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Prevalence of Fish Diseases in Sambalpur, Orissa, India

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Abstract

An assessment on prevalence of fish diseases in Sambalpur, Western Orissa, revealed the occurrence of six major diseases *viz.*, fin rot, saprolegniosis, gill rot, *Ichthyophthirius* (white spot), costiasis and argulosis in this region. The percentage of occurrence varied from 4% to 16% in different fish farms surveyed. The study of physicochemical parameters of stocking and rearing ponds at Bomlai and Chiplima (Sambalpur, Orissa) revealed variations between the ponds. The bacteriological analyses of sediments collected from different points indicate occurrence of hygiene indicator bacteria at lower level. There is an increasing trend in counts of different bacterial groups in both sediments and in fish of stocking ponds from February to March correlating with increase in temperatures. The difference in bacterial counts of mesophilic aerobes, coliforms, faecal streptococci and group D streptococci and staphylococci varied from <1 to 2 log cfu.g-1 of the sample. The antibiotic susceptibility of aeromonads isolated from diseased *Cyprinus carpio* varied among and between the isolates from kidney, liver and skin lesions. Among the 17 antibiotics tested, all the isolates were susceptible to erythromycin and nitrofurantoin and resistant to cefaclor and vancomycin. This study inquires for better management of fishponds to avoid problems in future.

Introduction

Many Asian countries have a long tradition of aquaculture, and over 80% of fish produced by aquaculture comes from Asia where the production was 31.07 million metric tons valued nearly US \$ 38.85 billion (FAO 2004). Nine of the top fourteen-aquaculture producers in the world are from Asia. Aquatic animal disease and environmental-related problems may cause annual losses of more than US \$ 3 billion annually to aquaculture production in Asian countries (Shankar & Mohan 2002).

The freshwater aquaculture in India is mainly the cultivation of Indian major carps (IMC). The advent of 1980s has seen expansion of commercial carp culture in various parts of the country. Besides carp, small-scale cultivation of catfish (*Clarias batrachus*) and murrells (*Channa punctata*) is also practiced, and the aquarium

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trade is also attempting to establish itself as an industry. The main aim of commercial aquaculture is to boost production by intensification; hence, increased stocking density, fertilisation, feeding and use of chemicals and antibiotics have become common (Khatri 2004). Fish in freshwater systems are susceptible to a number of bacterial, viral and parasitic diseases. Large-scale mortalities of fish due to bacterial diseases reported all over the country. Among these diseases of bacterial origin, motile aeromonads play an important role in freshwater systems. Mass mortalities in India in major carps *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* due to motile aeromonad infection are reported (Karunasagar et al. 1986). Among these fishes, catla is most susceptible followed by mrigal and rohu. Aquaculture ponds receive inputs of organic matter such as uneaten feed, fertiliser and faeces. To assess the sediment quality, the techniques that are simple, rapid and practical on-site such as redox potential, pH, the hydrogen sulphide activity potential (pH₂S) and soluble ammonium nitrogen are used (Hussenot & Martin 1995). Measures such as sediment removal and water exchange only transfer pollution problems from pond environments to surrounding environments (Boyd 1997).

Reports on the prevalence diseases in freshwater fish and on physicochemical and the bacteriological quality of the pond sediments and fish of Western Orissa are scant. Hence, this study is undertaken to assess prevalence of different fish diseases in Sambalpur, Western Orissa. The bacteriological and physicochemical quality of freshwater fish rearing and stocking ponds located in different areas of Sambalpur districts, Orissa, was assessed. The physicochemical quality parameters were assessed for stocking ponds in the months of February and March to find whether there are any changes during the transition period and their bearing on the bacteriological quality of the sediments and fish. The aeromonads isolated from the diseased fish were tested for antibiotic resistance against 17 antibiotics.

Materials and Methods

Studies on prevalence fish disease in Sambalpur

A total of 25 fish farms (of both commercial and state government) were selected for disease assessment. From each pond, fish were harvested from different areas (average of four) and fish with symptoms of disease were brought to the laboratory for further examination.

