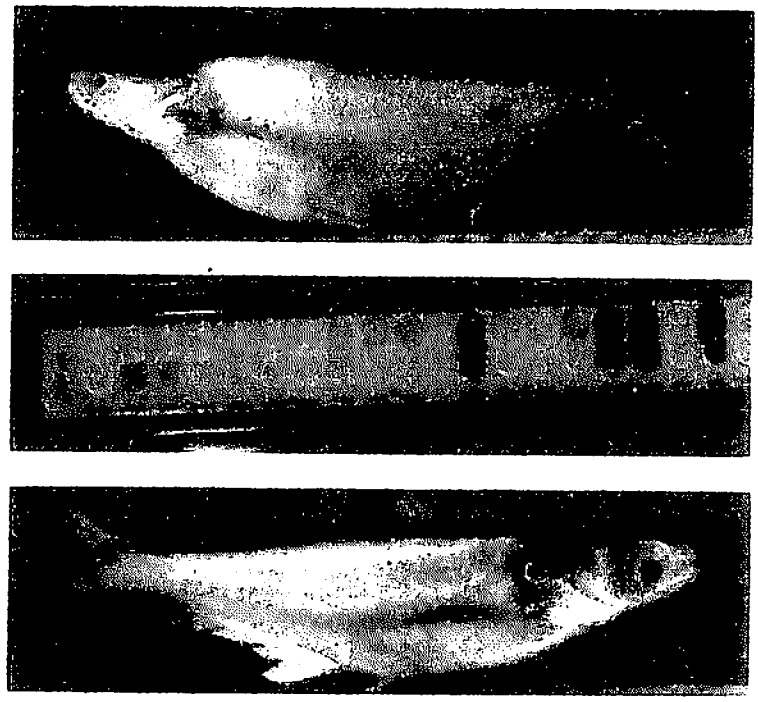
Investigation of Diseases and Development of Strategies for Improved Health Management of Thai pangas (*Pangasius sutchi*) Cultured in Rural Ponds in Mymensingh District

A WorldFish Center Funned Research Project



Final Report

By

Dr Md. Ali Reza Faruk
Associate Professor
Department of Aquaculture
Bangladesh Agricultural University
Mymensingh-2202, Bangladesh
E-mail: farukmar@royalten.net

August 2004

WorldFish CENTER

Investigation of Diseases and Development of Strategies for Improved Health Management of Thai pangas (*Pangasius sutchi)* Cultured in Rural Ponds in Mymensingh District

Final Report

By

Dr Md. Ali Reza Faruk
Associate Professor
Department of Aquaculture
Bangladesh Agricultural University
Mymensingh-2202, Bangladesh
E-mail: farukmar@royalten.net

WorldFish
CENTER

Contents

Investigation of Diseases and Development of Strategies for Improved Health Management of Thai Pangas (*Pangasius sutchi)* Cultured in Rural Ponds in Mymensingh District

Executive Summary

Thai pangas, *Pangasius sutchi* (Syn. *P. hypophthalmus*) is one of the important species in aquaculture of Bangladesh. Over the last few years, spectacular development of has been taken place in Thai pangas farming in Mymensingh district. Due to quick profit, peoples are converting their rice field into pangas farm. However, when the expansion of pangas farming is almost in its peak, farmers are facing serious disease problems and mortality in their fish. Therefore, to examine health and disease status of Thai pangas, clinical, histopathological and bacteriological techniques were employed. In addition, to assess the financial losses of farmer due to fish disease, questionnaire interview and participatory rural appraisal (PRA) tools were used with selected farmers. A total of 100 fish farmers were interviewed and several PRA sessions were conducted in three upazilas in Mymensingh districts. This study indicated that there are economic losses of approximately 3.6% to farmers from diseases. These losses varied among the different farm categories. The most prevalent disease or clinical signs as reported by the farmers was red spot, followed by anal protrusion, tail and fin rot, pop eye, dropsy and gill rot. Other conditions like cotton wool type lesion, ulceration and white spot were reported but with lower incidence.

Potential pangas farmers having disease problems in their ponds, were identified during the field survey. Twelve fishes were routinely sampled once in a month for laboratory analysis. Fishes were found susceptible to disease during the winter months of the year. The clinical signs of fish were found quite similar as was reported by the farmers during the field survey. Four isolates of *Aeromonas hydrophila* were recovered from kidney and lesion of disease affected fish. Hemorrhage over the body especially near mouth and caudal region was noticed in the fishes associated with Aeromonad infection. Internally, kidney, liver and spleen were swollen and enlarged. The isolates varied with their pathogenicity. All the four isolates were sensitive to Nitrofurantoin, Cortimoxazole and Tetracycline but were resistant to Amoxycilline. An attempt was made to treat diseased fish with extracts from neem leaf, garlic and turmeric. Recovery of infection was monitored through mortality and histopathology. Extract from neem leaf gave better result. General histopathological changes of different organs were also studied. All the organs were found affected to some extent. Telangiectasis, lamellar hypertrophy and hyperplasia hemorrhage, lamellar fusion, necrosis of lamellar epithelial cells, presence of parasites and their cysts were the major pathology of gills. Hemorrhage lesion, pyknotic nuclei and melanomacrophage centers (MMC) were found in the liver of fish. Major pathologies in kidney of fish included presence of MMC, necrotic and ruptured kidney tubules, severe haemopoietic necrosis, and hemorrhage. The study also highlighted fish health management related problems and recommended further work for the development of farmer-oriented fish health management packages.

1. Background

Thai pangas, *Pangasius sutchi* (Syn. *P. hypophthalmus*) is a siluriform catfish, belongs to the family Pangasiidae. The fish was originated from the Mekong river of Vietnam (Roberts and Vidthayanon, 1991) and is available in almost all countries of South-East Asia (Pan and Heng, 1983). It was introduced into Bangladesh in 1990 under the Ministry of Fisheries and Livestock (MOFL) from Thailand. Presently this fish is one of the important species in aquaculture of Bangladesh. It occupies one of the top positions with respect to growth, production and nutritional composition, and that also makes this species widely popular among Bangladeshi people. Two type of culture system have been practiced in Bangladesh: monoculture (following intensive culture strategy) and polyculture (following semi-intensive culture strategy). In polyculture system the production of Thai pangas is about 10-12 tons/ha/year. In case of intensive commercial culture, production is about 25-30 tons/ha/year with animal protein rich diets and water exchange (BFRI, 1998). Over the last few years there has been spectacular development on Thai pangas culture in Mymensingh district. Farmers are converting their rice field into pangas farm for quick profit. Presently, in Mymensingh district, there are about 1,364 pangas farm covering an area of 774 ha and which produce 19,203 mt. fish per year (DoF, 2003).

Fish disease is the interaction among host fish, active pathogen and the environment where they live. Diseases are having an ever greater influence on fishes in a globally expanding and intensifying aquaculture system (Plumb, 1997). In Bangladesh, fishes have been suffering from many types of diseases such as ulcer type disease including epizootic ulcerative syndrome (EUS), bacterial hemorrhage septicemia, tail and fin rot, bacterial gill rot, dropsy, columnaris disease, various types of fungal and parasitic diseases (Faruk et al., 2004; Chowdhury, 1997). Bacterial diseases have been found responsible for heavy mortalities in both wild and cultured fishes. Among the bacterial genera, *Aeromonas* spp. are one of the major bacterial pathogens which are widely distributed in aquatic environment (Banu, 1996).

Although Thai pangas is very tolerable to an adverse environment such as low O_2, pH and fluctuation of turbidity, farmers have already experienced serious disease problems and mortality of this fish under farming condition. Currently, disease is one of the major problems in Thai pangas culture in Mymensingh district especially at their early stage. As a consequence, price of this fish is decreasing, farmers are financially losing, and livelihood of farmers, their dependants, traders and many others are depending on the pangas industry are in great threat. Therefore, there is a need to the diseases of Thai pangas and formulate their improved health management strategies to sustain the industry.

2. Purpose

Culture of Thai pangas in freshwater pond has become very popular and economically beneficial among the rural fish farmers of Bangladesh. However, diseases are among the most common and serious problem in Thai pangas farming of the country and is responsible for substantial source of monetary loss to farmer due to death of fish, poor growth and food conversion, increased production costs and interruption of production schedule. But there is no systematic research on this particular area and no data exists in the published literature from which rural farmers could be benefited. Therefore, the purposes of the proposed research are (i) to investigate the impact and occurrence of disease outbreaks of Thai pangas (ii) to know the nature and extent of diseases with their clinical and histopathological techniques. (iii) to characterize the pathogens involved and (iv) to determine the problems in fish health management. Such basic information is important for the development of guidelines to help increase production yields, improve health management strategies, reduce disease risks and improve the livelihoods of farmers.

3. Materials and methods

The study consisted of both field investigation and laboratory study.

3.1 Field survey

For field study, data on status of pangas farming, production, diseases and economic loss due to disease were collected. The study included three upazila (Trishaw, Bhaluka and Muktagacha) of Mymensingh district. These were selected because maximum pangas culture is practices in these Upazilas.

Collection of data

Data was collected through the questionnaire interview and participatory rural appraisal (PRA) with fish farmers. For questionnaire interview, a set of preliminary questionnaire was prepared. A total of 100 farmers having different farm size were interviewed. Prior to field survey, background information on the number, location and distribution of fish farms and aquaculture activities was collected. The questionnaire was divided into several sections. The first section focused on general farming and farmers information, the second section on pond preparation information, the third one covered information related to fish stoking and pond management and the final section focused on fish health and disease problems, their economic loss, management interventions used to control disease. This section was used only when the farmers reported disease problems in their ponds.

PRA tools including focus group discussion (FGD) was conducted with rural fish farmers to get an overview of particular issues of fish health and disease. Several FGD sessions in different districts were conducted where each group size was between 6 and 12 farmers. Ten farmers were selected from each village of the selected Upaziala. Cross check interviews were conducted with key informants such as District Fisheries Officer, Upazila Fisheries Officer, NGO workers working with aquaculture, fishermen leader, village old man, school head master and village head-man where information was contradictory.

Analysis of data

After collection of data from the study areas, data were coded and entered into a computer for analysis. Some of the data were collected in local units such as *bigha, katha* etc. due to familiarity for respondent. These were converted into international units before transfer in to computer. Data were processed using Microsoft Excel and SPSS programme. Preliminary data sheets were compared with the original coding sheets to ensure the accuracy of the data entered. The data was analyzed by using tabular and descriptive statistical techniques. The summary tables were prepared in accordance to the objective of the study. The technique of analysis included the classification of tables into meaningful result by arithmetic mean, percentage and ratios.

3.2. Laboratory study

3.2.1 Sample collection: For examining the disease status of Thai pangas, diseased fish were collected on monthly basis from selected disease affected farms and brought to the laboratory for detailed studies. After gross diagnosis on the spot, clinical signs including behavior and appearance were made and recorded.

3.2.2 Clinical observation

The sampled fishes were examined by necked eyes to observe external signs and colour changes, any injury, lesion, fin/appendage damage and other abnormalities of fish body.

3.3.3 Histopathological study

Muscle tissues of affected fish were collected from various organs such as skin, muscle, gill, liver and kidney by sharp scalpel and force. Tissue samples were fixed in 10% buffered formalin solution, embedded in paraffin, and sectioned at 5µm. The sections were stained with haematoxylin and eosin (H & E). After staining the sections were mounted with Canada balsam and covered by coverslips. The prepared sections were left over a clean platform to hold the coverslips permanently and then examined under a compound microscope (Olympus). Photomicrographs of the stained sections were done by using a photomicroscope.

3.3.4 Bacteriological Studies

Collection of bacterial swabs

Bacterial swabs were taken aseptically from the lesions, kidney and liver of the diseased affected fish on the pre-prepared Tryptone Soya Agar (TSA) and incubated at 22°C.for 24 h. The isolates were maintained in TSA slant at 4^0C for further studies.

Characterisation and identification of bacteria

Various morphological characteristics of bacterial colonies such as shape, size and color were recorded. The shape of individual bacterium was determined after Gram's staining taking fresh culture of 24 h. Motility test was performed by taking dilute suspension of fresh bacterial culture on clean glass slide with coverslip and observed under a microscope with closed circuit camera adjusted with a TV- monitor. Oxidase test, OF-test (Hugh and Leifson's test), 0/129 sensitivity was performed following the usual microbiological procedure as described below. Suspective bacterial pathogens were identified up to genus level on the basis of the above characteristics according to following flow chart diagram modified after Tonguthai, *et. al.*, 1999 (Fig. 1). Species determination of Aeromonads bacteria was performed following the Aerokey-II system of schematic diagram described by Carnahan *et. al.*, (1991). All of the necessary characterizations were accomplished using API-20E system (Table 1).

