.0×10^4	13.95	<0.00005
4	5.0×10^5	14.58	<0.00005
5	5.0×10^6	11.32	<0.00005
6	5.0×10^7	8.76	<0.00005

Table 6.10. Results of Tukey multiple comparisons of the respiratory burst of PMA-stimulated and unstimulated macrophages inoculated with different concentrations of *A. hydrophila*, expressed as CFU ml^{-1}

PMA-stimulated	0	5.0×10^2	5.0×10^3	5.0×10^4	5.0×10^5	5.0×10^6
5.0×10^7	**-3.57** **0.031**	**-3.80** **0.014**	-3.0 6 0.13	-3.09 0.12	-3.22 0.08	-2.68 0.30
5.0×10^6	-0.89 1.00	-1.12 1.00	-0.38 1.00	-0.42 1.00	-0.55 1.00	
5.0×10^5	-0.34 1.00	-0.57 1.00	0.17 1.00	0.13 1.00		
5.0×10^4	-0.47 1.00	-0.70 1.00	0.04 1.00			

5.0 x 10^3	-0.51 1.00	-0.74 1.00				
5.0 x 10^2	0.23 1.00					
Unstimul -ated						
5.0 x 10^7	**3.93** **0.009**	2.02 0.75	2.29 0.57	2.37 0.51	2.61 0.34	-0.06 1.00
5.0 x 10^6	**3.99** **0.007**	2.08 0.72	2.34 0.52	2.43 0.46	2.68 0.30	
5.0 x 10^5	1.43 0.98	-0.48 1.00	-0.21 1.00	-0.19 1.00		
5.0 x 10^4	1.59 0.95	-0.29 1.00	-0.03 1.00			
5.0 x 10^3	1.58 0.95	-0.26 1.00				
5.0 x 10^2	1.84 0.85					

6.4 DISCUSSION AND CONCLUSIONS

The results of this study suggested that the presence of *A. hydrophila* within the macrophages was able to inhibit the respiratory burst activity of the cells. The highest amount of superoxide anion produced was found in wells containing macrophages, which had not been previously exposed to bacteria. Cells incubated with *A. hydrophila* for 60 min prior to measuring their respiratory burst in the presence of PMA had higher levels of respiratory burst than cells incubated with bacteria and their respiratory burst measured in the absence of PMA. However, this may have been due to the presence of the PMA which is a well-known and frequently used immunostimulant to induce superoxide anion production in the macrophages of a number of different fish species (Secombes 1990). The respiratory burst activity measured in the absence of PMA was slightly increased by *A. hydrophila* at ratios of approximately 10-100 bacterial cells per macrophage cell. However it was not possible to establish the concentration of bacteria which was inhibitory for respiratory burst, since cells with bacteria not treated with PMA were not included in Experiment 6.2.5.

There were no significant differences between uninoculated and inoculated macrophages when superoxide anion production was measured over a 24 h time period. The amount of superoxide anion produced in the presence of PMA was similar whether the macrophages had been pre-incubated with unopsonised bacteria, bacteria opsonised with serum or with bacteria incubated with heat inactivated serum. Although the respiratory burst was significantly higher in cells without bacteria, reiterating the suggestion that *A. hydrophila* can inhibit the intracellular respiratory burst activities of the macrophage. The ability of *A. hydrophila* to inhibit respiratory burst is only present when the cells are alive, suggesting that this inhibition is an active process, possibly related to the virulence of the bacterium, however the ECP of the bacterium did not affect levels of superoxide anion production.

Acknowledgements

Sections 6.2.3, 6.2.4 and 6.2.5 of this report were performed by Dr David Miles, IOA, Stirling.