Selection of fishponds for physicochemical and bacteriological quality assessment

Extensive survey was carried out in different farms located in Sambalpur district for assessment of physicochemical and bacteriological quality parameters. Among which pond selection was made from Bomlai and Chiplima fish farms. The selection of the farms ensured that both are far from each other (90 km), and neither the physicochemical

parameters nor the bacteriological quality parameters have any bearing on each other in view of the distance as well the source of water.

Description of the ponds

The Bomlai has built in area of 97.10 acres, whereas Chiplima has 151.06 acres and out of which Bomlai has 28.11 acres water spread area with 46 tanks. Among these tanks, 29 are nursery tanks in 5.01 acres, 7 rearing ponds in 3.01 acres and 10 stocking ponds in 20 acres. The Chiplima farm holds 62.5 acres of water-spread area that includes 7.5 acres of canals. The total numbers of tanks in the farms are 44 and out of which 12 are breeder tanks and 32 numbers nursery tanks. Each tank covers an area of 1.25 acres. Both farms are active in production in all spears from the inception. The study revealed that for the last 10 years both farms are active in production of spawn, fry and fish. In commercial farms, the tank size varied from half acre to one acre and number of tanks ranged from two to six in each farm.

Physicochemical quality parameters of the fishponds

Physicochemical parameters were obtained from rearing and stocking ponds of both the areas of study, i.e. Bomlai and Chiplima: oxidation-reduction potential $(ORP-E_h)$, total dissolved solids (ppm), dissolved oxygen (ppm), temperature (°C), conductivity (mho) and salinity (ppt). All the parameters were analysed using Potable-Water and Soil Analyzer Kit (Naina Enterprises, Agra, India) (Khatri 2004).

Collection of fish samples

Fish sample was collected from the Bomlai and Chiplima rearing ponds in live oxygenated condition and was brought to the laboratory for observation and immediate analyses.

Diseased fish

Infected common carp was cleaned of the surface contaminants with sterile cotton swabs soaked in a chloroxylenol-based antiseptic. Skin lesions, gall bladder and kidney regions were sampled as described by Karunasagar et al. (1989). Samples were ground with sterile nutrient broth using a sterile mortar and pestle under aseptic conditions. A loop full of the sample was spread on M-Aeromonas selective agar plates and was incubated at 20° C and $36 \pm 1^{\circ}$ C for 24 h (Niewolak & Tucholski 2000).

Collection of sediments of fish rearing and stocking ponds

Top layer of the sediment was collected from different spots of different ponds. From each pond, samples were drawn from peripheral, mid and central regions in sterile polythene bag (200 gauge) and were mixed thoroughly before making serial dilutions. Sterile normal saline (0.85% NaCl) was used as diluent throughout the study.

Microbiological methods

Miles—Mishra method was used for screening the sediment and fish samples using sterile (gamma irradiated) disposable pipettes (Volac-John Poulten Ltd, Barking, England) for quantitative estimation of different groups of bacteria (Khatri 2004). Spread plate method was also used for selective isolation of suspected bacteria for further purification and characterisation (Khatri 2004).

Suitable dilutions of the sediment and fish meat samples in sterile normal saline were surface plated on plate count agar, violet bile salt glucose agar, kanamycin aesculin azide agar and Baird-Parker agar for the enumeration of mesophilic aerobic bacteria, faecal coliforms, faecal streptococci and staphylococci according to the standard procedures (Bennet, 1984). Inoculated plates were incubated at 37°C for 24 h for mesophilic aerobes and faecal coliforms, 30 h for staphylococci and 48 h for faecal streptococci. In case of faecal streptococci, the incubation period was extended to 96 h till the very small colonies grew to a larger size for the count. M-Aeromonas selective agar was used (Havelaar et al. 1987) for screening the fish Cyprinus carpio based on the symptoms of the disease. In this medium, yellow (dextrin fermentation) colonies were picked and further tested for oxidase and trehalose fermentation. All the positive isolates were further characterised to confirm species level identification (Havelaar et al. 1987; Prasad et al. 1998).

Antibiotic sensitivity of the bacterial isolates

The antibiotic sensitivity of the *Aeromonas* spp. from the diseased fish was tested by agar diffusion method as described (Baur et al. 1966; Prasad et al. 1998). The bacteria were isolated from skin lesions: SSA⁴B, SSA⁴B (D), SSA¹B, SSA¹B (D), SSA³S, SSA³S (D), SSA²S, SSA²S (D); liver: LSA¹B, LSA¹B (D), LSP¹S, LSP¹S (D) and kidney: KSA¹S, KSA¹S (D), KSA²B, KSA²B (D) of the diseased fish.