Oxidase test

An oxidase strip (Oxoid) was placed in a clean Petri dish and a substantial bacterial inoculum, taken directly from a 24 h culture, was smeared onto the end of the strip containing the oxidase reagent, N,N-dimethyl-1,4-phenylene di-ammonium chloride. The strip was left for 30 sec and then examined for any colour change; a deep purple/blue colour indicated oxidation of the reagent and a positive reaction.

Oxidative-Fermentative test

Oxidative or fermentative glucose metabolism was examined using Hugh-Leifson (O-F) medium (Difco) containing 1% (w/v) glucose. Briefly, a culture of bacteria was inoculated into freshly prepared tubes of O-F medium by a single stab with a straight wire. One tube was incubated in the presence of air (aerobic tube), the other was covered with a thin layer of liquid paraffin to exclude air (anaerobic tube). The medium also contained bromothymol blue pH indicator to detect acid formation as a result of glucose metabolism. The colour of the medium was examined after incubating the tubes at 22°C for 48h. If both tubes remained green, this indicated that no reaction had taken place. If the aerobic tube was yellow, but the anaerobic tube was green this indicated than an oxidative reaction had occurred, whereas if both tubes had turned yellow this indicated the presence of a fermentative reaction.

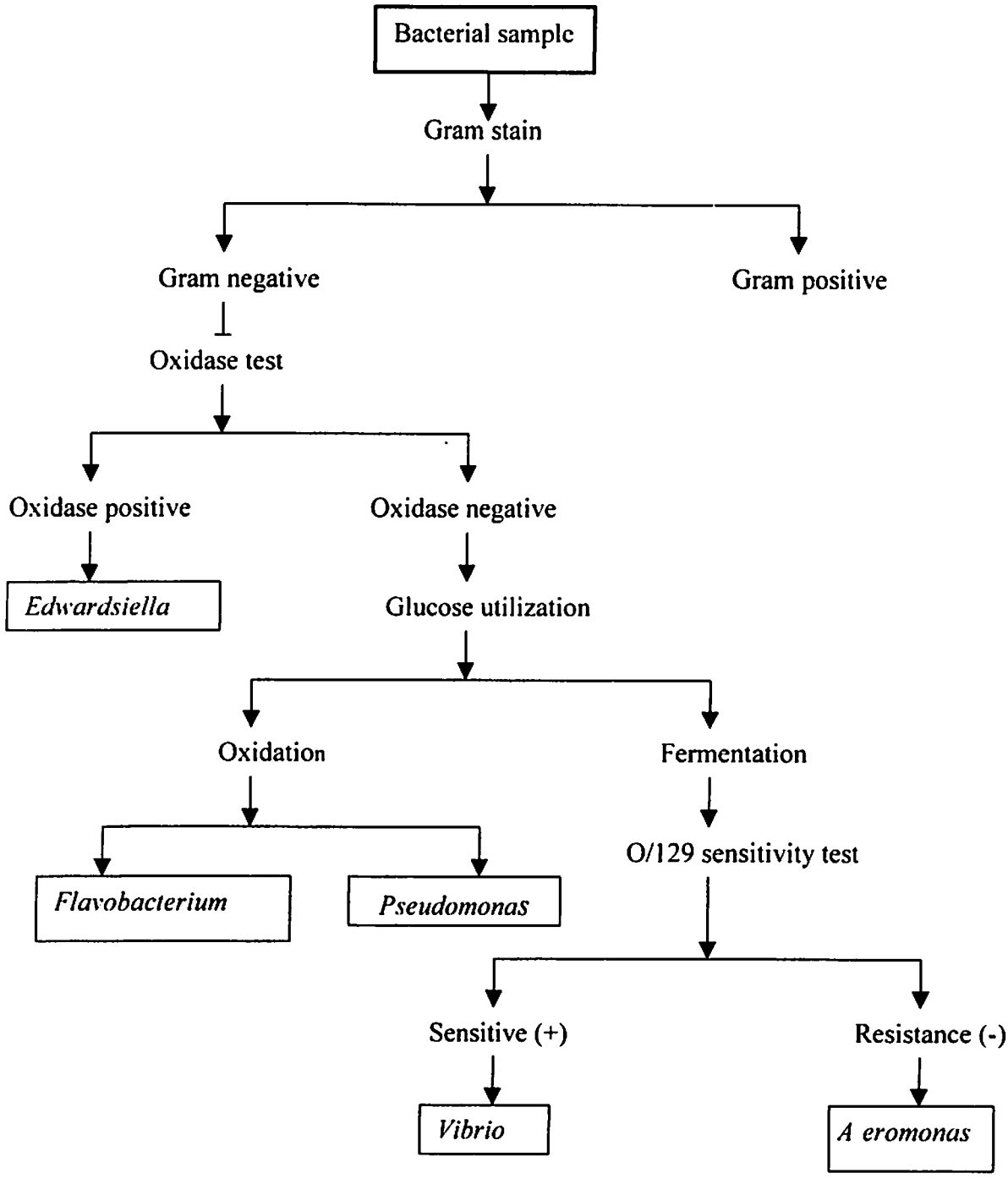


Fig. 1. Schematic diagram for primary identification of bacteria (modified after Tonguthai et al., (1999)

O/ 129 Sensitivity test

O/129 (2,4-Diamino-6,7-di-iso-propyl pteridine phosphate) is a bacteriostatic agent effective against *Vibrio* species. This test is important in differentiating *Vibrio* species from other Gram-negative rods and particularly from *Aeromonas* species, which are resistant to 0/127. Several colonies of growth were removed using sterilized loop and inoculated in a small quantity of sterile saline. The culture was suspended in the saline by gentle shaking. A small volume of the suspension was removed using a sterile pipette and carefully pipetted 5 drops onto the surface of the agar. The suspension was then spread over the whole surface of the agar plate by a glass spreader and the lid of the petridish was replaced. The plate was allowed to dry for approximately one minute. Then one 10µg disc and one 150µg disc of O/129 was placed onto the plate. The plate was inverted and incubated at 25°C for 24h.

API 20E microbiological identification kit

The API 20E system is standardized miniaturized version of conventional procedures for the identification of certain Gram-negative bacteria. It is a micro-tube system enabling 23 standard biochemical tests to be carried out on a bacterial culture. A small volume of tap water was dispensed into the incubation tray and API strip was placed in the tray. A single well- isolated colony of bacteria from a pure culture was picked up using a sterile loop and inoculated in 5 ml of sterile saline by tilting the bottle and gently rubbing the culture from the loop on the inner surface of the bottle. The culture was suspended in the saline by gentle shaking. A quantity of the culture suspension was pipetted using a sterile pipette. The API incubation tray was tilted and carefully filled the tube section of the microtubes by placing the pipette tip against the side of the couple. The cupule section of the CIT, VP and GEL tubes were filled up with the bacterial suspension. After incubation, the cupule section of the ADH, LDC, ODC , H2S and URE tubes were filled completely with sterilized liquid paraffin using a sterilized pipette to create anaerobiosis. The lid of the strip was replaced and then incubated at 22°C for 2 days. After examining the strip, all reactions were recorded in a table and the results obtained were compared with the summery of results. For TDA, VP and IND tubes particular reagents were added according to the manufacturers instructions.

Table 1. API 20E: summary of results

Test	Substrate	Reaction/enzyme	Negative	Positive
ONPG	Ortho-nitrophenyl galactosidase	beta-galactosidase	Colourless	Yellow
ADH	Arginine	arginine dihydrolase	Yellow	Red/Orange
LDC	Lysin	lysine decarboxylase		YellowOrange
ODC	Ornithine	ornithine decarboxylase	Yellow	Red
CIT	Sodium Citrate	citrate utilisation	Yellow	Blue/Green
H2-5	Sodium Thiosulphate	H2S production	Colourless	Black Deposit
URE	Urea	urease	Yellow	Red/Orange
TDA	Tryptophane	tryptophane deaminase		YellowDark Brown
IND	Tryptophane	indole production	Yellow Ring	Red Ring in 2 minutes
VP	Sodium Pyruvate	acetoin production	Colourless	Pink/Red in 10 minutes
GEL	Gelatin	gelatinase	No Black Diffusion	Black Diffusion
GLU	Glucose	fermentation/oxidation	Blue/Green	Yellow
MAN	Mannitol	fermentation/oxidation	Blue/Green	Yellow
INO	Inositol	fermentation/oxidation	Blue/Green	Yellow
SOR	Sorbitol	fermentation/oxidation	Blue/Green	Yellow
RHA	Rhamnose	fermentation/oxidation	Blue/Green	Yellow
SAC	Sucrose	fermentation/oxidation	Blue/Green	Yellow
MEL	Melibiose	fermentation/oxidation	Blue/Green	Yellow
AMY	Amygdalin	fermentation/oxidation	Blue/Green	Yellow
ARA	Arabinose	fermentation/oxidation	Blue/Green	Yellow

Antibiotic sensitivity test

Several colonies of growth was removed using a sterilise loop and inoculated a small quantity of sterile water. The culture was suspend in the saline by gentle shaking. A small volume of the suspension was removed using a sterile pipette and carefully pipetted 5 drops onto the surface of the agar. The suspension was then spread over the whole surface of the agar plate by a glass spreader and the lid of the petridish was replaced. The plate was allowed to dry for approximately one minute. Then one 300µg nitrofurantoin, one 25µg cortimoxazole, one 30µg tetracycline and one 10µg amoxyciline disc was placed onto the plate. The plate was inverted and incubated at 25°C for 24h.

Pathogenicity study

Isolates of *Aeromonas hydrophila* were cultured in TSA plate. The bacteria were gently scraped off the agar and suspended in physiological saline. The concentration of bacteria in each bacterial preparation was confirmed by determining the number of cfu ml^{-1}. Young Thai pangas were obtained from healthy stocks of fish reared at a commercial farm (Trishal) with no known history of any disease. Fish were acclimatized for 4 days, fed with a commercial pellet diet 2-3 times daily. Duplicate groups of Thai pangas were placed in 10l liters of tap water in a 15-litre capacity glass aquarium. Each tank was aerated and the water temperature was maintained at between 22 and 25°C. Details of the set up of each challenge are shown in Table 2. Fish were injected either intramuscularly (below the left dorsal fin of the animal) 0.1 ml of bacterial suspension contains 1.2 $x10^8$ cfu ml^{-1}. Two control groups of 10 fishes were injected IM with 0.1 ml of sterile saline in place of the bacteria. One-third of the water was replaced daily, dead fish were removed and debris siphoned from the bottom of the tank. Fish were monitored for 14 days, mortality and morbidity were recorded daily and necroscopy involved gross external and internal examinations.

Table 2. Set up of challenge experiment

A. hydrophila isolate	[a]No. of experimental fish	[a]No. of control fish	Average weight (g)	[b]Challenge dose (cfu $fish^{-1}$)	Routes of administration
PK1	20	20	10g	$1.2 x10^7$	IM
PK2	20	-		$1.2 x10^7$	IM
PK3	20	-		$1.2 x10^7$	IM
PL2	20	-		$1.2 x10^7$	IM

[a]Fish were divided into two replicate tanks, [b]Control groups of fish treated with were included in each challenge using corresponding routes administration and identical numbers of fish . [c]cfu ml^{-1}. Abbreviations: IM: intramuscular

3.2.5 Effect of herbal medicine against Pangasius disease

An attempt was made to observe the effect of some herbal products in healing process of affected Thai pangas. Weak and affected fish (10g) were collected from Trishal upazila of Mymensingh district and brought to the Laboratory by ploythene bag filled with water and oxygen. The fish were conditioned in aquaria for four days. Aeration and commercial catfish pellet was supplied the aquaria. Record of length weight, clinical and pathological observation was made at the beginning of the experiment. For pathological observation skin, muscle, gill and kidney were collected and processed for histology ant the beginning and at the end of the experiment.

Extracts of three herbal products such as neem (*Azadirecta indica*) leaves, garlic (*Allium sativum*) and turmeric (*Curcoma longa*) were prepared in the Pharmacy Lab of BAU maintaining 10% stock solution. Thus for treatments (T) were selected such as T1 with neem leaves, T2 with garlic, T3 with turmeric and T4 as control. Eight fish were introduced to 20 liters of each

treatment. Concentration of herbal extract for the first three treatments was maintained at 50 ppm. Continuous aeration was maintained to all the aquaria. Half of the water was changed in every morning with removal of waste products (faces and uneaten food). Herbal products were added to maintain concentration of drugs. Daily observation of mortality and clinical healing process were made. DO, pH and temperature were recorded weekly. The experiment was continued from four weeks. Clinical and pathological record were also made at the end of the experiment.