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Appendix 7

This document is an output from a project funded by the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID'

POND STUDIES

Dr. Supranee Chinabut, Dr. Temdoung Somsiri and Ms. Yolprapa Dumrongphol

Aquatic Animal Health Research Institute, Dept. of Fisheries, Kasetsart University Campus. Jatuyak. Bangkok. 10900 Thailand

BACKGROUND

High bacterial loads have been reported within the aquatic environments of the fish farms in Thailand, and a statistically significant relationship was been found between high bacterial load in the pond and the occurrence of bacteria within macrophages (Thompson and Crumlish 1999). The levels of bacteria within the isolated macrophages were generally low (<1%), but were observed in a variety of fish species cultured in a range of freshwater farming systems in Thailand. Bacteria identified as Aeromonads and Pseudomonads were predominantly recovered and identified from the macrophage cell suspensions isolated from the anterior kidney of clinically healthy farmed fish (Inglis and Crumlish 1997, Thompson and Crumlish 1999, and Crumlish *et al* 2000). The clinical significance of bacterial loading within the tissue dwelling macrophages of farmed fish was not determined, however. One hypothesis was that the fish were physiologically able to cope with the constant microbial interaction and the immune response was robust enough to clear the microbes from the fishes system before without disease occurred. However, it was unclear whether the consistent clearing of these opportunistic microbes found ubiquitously in the farming aqueous environment, left the fish stressed and more likely to succumb to bacterial infections/disease.

A four month pond study was conducted in Thailand in which some of the potential risk factors identified from the data collected during the fish farming survey in Appendix 1 were examined. The variables tested were polyculture *vs.* monoculture and the influence of pond preparation on the outcome variable identified as fish disease occurrence. Furthermore, samples were taken every month for water quality analysis, and to assess the bacterial load in the water and the presence of bacteria within macrophages.

THE STUDY

The experiment was designed (Table 1) and conducted by AAHRI staff, the results of which will contribute to the MSc thesis of Ms Dumrongphol. Only the experimental design is presented here as the results are still being analysed and will be available when the thesis is completed in September 2003.

A commercial freshwater fish farm (Wan Mat Cha) located in Minburi Province was chosen as the study site because it was large enough to provide replicate ponds of 800 m^2 and was within daily travelling distance of AAHRI.

10 Table 1 Experimental design of the study

No.	Date	Pond	Size (cm.)		Weight (g)	
			Tilapia	Puntius	Tilapia	Puntius
0	30/11/00	1 to 6	3.89	4.37	2	2.2
1	18/01/01	1	11.52	-	30	-
		2	-	13.13	-	36.5
		3	9.03	14.13	18	37
		4	10.65	-	22	-
		5	-	12.18	-	30
		6	13.23	10.4	45	16
2	01/02/01	1	12.67	-	50	-
		2	-	13.42	-	42.5
		3	14.27	14	50	51.67
		4	13.05	-	31.67	-
		5	-	12.98	-	31.67
		6	11.57	14.53	33.33	38.33
3	15/02/01	1	16.1	-	46	-
		2	-	16.02	-	42.16
		3	14.83	14.87	42.37	39.13
		4	15.72	-	44.91	-
		5	-	15.58	-	41
		6	15.57	16.27	44.48	42.82
4	01/03/01	1	15.23	-	82.5	-
		2	-	14.15	-	48.33
		3	13.9	14.77	65	55
		4	14.28	-	65.83	-

		5	-	14.02	-	38.33
		6	14.37	17.47	73.33	75

11 MATERIALS AND METHODS

In total 6 ponds were used and stocked with tilapia and/or puntius. These species were routinely cultured and therefore available on the farm. Three of the 6 ponds were prepared by the fish farm following his "regular" protocol whereas the remaining ponds were not prepared and only water and fish were added. The ponds were prepared by draining the pond and adding lime at 16kg per pond and allowing the pond to dry for 2 weeks, before draining the pond further and inserting new water.

Water was sampled weekly and pH, alkalinity and transparency measured. Fish were sampled every 2 weeks to establish the number of macrophages that contained bacteria. The levels of bacteria that could be cultured from the fish and from the water were also measured at this time on tryptic soya agar (TSA) and Aeromonas selective medium.

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