The dehydrated media, reagents and antibiotic discs used in this study were from Hi Media (Mumbai, India) and chemicals from Qualigens (India).

Results and Discussion

Occurrence of fish disease

The major diseases identified were included fin rot, saprolegniosis, gill rot, *Ichthyophthirius* (white spot), costiasis and argulosis. The percentage of occurrence in different fish farms surveyed varied from 4% to 16% in this region.

The fin rot was seen in 12% of the farms from which samples were drawn. Saprolegniosis is prevalent mainly in IMC, especially in *Catla catla*, in Chiplima farm. This is seen in all stages of fish that included eggs, fry, fingerlings and fishes. Percentage of occurrence is seen in 4% of the farms. Gill rot is seen in farmed fish of both areas namely Bomlai and Chiplima, especially in the ponds polluted with organic matter. The percentage of occurrence is limited to 8% of the farms. *Ichthyophthirius* (white spot) and costiasis are seen in 16% of the farms of this region. The most affected fish varieties of costiasis were *L. rohita*, *C. catla*, and *C. mrigala*. This infection is seen in 16% of the

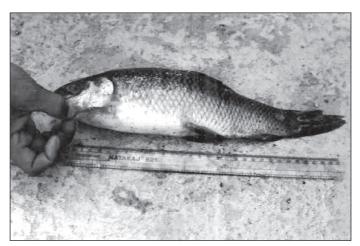


Figure 1. *Labeo rohita* infected with argulosis, Chiplima Fish Farm, Sambalpur

farms surveyed. The prevalence of argulosis is seen in 12% of the farms surveyed (Figure 1). Argulosis infection is also seen in farmed freshwater carp, Rohu (L. rohita) in Andhra Pradesh. Ornamental fish viz., twin tail barb and brass gold appear to be useful as biological agents for the control of argulosis in freshwater carps (Anon 2002).

This study indicates that disease problem is prevalent in the breeding tanks of Bomlai right from the beginning of the production, i.e. 1975. Similar problems are associated with farm at Chiplima too from the beginning of production, i.e. 1986. Besides these problems, Epizootic Ulcerative Syndrome (EUS) was major problem 10 years before, but the severity of the problem drastically reduced for the last few years. With regard to commercial fish farms too the disease problems were noted for last two decades, but the intensity of the diseases decreased. No large-scale mortalities were observed.

Studies on fish ponds located at Bomlai and Chiplima

Physicochemical and bacteriological quality of Bomlai and Chiplima fish rearing ponds

The physicochemical parameters of two rearing ponds of Bomlai and Chiplima (Table 1) indicate difference in temperature 4.22°C, pH 0.01, ORP 1.92, TDS 21.57, conductivity 0.13, DO₂4.02, but the salinity of both farms found to be same. Presence

of less TDS in Chiplima resulted in high DO_2 content that is providing congenial conditions for growth of fish.

Table 1. Physicochemical and bacteriological quality parameters of fish rearing ponds at Bomlai and Chiplima (Data collected in Feb 2004)

Physicochemical		Bomlai		Chiplima					
parameters	Peripheral Mid region Centr		Central poin	nt Peripheral	Mid regio	Central			
						point			
Temperature (°C)	21.31 ± 0.22	21.02 ± 0.35	21.2 ± 0.14	16.68 ± 0.46	17.0 ± 0.62	17.2 ± 0.14			
pН	6.86 ± 0.31	7.07 ± 0.17	7.03 ± 0.01	6.54 ± 0.37	6.43 ± 0.02	7.95 ± 0.07			
$ORP(-E_h)$	80.75 ± 3.77	80.75 ± 3.3	92.0 ± 1.41	84.75 ± 1.26	82.5 ± 1.29	80.5 ± 0.71			
TDS (ppm)	34.2 ± 0.40	34.75 ± 2.36	32.0 ± 0.00	12.5 ± 1.29	$11.75\ \pm0.96$	12.0 ± 0.00			
Conductivity (mho)	0.83 ± 0.10	0.93 ± 0.1	0.90 ± 0.00	0.70 ± 0.00	0.78 ± 0.05	0.80 ± 0.00			
Salinity (ppt)	0.88 ± 0.10	0.88 ± 0.1	0.85 ± 0.07	0.80 ± 0.00	0.80 ± 0.00	0.80 ± 0.00			
DO ₂ (ppm)	7.43 ± 1.01	6.93 ± 0.26	7.25 ± 0.07	11.4 ± 0.18	11.33 ±0.05	11.95 ± 0.07			