4. Results

4.1 Field survey

Farmers category

All together, data from 100 farmers were analysed. The survey split farmers into three catagories depending on their pond area. The first category comprised of small farmers having less than 0.5 ha pond area which represented about 45% of the total farmers intervied (Table 3). The second category was medium farmers who had pond area between 0.5 and 1.0 ha, and the third category was larger farmers having pond area over 1.0 ha. The medium and large category farmers represented 35% and about 20% respectively.

Table 3. Summary of the respondent with different pond categories in the study area

Pond Category (ha.)	Trishal	Bhaluka	Muktagacha	Total
<0.5	20	10	15	45
0.5-1.0	12	13	10	35
> 1.0	8	10	2	20
Total	40	33	27	100

Farmer's age, family, experience and education

All the farmers interviewed were male with an average age of 37 years and an average family size of 6 (Table 4). The average experience of fish culture by the farmers was 5 years. About 44% farmers had education up to high school level but under SSC follwewd by SSC (29%) and HSC (20%). Only 7 % of the respondent had gradutuation degree. Majority of the farmers (71%) reported that that they culture fish for income and 29 farmers reported that they culture fish for both income and food.

Table 4. Average age, family size and experience of the respondent

Pond Category (ha)	Age (yr)	Family size (No)	Experience of fish culture (yr)
<0.5	37	6	4
0.5-1.0	36	5	5
> 1.0	38	7	6
Average	37	6	5

Pond preparation and stocking

During pond preparation, 70 respondent said that they used only lime, 19 respondent used both lime and cowdung, 6 respondent used lime, cowdung, Urea & TSP, 5 respondent used lime, cowdung, oil-cake, Urea & TSP. About 62 farmers reported that they do not remove undesirable fish species from their ponds, 38 farmers remove undesirable fish species by using netting. The average pond preparation cost was about Tk.1,910/ha. Average stocking density of fish was 39,581/ha and the average stocking cost was Tk 55,852/ha (Table 5).

Table 5. Stocking density (fish/ha) and stocking cost (Tk/ha)

Pond Category (ha.)	Stocking density (fish/ha)	Stocking cost (Tk/ha)
<0.5	37,213 ± 7193	52,226 ± 18170
0.5-1.0	41,515 ± 7017	55,555 ± 9265
> 1.0	40,014 ± 3757	59,774 ± 12516
Average	39,581 ± 5989	55,852 ± 13317

± Indicates standard deviation

Treatment used by the farmers before fry stocking

About 84 respondent reported that they do not used any pretreatment prior fish stocking, only 16 respondent reported that they dip their fish fry in potassium permanganate or salt before releasing it in the ponds.

Feed and feeding

About 50 respondents used commercial feed and 50 respondent used own made supplementary feed in their ponds.

Fish disease and health management

During the present survey, the areas for data collection were selected mostly on the basis of having previous disease history. When farmers were asked whether they had disease problems in their ponds, the majority (98 %) of the farmers said they had disease problems durring last years or year before and 2 respondent that they do not found any disease in their ponds. The average prevalence of disease was about 7.2% with highest was found with small-scale farmers (7.6%) followed by medium (7.2%) and large scale farmers (6.7%). Farmers reported about 3.5% mortality of their fish due to disease. About 96 farmers reported that they found disease during winter season in their ponds and 2 farmers reported that they found disease in winter & rainy season.

Fate of diseased fish

Majority (81.4%) of the farmer said that they through away the diseased fish, only 3.8% said that they eat it, 5.0% reported that they sale the fish and 10.2% farmer reported that they do not do anything with the diseased fish.

Types of disease problems

When farmers were asked about kind of diseases in their ponds, a range of diseases and conditions was reported by the farmers according to their occurrence. The most prevalent disease was red spot (19.1%), followed by anal protrusion (18.9%), tail and fin rot (14.3%), pop eye (12.5%), dropsy (10.9%) and gill rot (9.0%). Other conditions like cotton wool type lesion, ulceration and white spot were also reported by the farmers but with lower incidence (Fig. 2).

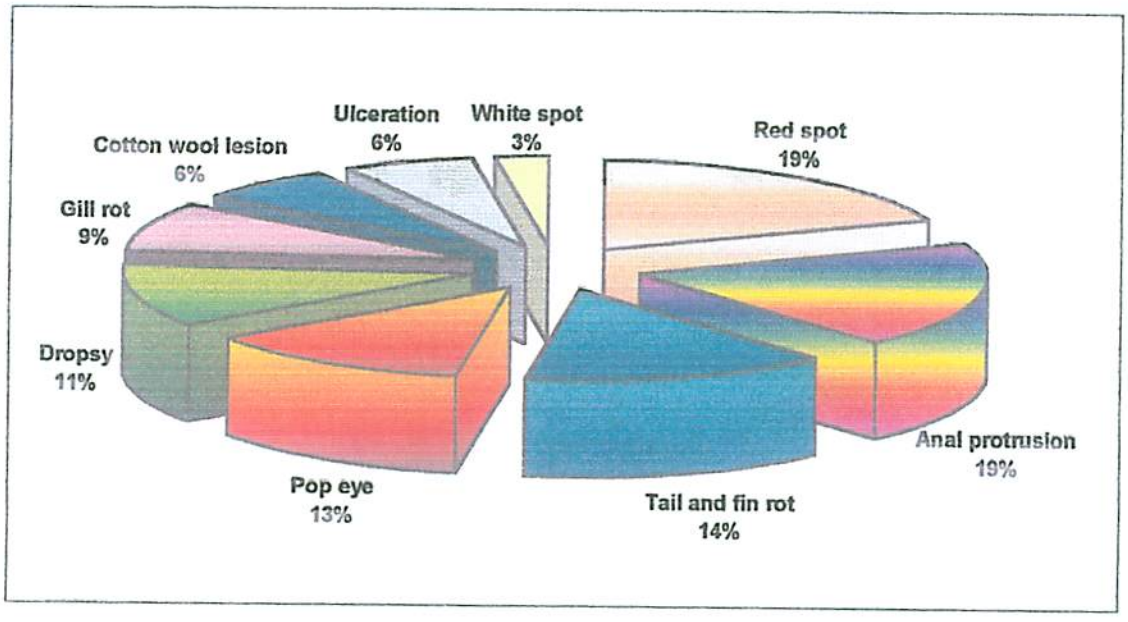


Fig. 2. Types of Diseases of Thai pangas in Mymensingh district

Response of farmers to disease problems

Most of the farmers turned to other farmers for advice when disease occurred in their ponds and applied range treatments. About 82% farmers treat their fish after occurrence of fish diseases in their ponds. A diverse number of treatments were reported, many in multiple combinations. Liming was the most common treatment followed by application of salt, potassium permanganate, antibiotics, pesticides and insecticides. It was found that highest number of farmers (39%) used lime and salt followed by lime, potash and vitamins (21%), lime, salt and potash (10%), and lime and potash (8%). Apart from the above treatments, farmers were found to some antibiotics like Renamycine (37%) and Tetracycline (11%).

Disease control cost

Average disease control cost was Tk4,285/ha/yr which included prevention cost (Tk.2,827/ha/yr) and treatment cost (Tk.1,458 ha/yr) (Table 6). The preventive measures include the cost of pond drying, addition of water, use of lime before disease outbreak, removal of water turbidity etc. While treatment cost include the cost of chemicals used for treating after occurrence of fish diseases.

Table 6. Disease control cost (Tk/ha/yr)

Pond Category (ha.)	Prevention cost (Tk)	Treatment cost (Tk)	Total (Tk)
<0.5	2342 ± 786	1220 ± 1106	3,562 ± 1892
0.5-1.0	2433 ± 548	1447 ± 847	3,880 ± 1395
> 1.0	3705 ± 753	1707 ± 709	5,412 ± 1462
Average	2827 ± 696	1458 ± 2662	4,285 ± 3358

Fish production

Fish production varied with different farmers category. Farmers were asked about their expected production when they had no disease problems and the actual production obtained due to disease problems at the end of the production cycle. Large category farmers had the highest average expected production (Tk.656,031/ha/cycle) followed by medium (Tk.620,649/ha/cycle), and small farmers (Tk.588,126/ha/cycle). Average actual production, that the farmers received after selling fish at the end of the production cycle was also highest in large category farmers (Tk. 633,636/ha/cycle) and the lowest was in small-scale farmers (Tk.565,022/ha/cycle) (Table 7).

Economic loss due to fish disease

The results of the study indicated that there are economic losses of Tk 21,500/ha/cycle to farmers from disease. These losses varied with different districts and with the size of farms. The economic loss was estimated by the differences between the expected production and actual production, here prevention and treatment cost of fish diseases was not calculated. The highest

average loss as high as Tk. 23,104/ha/cycle was found with small-scale farmers followed large (Tk.22,395) and medium scale farmers(Tk.18,999) (Table 7 and Fig. 3). The average percentage of loss of actual production was 3.6%.

Table 7. Expected and actual fish production (Tk/ha/cycle) and economic loss (Tk/ha/cycle) in the study area

Farm category (ha)	Expected production (Tk/ha/cycle)	Actual production (Tk/ha/cycle)	Economic loss (Tk/ha/cycle)	Percentage of actual production
<0.5	588,126	565,022 ± 126938	23,104 ± 10435	4.1
0.5-1.0	620.649	601.650 ± 116008	18.999 ± 5424	3.1
> 1.0	656,031	633,636 ± 101092	22,395 ± 7892	3.5
Average	621,602	600,102 ± 114679	21,500 ± 7917	3.6

± Indicates standard deviation

Problems in fish health management

Farmers faced several problems when they encounter particular disease in their ponds. These are lack of appropriate advice'suggestion from any GO or NGOs (49%), lack of knowledge on fish health and disease (35), unavailability of medicine (10%) and lack of training facility about fish disease treatment (6%).

Reporting of diseases by the farmers

There were severe under reporting of diseases by the farmers was found and only 9.6% farmer said that they go to the Upazila Fisheries Officer (UFO) and about 79% farmer do not usually inform the UFO or elsewhere for advice. Assistance of fish farmers on disease prevention and health management from non-govemmental organization (NGOs) and government extension officers ranked very low.

Problems of farmers other than disease

When asked their problems other than fish diseases, 56% said high input cost, 21% said lack of training on fish culture, 11% said lack of marketing facilities. Other problems included quality seed and financial problems.

Importance of fish disease to farmers

About 46% farmers mentioned disease as a major problem in fish culture while 22% considered it as a moderate problem, 21% farmers as minor and 11% farmers mentioned that they do not think disease a problem.

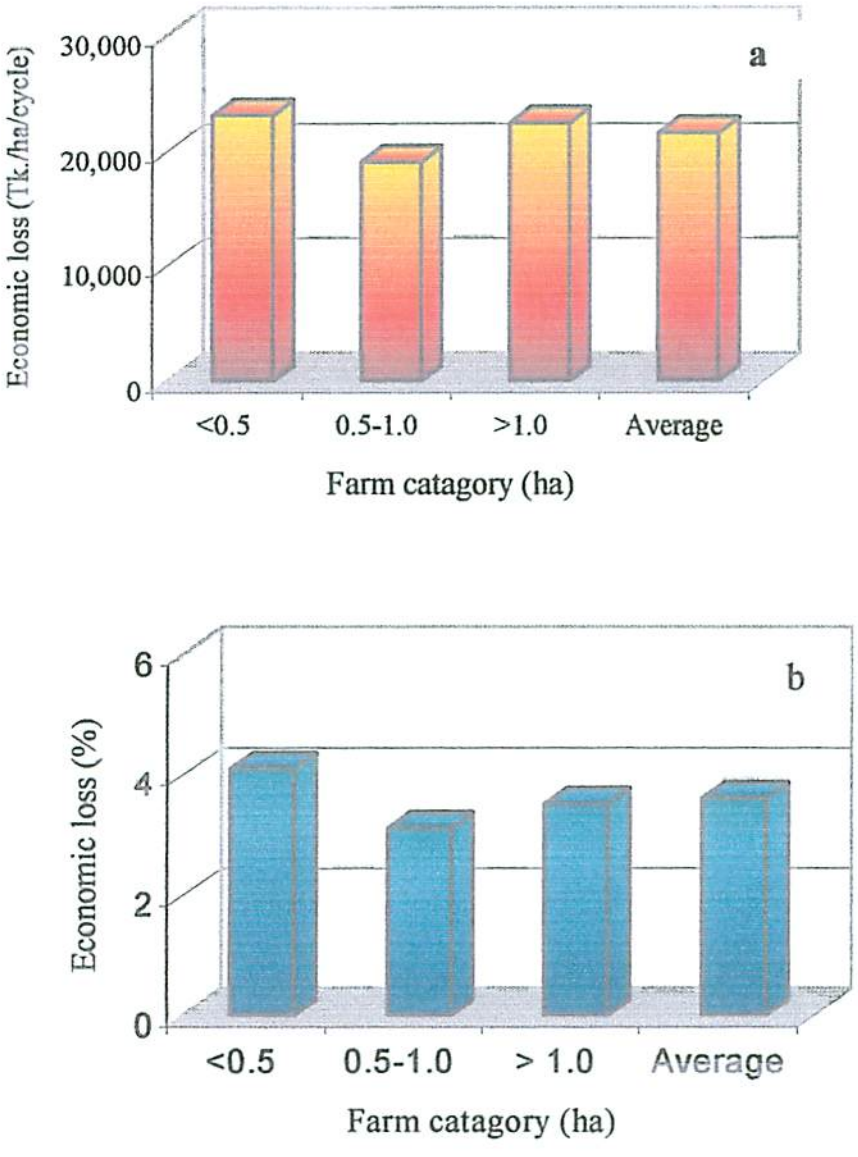


Fig. 3. Economic of fish farmers due to diseases of Thai pangas. (a) Economic loss (Taka/ha/cycle) (b) Economic loss percentage of actual production.