ORP: Oxidation Reduction Potential; TDS: Total dissolved Solids. Data presented are averages of 4 ponds.

Bacteriological quality (counts in log cfu per g of the sample)										
	Fish	Sediment	Fish	Sediment						
Mesophilic aerobes	3.52	3.4	4.6	5.2						
Fecal coliforms	2.2	4.26	ND	ND						
Streptococci	ND	ND	ND	ND						
Group D fecal										
streptococci			4.00	2.96						
Staphylococci	ND	4.08	4.23	ND						

The bacteriological analysis of the sediments of the fish rearing ponds at Chiplima indicates that the tanks are free from hygiene indicating faecal coliforms but harboured group D faecal streptococci in the range of 3.08-3.30 log cycles with an average of 2.96 log cycles.g⁻¹ of the sample. The mesophilic aerobic bacterial count ranged from 4.45 to 5.72 with an average of 5.20 log cycles.g⁻¹ of the sample. The fish from the same pond contained group D faecal streptococci and staphylococci at 4.0 and 4.23 log cycles g⁻¹ of the sample, respectively (Table 1).

The bacteriological analyses of the sediments of the fish ponds at Bomlai contained mesophilic aerobes in the range of 2.60-4.20 log cycles with an average of 3.52 log cycles.g⁻¹ of the sample. The faecal coliforms occurred in the range of <1-2.60 log cycles were with an average of 2.20 log cycles.g⁻¹ of the sample. However, all the sediment

samples were free from faecal streptococci and staphylococci. Overall load of different groups of bacteria is slightly higher in Chiplima than in Bomlai. Occurrence of coliforms in Bomlai fish rearing ponds could be due to external contamination.

Bacteriological quality and physicochemical parameters of the sediments and fish samples of Bomlai and Chiplima fish stocking ponds during transition period

The physicochemical parameters of the stocking ponds of two farms, i.e. Bomlai and Chiplima, recorded during transition period of winter to summer, i.e. February and March 2004, are provided in Tables 2 and 3.

Table 2. Physicochemical and bacteriological quality parameters of fish stocking ponds at Bomlai during transition period from winter to summer

Physicochemical parameters		Bomlai (l	Chiplima (March 2004)				
	Peripheral	Mid region	Central poin	nt Periphera	l Mid region	n Central point	
Temperature (°C)	21.05 ± 0.79	20.8 ± 0.45	20.1 ± 0.00	25.98 ±1.04	26.33 ± 1.04	26.55 ± 0.07	
pH	6.57 ± 1.94	5.76 ± 0.78	9.8 ± 0.14	6.25 ±1.33	7.65 ± 1.33	9.0 ± 0.14	
$ORP(-E_h)$	54.75 ± 11.18	55.75 ± 6.70	42.0 ± 1.41	79.5 ±10.4	68.5 ± 10.41	63.5 ± 2.12	
TDS (ppm)	27.5 ± 2.38	26.75 ± 0.96	27.1 ± 0.85	15.0 ±0.00	15.0 ± 0.00	15.0 ± 0.00	
Conductivity (mho)	1.43 ± 0.49	1.4 ± 0.14	1.6 ± 0.00	0.2 ±0.00	0.2 ± 0.00	0.2 ± 0.00	
Salinity (ppt)	1.15 ± 0.13	1.13 ± 0.15	0.8 ± 0.00	0.1 ±0.00	0.1 ± 0.00	0.1 ± 0.00	
DO ₂ (ppm)	8.05 ± 0.44	8.03 ± 0.41	9.00 ± 0.14	5.28 ±1.18	4.8 ± 1.18	6.15 ± 0.07	