4.2 Laboratory study

4.2.1 General clinical observation

Clinical signs of fish varied with seasons and it was found more sever in winter months of the year. Fishes were almost healthy in June and July. Weak body and rough skin were found in August. Red and pop eye was evident in August (Fig. 4a). Hemorrhages or red spot, lesion in mouth and red ventral reddening was observed in September (Fig. 4b and 4c). Anal protrusion (Fig. 4d and 4e) was found to a major clinical problem in fish. Other signs included lumps over body surface (Fig. 4f), ulceration and eroded tail (Fig. 4g), cotton wool patches on skin and eroded tail (Fig. 4h) and dark coloration (Fig. 4i).

4.2.2 General histopathological observation

Major skin and muscle pathology included separation of epidermis from dermis (Fig.5), vacuoles, irregular arrangement and necorsis of myotome, vacuolation, pyknotic nuclei (Fig.7), necrosis of muscle tissue and presence of inflammatory cells (Fig. 6).

Major gill pathology of both of the farms included lamellar hypertrophy and hyperplasia (Fig.10), fusion of secondary gill lamellae, telangiectasis (Fig. 8 and 10), hemorrhage, necrosis (Fig. 9) and presence of parasites in the gill.

Liver pathology of fishes included hepatic degeneration and necrosis (Fig.11 and 14), vacuolation (Fig.12 and 14), presence of pyknotic nuclei (Fig.13), presence of melano-macrophage center (mmc), brown pigmentation, haemorrhages, ruptured blood vessel and granuloma formation.

Major common renal pathology included haemopoietic necrosis (Fig.15), vacuolation, hemorrhage in haemopoietic tissue, presence of mmc (Fig.16) and parasitic cysts and tubular granuloma (Fig 15).

4.2.3 Bacteriological observation

Four bacterial isolates were recovered from kidney (PK1, PK2 and PK3) and one isolate was recovered from the lesion (PL2) of disease affected Thai pangas. These isolates were identified as *Aeromonas hydroiphila* based on the primary conventional methods, following the Aerokey-II system of schematic diagram described by Carnahan *et. al.*, (1991) and API 20E microbiological kits. The reaction obtained from API 20E kit is shown in Fig 17. The characteristics of the recovered isolates is summarized in Table 8.

Clinical signs associated with Aeromonad infection

Harmorrhage over the body especially near mouth and caudal region was evident in the fishes associated with Aeromonad infection (Fig.19a). Internally, all the organs were swollen and enlarged especially, kidney, liver and spleen (Fig. 19b).

Histopathology associated with Aeromonad infection

Histopathologically, kidney and lever was found more affected due to Aeromonad infection. Renal tubules were totally lost and necrotic haemapoietic tissues could be seen (Fig.20a). Haemorrhages and vacuoles were also evident (Fig. 20c). Interestingly, colonies of bacteria was found in kidney of infected fish (Fig. 20a and 20b).

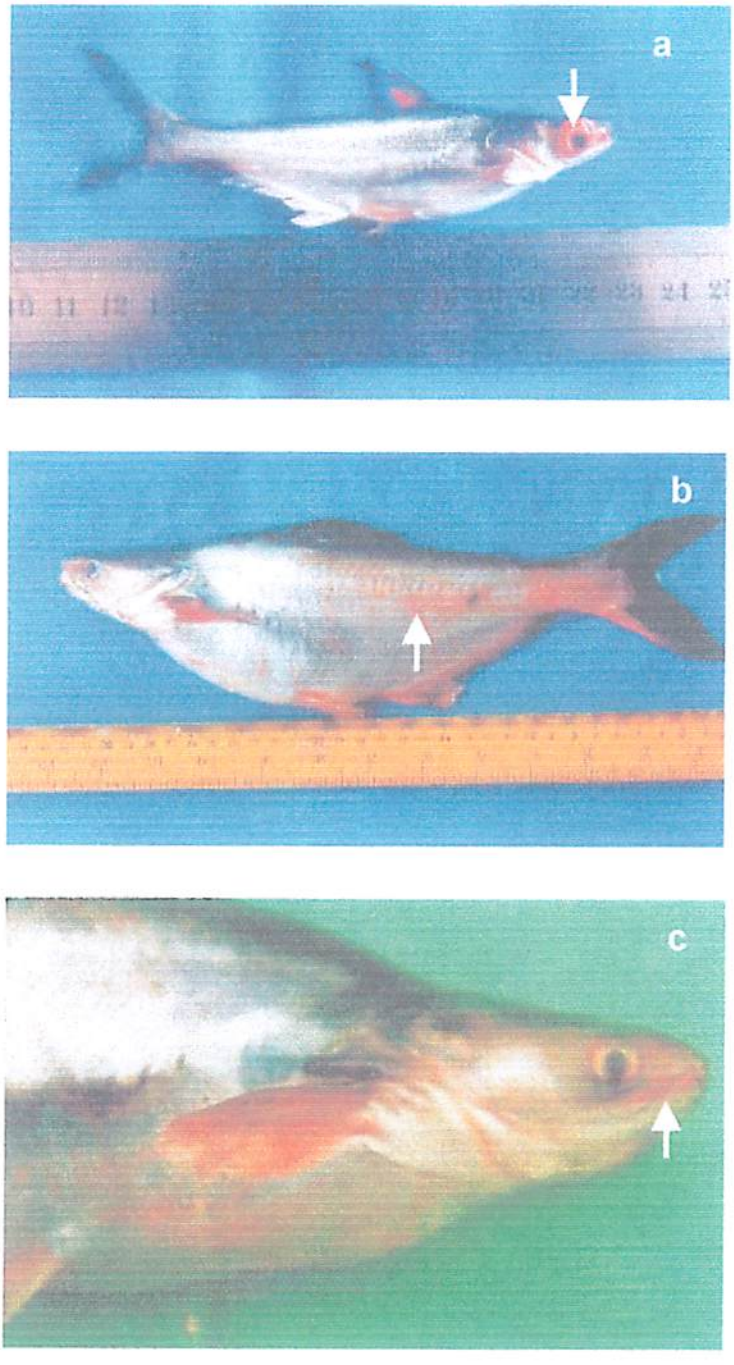


Fig. 4. Clinical signs of *Pangasius sutchi*. (a) Red and pop eye (b) Red spot or hemorrhage over body surface (c) Red mouth and body.

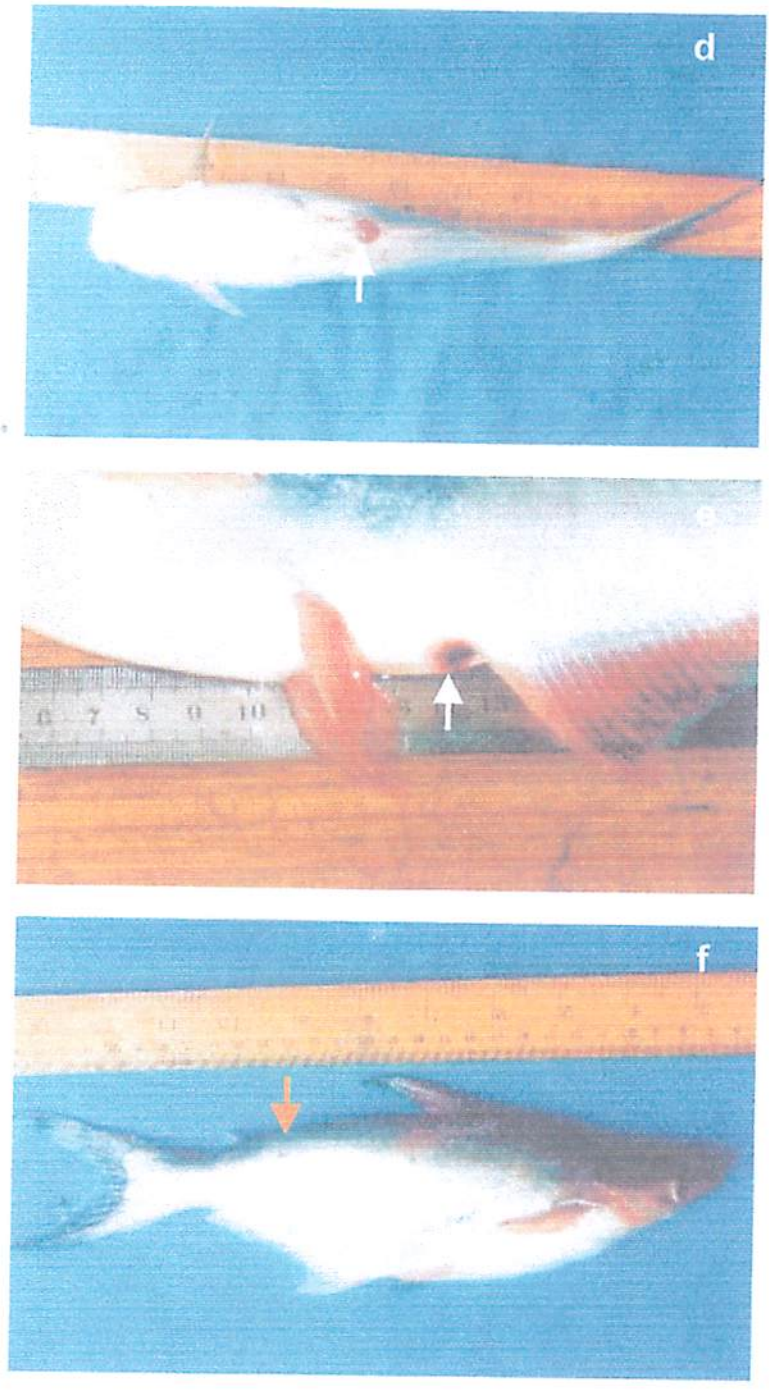


Fig. 4. Clinical signs of *Pangasius sutchi*. (d & e) Anal protrusion (f) Lumps over body surface and some white spot.