Bacteriological quality (counts in log cfu per g of the sample)

	Fish	Sediment	Fish	Sediment
Mesophilic aerobes	4.23	3.81	5.14	4.73
Fecal coliforms	2.22	ND	4.61	3.34
Streptococci	3.10	ND	4.15	ND
Group D fecal streptococci	3.40	2.86	3.60	2.92
Staphylococci	3.57	ND	4.12	ND

Table 3. Physicochemical and bacteriological quality parameters of fish stocking ponds at Chiplima during transition period from winter to summer

Physicochemical		Chiplima	(Chiplima (March 2004)				
parameters	Peripheral	Mid region	Central poin	nt Periphera	l Mid region	n Central point		
Temperature (°C)	19.18 ± 0.17	19.35 ± 0.13	21.05 ± 0.07	27.93 ± 0.38	28.03 ± 0.29	28.30 ± 0.14		
pН	7.64 ± 0.36	7.91 ± 0.35	6.0 ± 0.14	5.88 ± 0.50	5.78 ± 0.05	5.90 ± 0.00		
$ORP(-E_h)$	58.25 ± 1.26	62.5 ± 1.29	68.5 ± 0.71	74.5 ± 7.05	73.5 ± 2.52	78.0 ± 1.41		
TDS (ppm)	12.75 ± 1.71	16.75 ± 0.96	12.0 ± 0.41	15.25 ± 3.10	15.08 ± 2.56	20.0 ± 0.00		
Conductivity (mho)	0.58 ± 0.10	0.58 ± 0.10	0.6 ± 0.00	0.3 ± 0.00	0.3 ± 0.00	0.3 ± 0.00		
Salinity (ppt)	0.68 ± 0.05	0.63 ± 0.05	0.6 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00		
DO ₂ (ppm)	10.28 ± 0.13	9.95 ± 0.13	10.35 ± 0.07	4.53 ± 0.35	4.63 ± 0.10	5.85 ± 0.07		

ORP: Oxidation Reduction Potential; TDS: Total dissolved Solids. Data presented are averages of 4 samples.

Bacteriological quality (counts in log cfu per g of the sample)										
	Fish	Sediment	Fish	Sediment						
Mesophilic aerobes	3.67	3.26	5.15	4.63						
Fecal coliforms	3.93	3.34	4.45	3.51						
Streptococci	2.07	ND	4.1	ND						
Group D fecal streptococci	2.78	2.54	3.48	2.97						
Staphylococci	3.78	ND	4.29	ND						

During the period of February and March in Bomlai farm, increasing trend of temperature and ORP is seen and with the rest of the parameters a decreasing trend was observed which is marginal except for TDS. Similar trends were also observed in Chiplima except with TDS that has enhanced by nearly 3 ppm. Only the upper 5-10 cm of pond bottom soil influences water quality in ponds, and hence management standpoint, thus, the composition of this surface layer is of most importance (Boyd 1995). Low dissolved oxygen level is the major limiting water quality parameter in aquaculture systems. Chronically low-dissolved oxygen levels can reduce growth and feeding (Khatri 2004). In both areas under study the physicochemical quality of the pond water in both the stocking and rearing ponds, the TDS is high (>10 mg.L⁻¹). This can lead to low productivity of the ponds. The bacteriological quality of Bomlai showed an increasing

trend from February to March along with temperatures. The difference is more than 1 log cfu.g⁻¹ of the sample in case faecal coliforms and faecal streptococci, nearly 1 log cfu.g⁻¹ with mesophilic aerobes and less than 1 log cycle with group D faecal streptococci (Table 2). Similar trends were also seen in the study of the populations of indicator bacteria *viz.*, mesophilic, coliform and faecal streptococci together with relevant limnological parameters such as temperature, oxygen, BOD and chlorophyll-a revealed that during each season, populations of indicator bacteria increased with increasing water temperature, and maximal numbers of bacteria were recorded during the summer months (Markosova & Jezek 1994). This study corroborates the findings of the studies by Sivakami et al. (1996) and Al Harbi (2003) in which coliform organism found in sediment and fish samples.