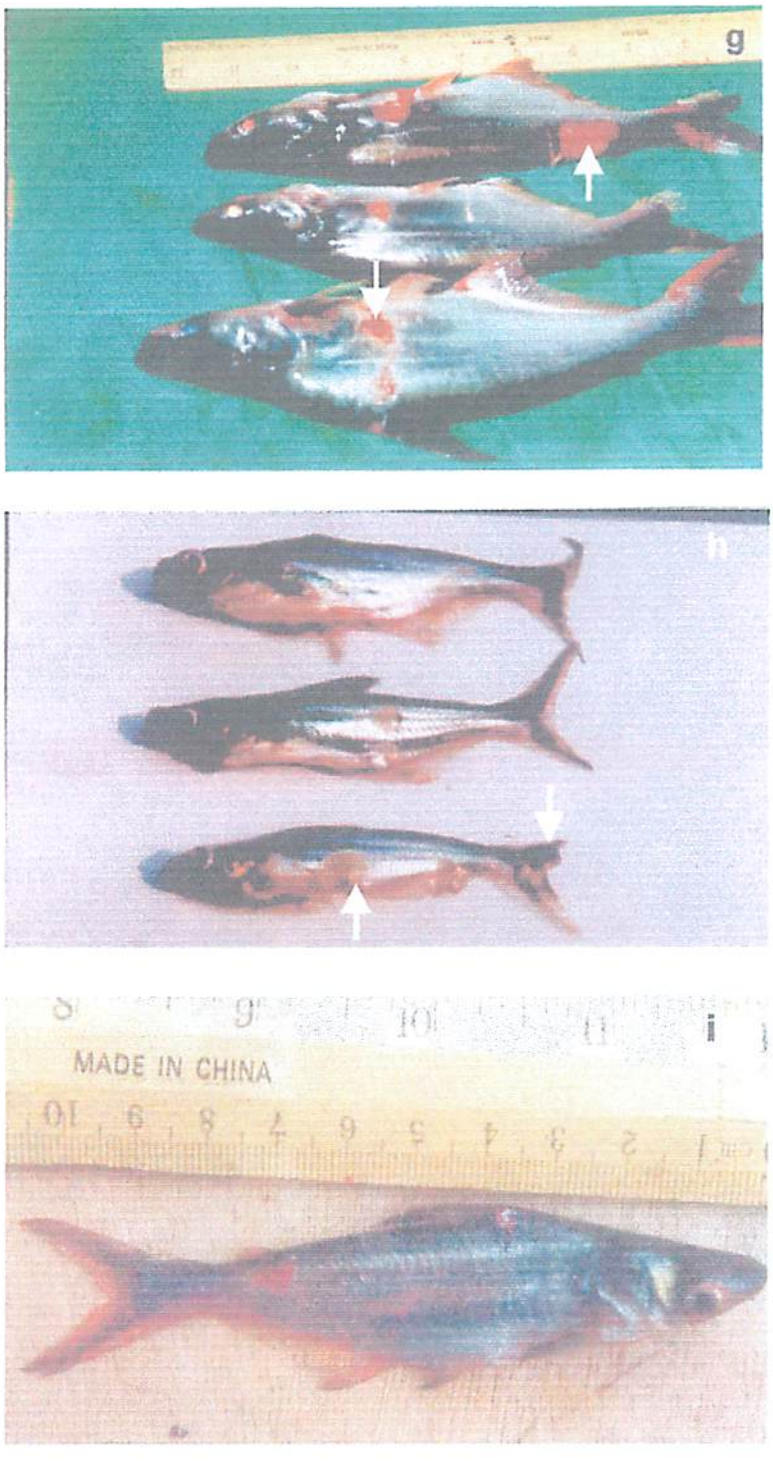


Fig. 4. Clinical signs of *Pangasius sutchi*. (g) Ulceration and fin rot of fish from farmers pond (h) Cotton wool type lesion and fin rot young fish (i) Dark coloration and ulceration.

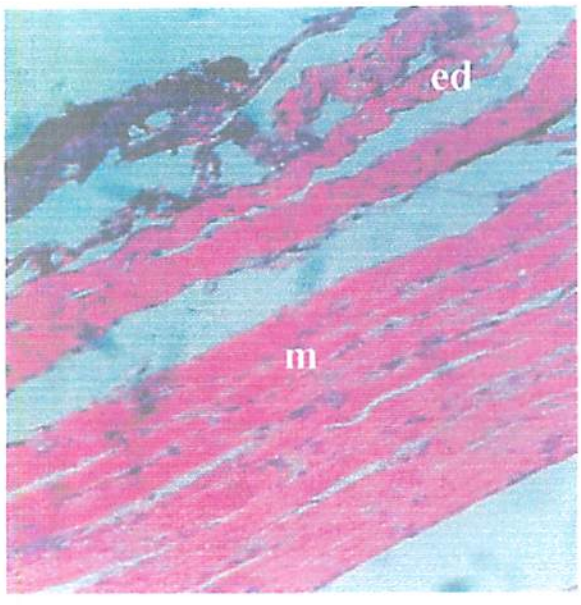


Fig. 5. Section of skin and muscle of *Pangasius sutchi* sampled farmer's pond in September showing epidermis (ed) separated from dermis and myotome (m) regularly arranged. (H & E × 420).

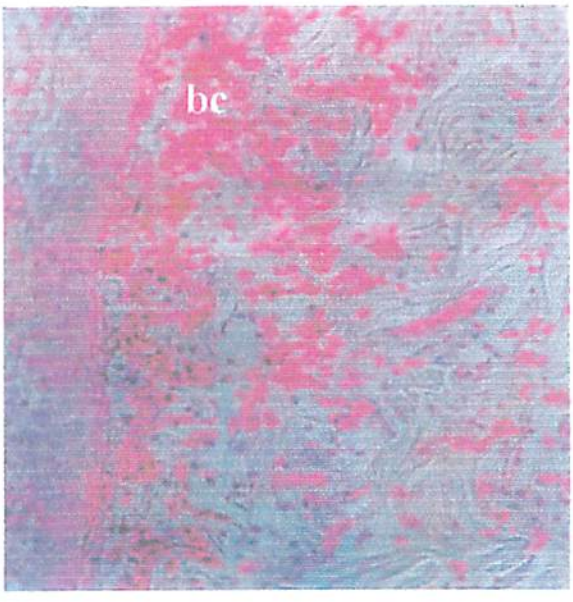


Fig. 6. Section of skin and muscle of *Pangasius sutchi* obtained from farmer's pond in October showing many blood cell (bc) in the dermis. (H & E × 420).

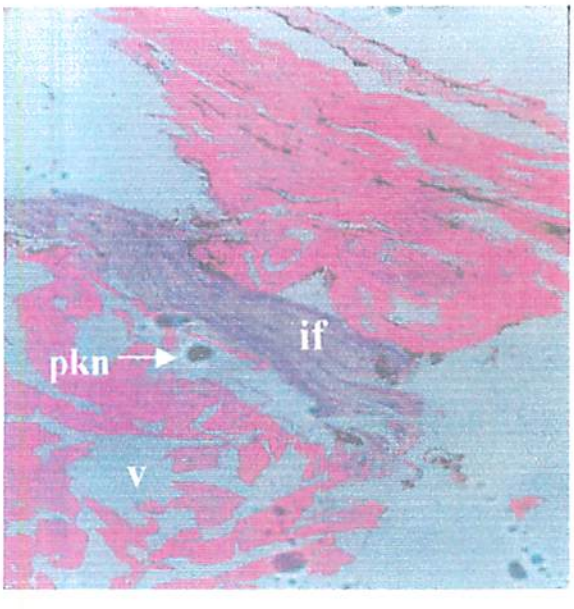


Fig. 7. Section of skin and muscle of *Pangasius sutchi* sampled from farmer's pond in November showing pyknotic nuclei (pkn), vacuolation (v) and infarcton (if). (H & E × 420).

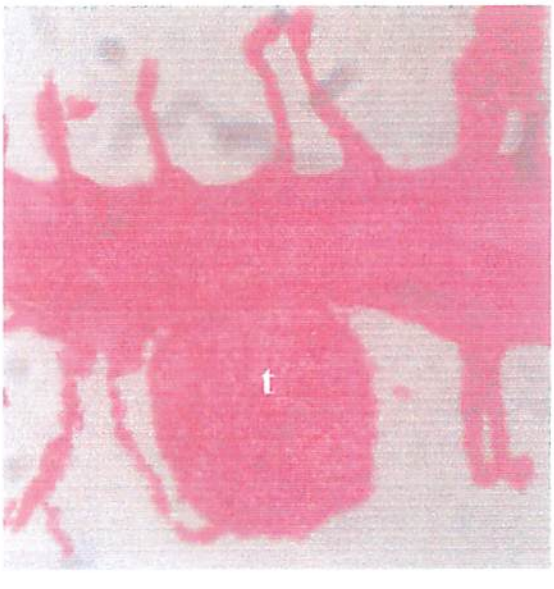


Fig. 8. Photomicrograph of gill of *Pangasius sutchi* obtained from farmer's farm in December showing telangiectasis (t) in the secondary gill lamellae. (H & E x 420).

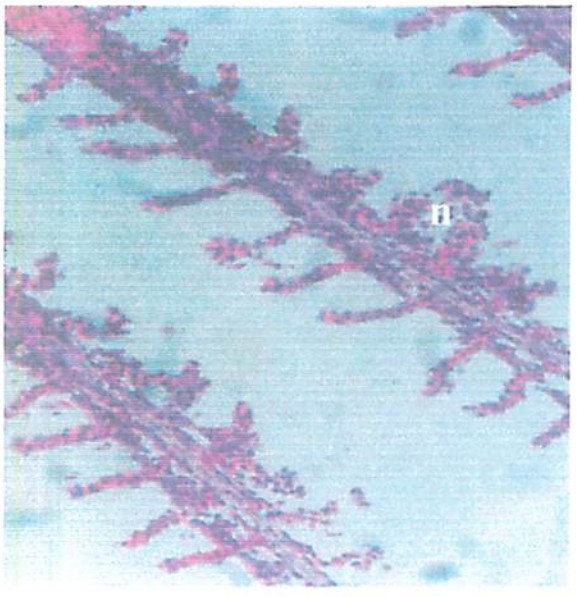


Fig. 9. Section of gill of *Pangasius sutchi* sampled from farmers pond in August showing lamellar necrosis (n) and blood cells (bc) in some places and secondary gill lamellae were missing partly (H & E × 420).

Fig. 10. Section of gill of *Pangasius sutchi* obtained from farmers pond in September showing telangiectasis (t) in secondary gill lamellae with hyperplasia (hyp) and hypertrophy (hyt) (H & E × 125).

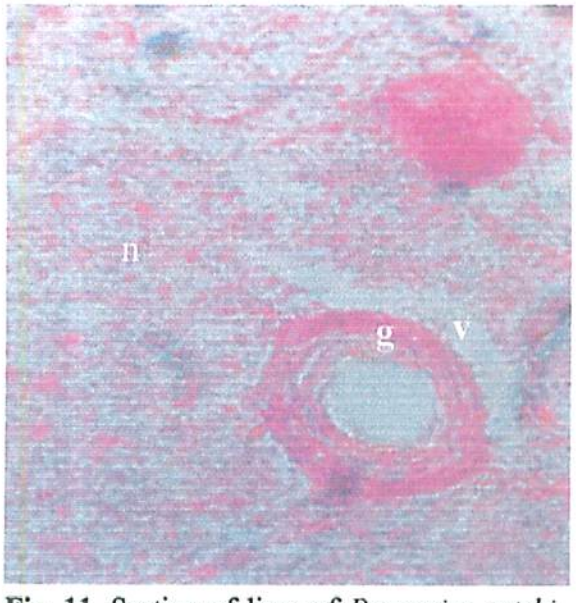


Fig. 11. Section of liver of *Pangasius sutchi* obtained from farmers farm in January showing necrotic hepatocyte, granuloma (g) and marked vacuolation (v). (H & E × 125).

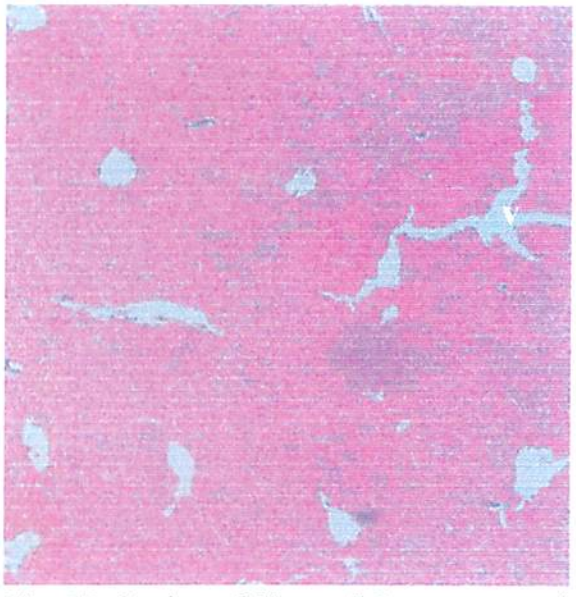


Fig. 12. Section of Liver of *Pangasius sutchi* obtained from the commercial farm in June showing vacuoles (v). (H & E × 125).

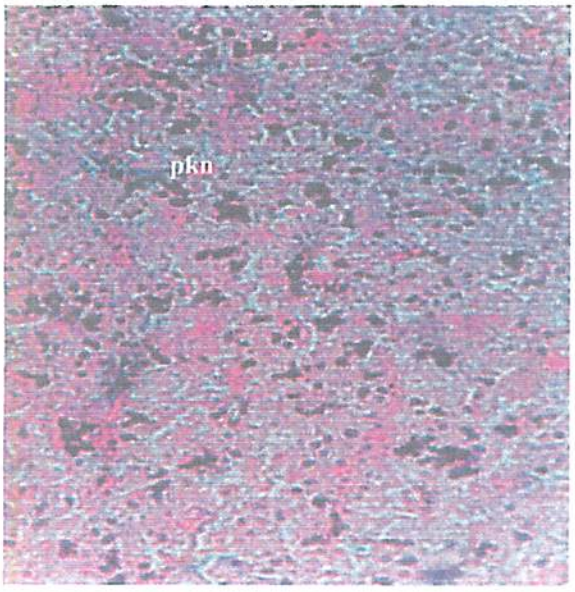


Fig. 13. Section of Liver of *Pangasius sutchi* obtained from the commercial farm in July showing necrotic hepatocyte, having pyknotic nuclei (pkn) (H & E ×420).

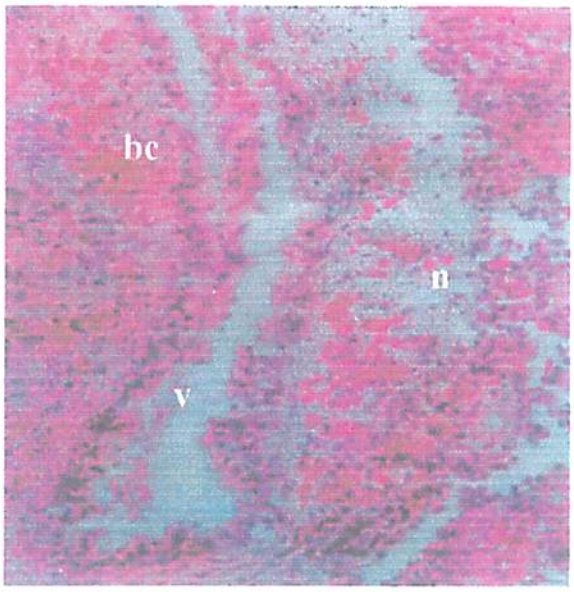


Fig. 14. Section of Liver of *Pangasius sutchi* obtained from commercial farm in October showing necrotic (n) hepatocyte, blood cell (bc) and vacuoles (v). (H & E ×420).

Fig. 15. Section of kidney of *Pangasius sutchi* obtained from farmers farm in September showing necrotic (n) haemopietic tissue having vacuolation (v), blood cell (bc) and tubular granuloma (tg). (H & E ×125).

Fig. 16. Section of kidney of *Pangasius sutchi* obtained from farmers farm in November showing ruptured kidney tubules (kt), vacuoles (v) and melanomacrophage centre (mmc). (H & E ×420).

Table 8. Characteristics of *Aeromonas hydrophila* determined by conventional methods, Aerokey-II and API 20E system

Characteristics	*Aeromonas hydrophila*
Gram reaction	-
Rods in singles and pairs	+
Motility	+
Oxidase	+
O/129 sensitivity	-
O/F	+
Esculin hydrolysis	+
TSI	+
Acid from arabinose	+
Resistance to cephalothin	+
Growth in TSB at 37^0C	+
ONPG	+
ADH	+
LDC	+
ODC	-
CIT	+
H_2S	+
URE	-
TDA	+
IND	+
VP	+
GEL	+
GLU	+
MAN	+
INO	+
SOR	-
RHA	-
SAC	+
MEL	-
AMY	+
ARA	+

Antibiotic sensitivity

The Aeromonads isolates were found to vary in their sensitivity to the four antibacterial agents tested (Table 9 and Fig.18). All the four isolates were sensitive to Nitrofurantoin, Cortimoxazole, and Tetracycline and resistant to Amoxyciline. However, Cortimoxazole was found less sensitive than to Nitrofurantoin and Tetracycline.

Table 9. Sensitivity of *Aeromonas hydrophila* isolates to various antibiotics.

Isolates	N	C	T	A
PK1	++	+	++	R
PK2	++	+	++	R
PK3	++	+	++	R
PI2	++	+	++	R

+: Sensitive, R: Resistance; N: Nitrofurantoin; C: Cortimoxazole; T: Tetracycline;A: Amoxyciline

Pathogenicity test

The percent cumulative mortalily (PCM) obtained upon termination of the trial at 14 days post-challenge were 50%, 60%, 80% and 80% for strains PK1, PK2, PK3 and PL2, respectively (Fig. 21). No deaths occurred in the control group over the course of the trial. Based on this trial, isolate PK3 and PL2 could be considered as highly pathogenic.

4.2.5 Effects of herbal products

At the start of the experiment the fish were weak with eroded fins, lesioned skin, injured mouth, haemorrhaged and red spotted body. Pathologically, the investigated organs had necrosis, pyknosis and parasites in gills and secondary lamellae were lost (Figs.26, 28 and 30). Recorded mortality were 25%, 38%, 38% and 75% in T1, T2, T3 and T4 respectively. Water temperature varied from 23 to 27°C in the morning and 25 to 29°C in the afternoon in all the treatments. Water pH ranged between 6.5 to 8.0 and DO from 5.0 to 6.5 ppm at all the treatments. An increased healing of the affected organs were recorded in T1 followed by T3 and T3 whereas, it was almost negligible in the control treatment (Figs. 27,29,31).

Pathologically, all the organs were healed to about normal structure in the treatment provided with neem (Fig. 27). In garlic and turmeric treated group (T1 and T2) there were some space in muscle and secondary gill lamellae were yet to be developed in normal for at the termination of the experiment (Fig. 30 and 31). Clinically, almost normal appearance was seen with T1 where as in T2 and T3 there were still few red spots over the body at the end of the experiment (Figs. 23, 24 and 25). However, in control treatment, healing of physical injury were very slow and as result 75% of fish were died at the end of the experiment. Thus from survival, clinical and pathological point of view, neem leaf seems to be better herbal product over garlic and turmeric in the external and internal healing of affected fish.

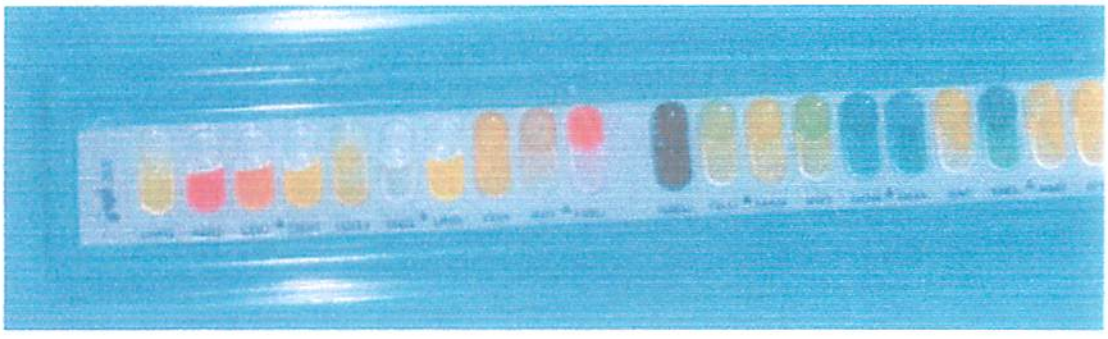


Fig 17. Result of APE 20E microbiological test with one of the Aeromonad isolates obtained from Thai pangas

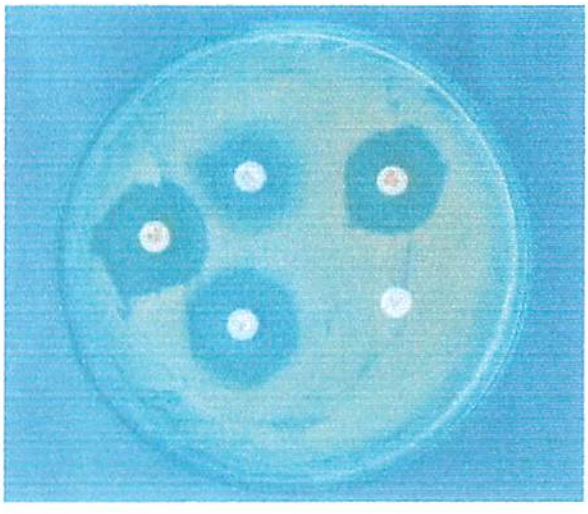


Fig. 18. Antibiotic sensitivity of Aeromonad isolates obtained from Thai pangas

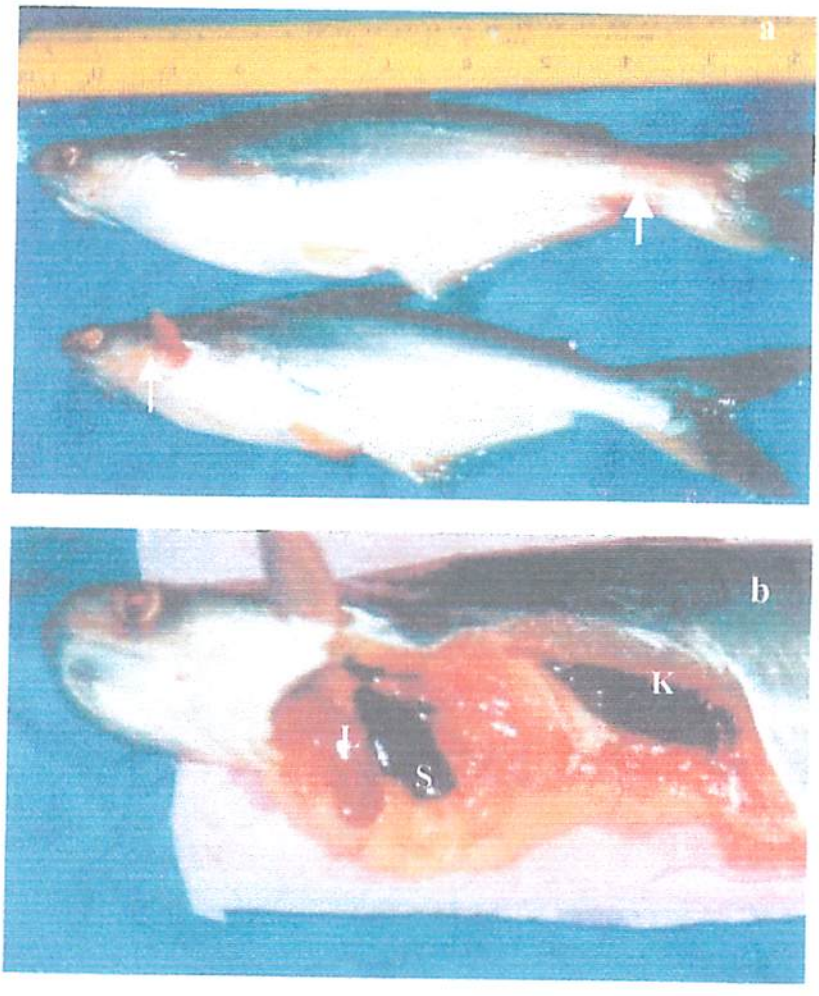


Fig. 19. Thai pangas infected with *Aeromonas hydrophila*. (a) Hemorrhages in the caudal and mouth region. (b) Enlarged internal organs: kidney (K), spleen (S) and liver (L)

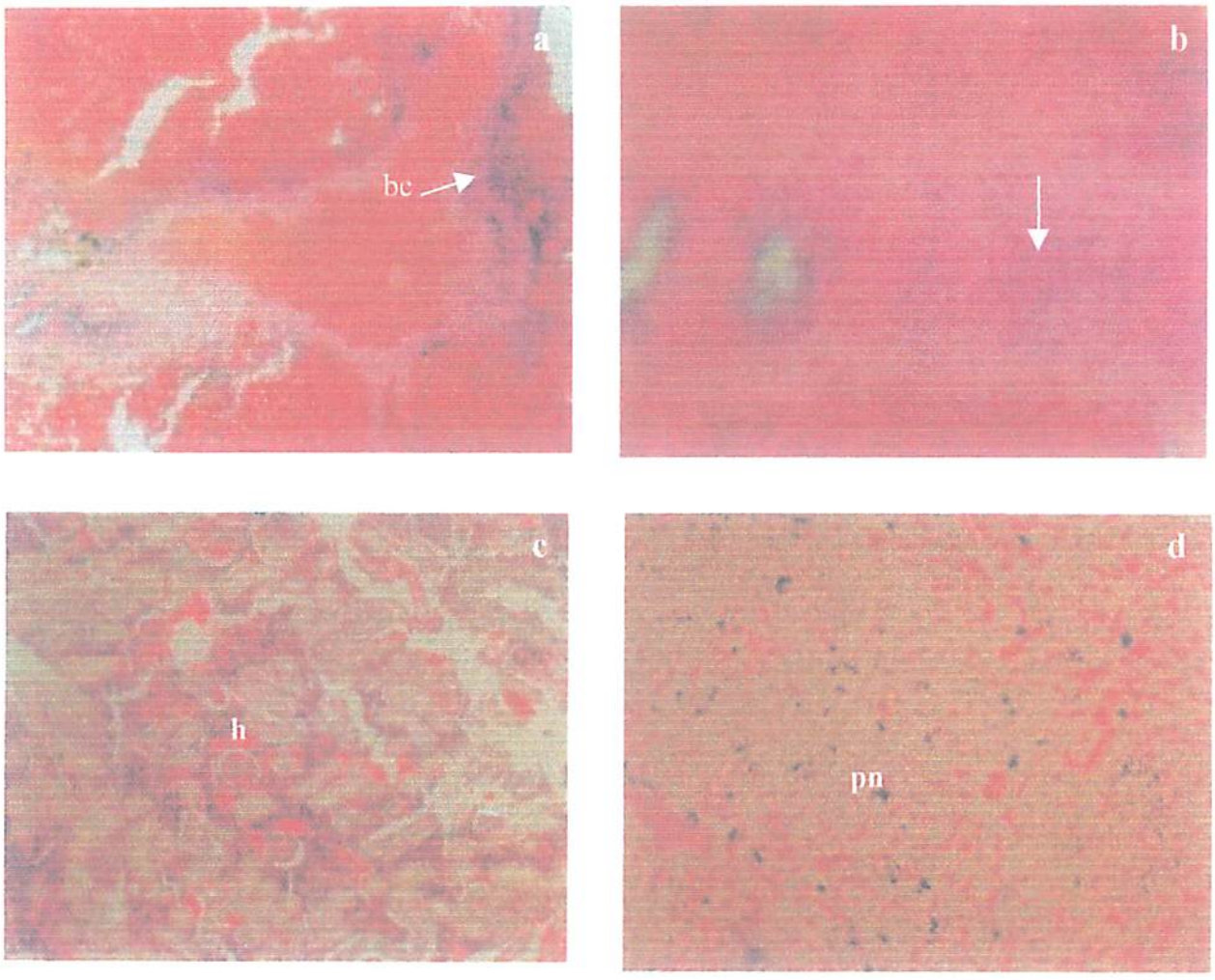


Fig . 20. Histopathology of Thai pangas associated with the *Aeromonas hydrphila* infection. (a) Massive necrosis in haemapoietic tissues and renal tubules with bacterial colony (bc) (H&E x125. (b) Kidney section showing bacterial cells (H&E x1420). (c) Kidney section with ruptured kidney tubules and haemorrgahes (h) (H&E x125). (d) Liver section with massive haemapoietic necrosis and pyknotic nuclei (pn) (H&E x125.

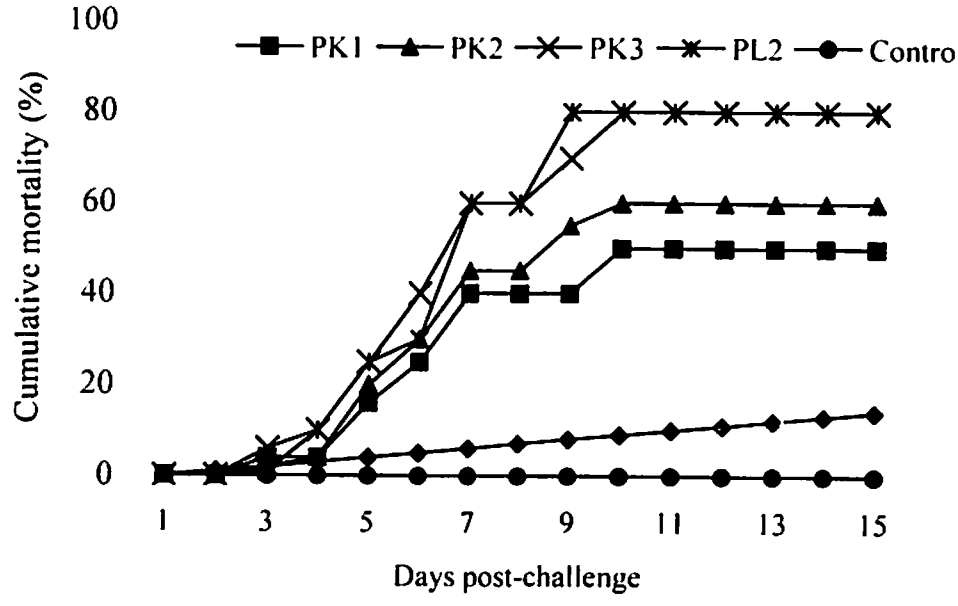


Fig. 21.Cumulative mortalities (%) of Thai pangas following intramuscular injection with different isolates of *Aeromonas hydrophila* at 1.2 x10^7 cfu $fish^{-1}$ (n = 20 fish).

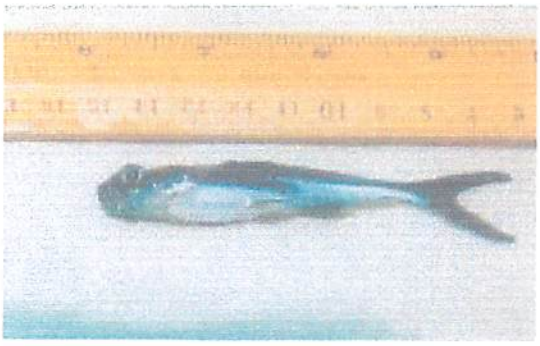


Fig. 22. Weak and disease affected fish at the beginning of the experiment.

Fig. 23. Normal fish in the group treated with neem leaf at the end of the experiment.

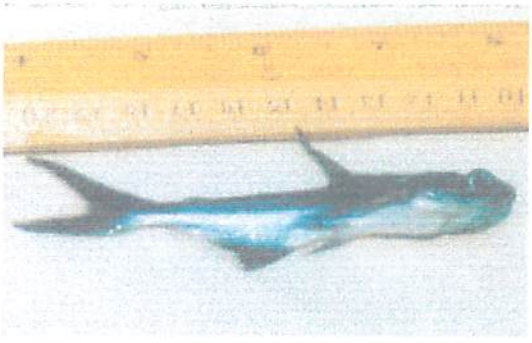


Fig. 24. Almost normal fish in the Treatment 2 at the end of the experiment

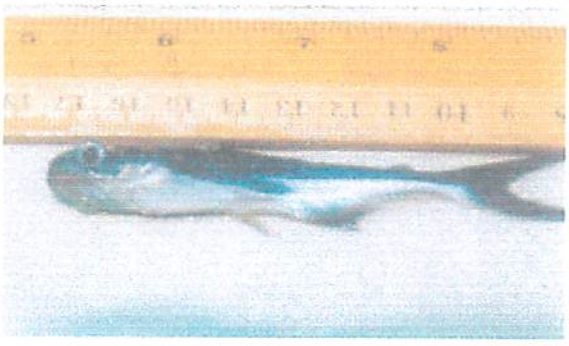


Fig. 25. There are few spots on head and ventral side in the Treatment 3 at the end of the experiment

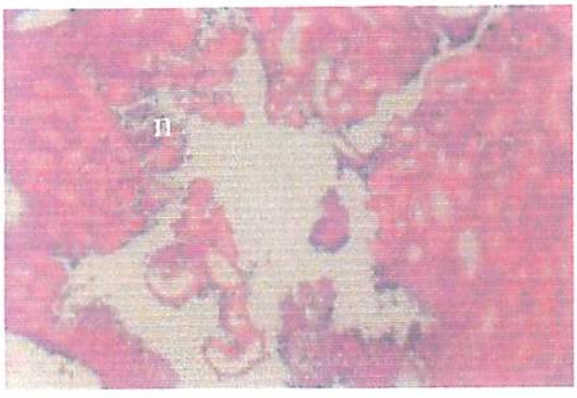


Fig. 26. Section of kidney showing necrosis (n), pyknosis and ruptured kidney tubule at the beginning of the experiment. H&E X 120

Fig. 27. Section of normal structure of kidney of the fish of neem treated group at the end of the experiment. H&E X 120

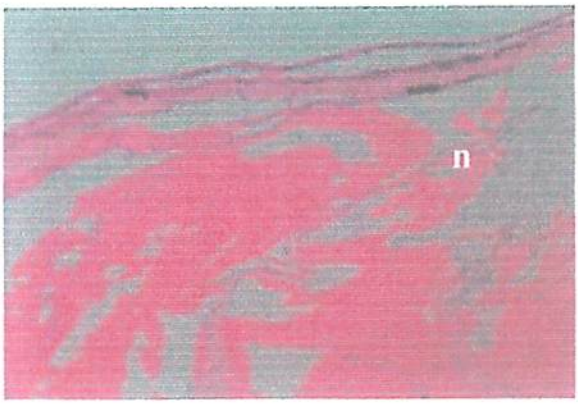


Fig. 28. Section of skin and muscle showing loss of eppidermis, necrosis (n) and empty space in muscle in fish at the beginning of the experiment. H & E x 120

Fig 29. Section of skin and muscle showing almost recovered skin and muscle in the garlic treated group at the end of the experiment. H&E x120

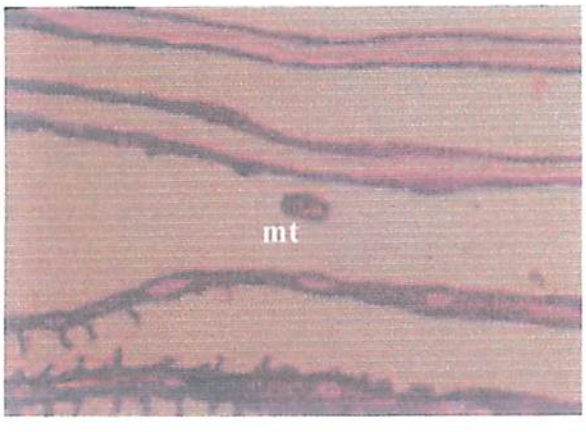


Fig. 30. Section of gill showing loss of secondary gill lamellae and monogenetic trematode (mt) parasite from fish at the start of experiment H&E x120

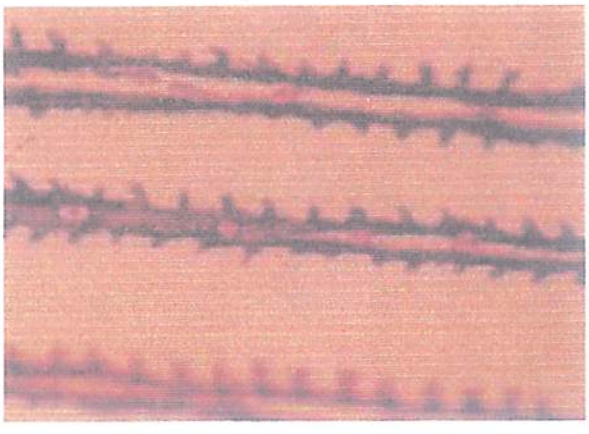


Fig. 31. Secondary gill lamellae almost developed in fish of turmeric treated group at the end of the experiment. H&E x120

5. Summary of Major Findings

Field survey

- Prevalence of diseases of Thai pangas had negative impacts in fish production
- Economic losses due to fish diseases could be as high as Tk 23,104 /ha/cycle.
- The rate of prevalence varied among farm categories and it was highest in samll-sacle farmers pond.
- Small-scale farmers suffered from highest average loss than the bigger size farms
- The most common diseases identified were the hemorrhage or red spot, anal protrusion, pop eye, tail and fin rot, ulceration and white spot.
- Average disease control cost was Tk.4,285 /ha/cycle
- The diseases were more prevalent in winter season.
- There were severe under reporting of diseases by the farmers.

Problems in fish health management

- Lack of assistance from GO and NGOs
- Lack of technical knowledge of fish farmers on fish health management
- Unavailability of appropriates therapeutic
- Lack of knowledge of application of therapeutic
- Indiscriminate use of chemicals
- Pressure on farmer from pharmaceuticals company and pesticide sellers
- Low quality chemicals (from India)
- High stocking density
- Financial problem

Laboratory study

- The study provided major clinical signs of Thai pangas
- The study highlighted major histopathology of different organs of diseased Thai pangas
- *Aeromonas hydrophila* was found to be the major causative agent of Pangasius disease.
- The bacteria could be isolated from kidney and lesion of fish.
- Histopathology of Aeromonad infection included necrosis of kidney tubules and haempoietic tissues.
- Nitrofurantoin and Tetracycline were found very effective against *A. hydrophila.*
- Neem leaf extract could have some therapeutic value against Thai pangas disease as was found by a laboratory trial.

6. Recommendations

- The farmers and the extension agents should be trained up on simple diagnostic procedure and effective therapy
- Awareness creation among the farmers on fish heaith management
- There should be legislation on the safe use of the chemotherapeutic agents for prevention and control.
- Establishment of mobile diagnostic centers and support service
- More research needed on the characterization of pathogens
- Herbal therapy could be a low cost and environmental friendly alternative of chemical treatment but more research is needed in this particular area.
- To minimize stress, stocking density and feeding should be carefully maintained
- Avoid stocking of stressed and unhealthy fry
- Disaffection of fingerling before stocking
- Disaffection of nets, container and other equipment used in the pond.
- Periodic toxic gas removal by manual dredge on the pond bottom.
- Maintain optimum level of water in the pond
- Controlling of fish pathogen carrying birds, aquatic animal and insect
- Regular monitoring of fish health by sampling of fish stock.

Acknowledgements

The study carried out with financial assistance from WorldFish Center. The author is grateful to Drs Johannes Janssen, Project Leader and Dr. Md. Ferdous Alam, Research Coordinator of DSAP, WorldFish Center for their kind support. The author is also grateful to all the farmers who participated in the survey and PRA activities.

References

Banu, G. R. 1996. Studies on the bacteria *Aeromonas* spp. in farmed fish and water in Mymensingh region. M. S. Thesis. Dept. of Fisheries Biology and Limnology, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. 95 p.

BFRI. 1998. Studies on the stress factor in relation to the disease of fresh water fishes. Research progress Report. Bangladesh Fisheries Research Institute. Fresh Water Station, Mymensingh, Bangladesh.

Carnahan, A. M., S. Behram and S. Joseph. 1991. Aerokey II: A flexible key for identifying clinical *Aeromonas* species. J. of Clinical Microbiology, 29: 2843-2849.

Chowdhury, M. B. R. 1997. Bacterial involvement in fish disease in Bangladesh. Presented at the International Symposium on Disease in Marine Aquaculture, October 3-6, 1997. Hiroshima, Japan. Abstract: III. 2: 24.

DoF, 2003. Fish Forth Night Sangkalan-2003. Department of Fisheries, Mymensingh. pp100.

Faruk, M.A.R., Islam, J and Sarkar, M.R. 2004. Economic impact of fish diseases on rural freshwater aquaculture of Bangladesh. Bangladesh J. Fish., Special Issue, 27:27

Plumb, J. A. 1997. Trends in Freshwater Fish Disease Research. In: Diseases in Asian Aquaculture III, Flegel, T. W. and MacRae, I. H. (eds.), Fish Health Section, Asian Fisheries Society, Manila. pp. 33-44.

Roberts, T.R. and C. Vidthayanon, 1991 Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. Proc. Acad. Nat. Sci. Philad. 143:97-144.

Tonguthai, K., S. Chinabut, T. Somsiri, P. Chanratchakool, S. Kanchanakhan. 1999. Diagnostic Procedures for Finfish Diseases. Aquatic Animal Health Research Institute, Bangkok, Thailand.

Appendix

Pangas Disease and Health Management Project

WorldFish Center and Department of Aquaculture, BAU

Questionnaire number: **Name of the Interviewer:** **Date:**

General Information

1. Farmer's name..... Age (years): .....

District: .... Upazilla: ..... Village:.....

Education: Under SSC..... .....Up to SSC..... .....HSC .....Graduate.....Other.....

Family information:

Name of the Member	Relationship with pond owner	Age	Sex	Education	Occupation/Souece of income	
					Main	Secondary

2. Description of Land (decimal)

- ❑ Homestead land: .....
- ❑ Crop land.....
- ❑ Ponds used for pangas culture.....
- ❑ Leased Land.....

3. Description of pond (s)

Type of Ownership	No of ponds	Area (Dec)	Depth (m)	Source of water	Perennial/seasonal	Have water inlet/outlet
Owned						
Leased in						
Other						
Total						

4. Catagory of Farmer

- ❑ Small (<0.5ha)
- ❑ Medium (0.5-1.0ha)
- ❑ Large (>1.0ha)

5. What type of farming are you doing here?

- ❑ Monoculture
- ❑ Other.....

6. Main reason of fish culture

- ❑ Income
- ❑ Food
- ❑ Hobby
- ❑ Status
- ❑ Other

7. Duration of fish culture:

8. Experience of fish production:

Pond Preparation Information

9. Did you dry your pond(s) during the last dry season? Y \ N

10. *(If no)* When did you last dry your ponds?

11. *(If yes)* For how many days did you dry the ponds for?

12. *(If the ponds were not dried)* Did you remove the undesirable species? Y \ N

13. *(If they were removed)* How did you remove the undesirable species? By netting (1), by poisoning (2)

14. *(If by poisoning)* Did you use Phostoxin (1), Rotenon (2), other .....(3)

15. What was the rate of application'? ..... kg/decimal

16. Have you seen wild fish in your ponds'? Y \ N \ DON'T KNOW

17. What species of wild fish have you seen in the ponds?

18. Pond preperation and the cost

Item	Rate	Amount (kg)	Price/kg	Total price (Tk)
Pond drying				
Lime				
Fertilizer (organic/inorganic)				
Phostoxin				
Rotenon				
Other				

Stocking and Pond Management

19. Name of fishes cultured in the ponds and stocking

Species	Source of fry (Hatchery/Wild/Both)	Size	Month of stocking	Duration of culture	Amount (kg) or No.	Price/Kg or/(100 fish)	Cost (Taka)
Pangas							

Total						

20. For the present stock, which month did you add water to your dried ponds? .....

21. Did you treat your fish before releasing in your ponds? Y\N

22.(*If yes*), what chemicals and doses do you use?

- ❑ Sodium chloride .....
- ❑ Potassium permanganate .....
- ❑ Copper sulphate .....
- ❑ Methylene blue.....

23. Do you fertilize the ponds during growing of fish? Y \ N

24. Do you lime the ponds during growing of fish? Y N

25. Do you feed your fish? Y N

26. Feeding:

Feed	Always	Usually	Sometimes	Never
Commercial feed pellets				
Supplements used (rice rean, oil cake, fish meal etc)				

27. Feed, fertilization, liming and their cost during grow out:

Subject	Rate	Amount	Interval	Price/Kg	Total cost (Tk)	Sources	
						Home made	Purchased
Copmmercial feed pillets							
Supplementary feed							
Organic fertilizer							
Inorganic fertilizer							
Liming							
Total							

28. Is there any aquatic weed floating or submerged? Y N

29. Do you remove them? Y \ N

30. What is the water colour?

- ❑ Greenish (phytoplankton)
- ❑ Reddish (zooplankton)
- ❑ Blackish (high organic debris)
- ❑ Transparent

Fish Health and Diseases

31. Do you check health of your fish by netting? Y\N.

32. If yes. how often?.....

33. Do you know why do disease occur in fish?.....

34. Whether your fish was infected by diseases or not? Y\N

35. *(If yes)* Give details about the diseases of your fish

Name of Fish	Age of affected fish	Kind of diseases with clinical signs	% of diseased fish	Pattern of death (%)	Frequency of disease occurrence	Disease occurring season
Pangas						
Total average						

36. Which of the following general signs you think you have noticed with your fish before it die?

☐ Changes in body surface:
- Loss brightness of body colour
- Excessive mucus production
- Lesion and ulcer on the body
- White spot on the body
- Ventral reddening and haemorrhage.
- Fin/appendage loss
- Fin rot
- Extended belly
- Cyst on the body
- Ectoparasite on the body

☐ Changes in gills
- Pale gill
- Black gill
- Gill rot
- Haemorrhaged gill.
- Cystic gill

☐ Behavior changes
- Rub body on hard objects
- Loss of balance during swimming
- Abnormal swimming behaves
- Jump over the surface of water

☐ Loss of appetite

☐ Loss of weight

☐ Asphyxia.

☐ Anemia

☐ Pop eye

37. Is there any price fall of fry during disease season? Y/N/Don't Know

38. Estimated number/weight of fish lost from present outbreak.....

39. Losses of fish from present outbreak e.g. 10-30%.....

40. What do you do with diseased fish ? eat.....throw away.....sale.....

41. Conditions 3-12 days before outbreak (e.g. temperature, rainfall).....

42. How was the pond water looks like before diseases outbreaks occur?.....

43. Have you ever had any unusually high mortality in your ponds?

44. *(If yes)* What year was that? .....

45. (If yes) What season was it? .....

46. *(If yes)* Roughly how many fish were affected? .....

47. Have you ever observed any parasite on the fish skin? Y \ N \ DON'T KNOW

48. *(If yes)* Could you identify the parasite? .....

49. *(If yes)* What year was this? .....

50. Do you confirm disease outbreak by a laboratory?.Y\N

51. *(If yes)* How do you carry fish to the laboratory? Live/Dead

52. Do you report to Upazilla Fisheries Office about any disease out break in you pond? Y\N

53. Is there any scope to pesticides/herbicides can come to your pond from agricultural activities?.....

54. Did you take preventive measures? Y\N.

55. *If yes*, what were those measures

☐ Pond drying
☐ Application of lime
☐ Weeding the pond
☐ Removing water turbidity
☐ Regular application of
☐ Addition of water
☐ Regular fish health checking

57. *If No*, why

☐ Lack of knowledge
☐ Unavailability of medicine
☐ Others, (please mention).....

58. Disease prevention and their cost:

Preventive measures used	Never	Usually	Always	Price (Tk)	Total cost (Tk)
Regular fish health checking					
Pond drying					
Application of lime					
Weeding the pond					
Removing water turbidity					
Regular application of					

Addition of water					
Other					

58. Disease treatment and their cost?

Name of Disease	Treatment measures used	Never	Usuall y	Always	Dose	Total amount	Price (Tk)	Total cost (Tk)
	Lime							
	Salt							
	Potassium per manganate							
	Formaline							
	Malachite Green							
	Dipterex							
	Copper sulphate							
	Vitamines							
	Antibiotics							
	Other							
					Total			

59. If you do not use any preventive/control measures, why?

☐ Lack of knowledge.
☐ Unavailability of medicine.
☐ Others (please mention).....

60. What problem do you face in control and treatment of fish diseases?

.....
.....
.....

61. Do you get any assistance from GO agencies for control and treatment of diseases? Y/N

61. *(If the answer is yes)* Types of assistance.....

62. Do you get any assistance from NGO agencies for control and treatment of diseases? Y/N

63. *(If the answer is yes)* Types of assistance.....

64. Fish Production and Market price:

Species	Estimated fish production (kg/cycle/acre)	Actual production due to disease (kg /cycle/acre)	Reduction of production due to disease kg /cycle/acre)	Price/kg of fish (Tk)	Total Price (Taka)	Financial Loss due to disease (Taka)
Pangas						
Total						

65. How do you categorize the disease problem in fish production?

- ☐ Not a problem (1)
- ☐ As a minor problem (2)
- ☐ As a moderate problem (3)
- ☐ As a major problem (4)
- ☐ DON'T KNOW (5)

66. Labour cost

Operation & activities	Man days	Wage rate (Tk)	Total labour cost (Tk)	Source(s)	
				Family	Hired
Excavation of pond					
Clearing & preparation of pond					
Lime application					
Feeding & Fertilization					
Pond watch					
Harvesting					
Marketing of fish					
Other					

67. Harvesting

Harvesting	Amount harvested (kg)	Price (Tk/Kg)	Sale		Consumption		Total Use
			Amount (kg)	Price (Tk)	Amount (kg)	Price (Tk)	
1st							
2nd							
3rd							
4th							

68. Income (Taka/year)

- ☐ Fish Production.....
- ☐ Field crop.....
- ☐ Poultry.....
- ☐ Livestock.....
- ☐ Small Business.....
- ☐ Wage labour.....
- ☐ Total Income.....

69. What is the contribution of aquaculture to your household income?.....

70. Expenditure (Taka/year):

- ☐ Agricultural Farming.....
- ☐ Food.....
- ☐ Aquaculture .....
- ☐ Clothing.....
- ☐ Housing.....
- ☐ Health care.....
- ☐ Education.....
- ☐ Total expendature.....

71. Subjective, Impression of Data Collector.

Environment of farm.	Good	1	2	3	4	5	Bad
Water quality.	Good	1	2	3	4	5	Bad

Thank you very much for your time

Signature of the data collector