The bacteriological quality of the sediment and the fish in Chiplima fish stocking ponds have also shown increasing trend from February to March with the increase in temperatures. In fish samples, the difference in increase is more than 1 log cfu.g-1 of the sample for mesophilic aerobes and streptococci. However, the difference in coliforms, group D streptococci and staphylococci from February to March is less than 1 log cfu.g-1 of the sample. In sediments of the Chiplima ponds, the difference in faecal streptococcal and group D streptococcal counts is also less than 1 log cfu.g-1 of the sample. This study agrees with other studies in which the counts of different groups of bacteria such as mesophilic aerobes, coliforms, streptococci and staphylococci of pond sediments and the fish tend to increase with increase in temperatures (Markosova & Jezek 1994; Kasai et al. 2002; Ogbulie & Cobiajuru 2003; Garcia et al. 2003).

Staphylococcus aureus is associated with eye infection in fish *Hypothalamichthys* molitrix (Shaw & Tyagi 1986). Catfish growing ponds contained faecal coliforms in the range of 14-1600 ml⁻¹ of pond water (Shireman & Cichra 1994). This is higher than what is observed in this study. Environmental stress can trigger spread of dormant disease into full bloom resulting in large-scale mortality of fish (Wedemeyer et al. 1999).

Characterisation of the bacterial isolates from diseased fish (*C. carpio*) (Figure 2) of this study revealed that they are *Aeromonas hydrophila* and *Aeromonas sobria*. Motile aeromonads also isolated from Epizootic Ulcerative Syndrome affected *Channa striatus* (Prasad et al. 1998). In this study, high temperatures (37°C) were used for detection of different groups of bacteria in sediments, fish and aeromonads from diseased *C. carpio* and all groups of bacteria under screening could be detected. These results agree with the study on evaluation of the bacteria identified on broth agar in 20°C and 37°C, *viz.*, coli forms, faecal coli forms, faecal streptococci, *Aeromonas* sp. and *Salmonella* sp. in the muscles, skin and digestive tract content of common carp (*C. carpio*) (Niewolak & Tucholski 2000) in which the high temperatures favoured growth of all bacteria more, so *Aeromonas sp.* motile aeromonads are inherently associated with freshwater bodies and cultured fish production. At present, 10 different species are

recognised, but in moribund tropical fish, most commonly isolated species are *A. hydrophila* and *A. sobria* (Khatri 2004). Motile aeromonads are most commonly associated with bacteria found in fish infected with EUS (Lio-Po et al. 1991; Karunasagar & Karunasagar 1994; Prasad et al. 1998; Karunasagar et al. 2003; Golas et al. 2004).

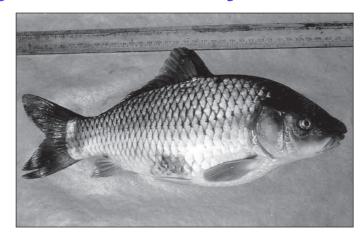


Figure 2. Cyprinus carpio with diseased symptoms

Antibiotic sensitivity of the bacterial isolates from diseased Common carp C. carpio

The sensitivity or resistance different isolates from different fish are shown in Table 4.

Table 4. Antibiotic sensitivity of the *Aeromonas hydrophila* isolated from the diseased fish

Antibiotic tested

Isolates	AN	ΑZ	CFC	СН	CLR	CL	Е	G	K	NF	NR	OX	PP	P	SC	T	V
Concentration of the antibiotics in µg per disc except NF 300ml and NR 10U per disc																	
	30	15	30	30	15	2	15	10	30	300	10	5	100	10	5	30	30
KSA1S	S	S	R	S	I	R	S	S	I	I	S	S	R	R	S	S	R
KSA2B	S	I	R	S	R	R	S	I	I	R	S	I	R	R	S	S	R
LSA1B	R	S	R	I	R	R	S	R	R	S	S	S	R	R	R	I	R
LSP1S	I	S	R	S	I	R	S	I	R	R	S	S	R	R	S	R	R
SSA1B	S	S	R	S	I	R	S	I	S	R	S	S	R	R	S	S	R
SSA2S	S	S	R	S	S	R	S	S	I	R	S	S	R	R	S	S	R
SSA3S	S	S	R	S	S	R	S	I	I	I	S	S	R	R	R	S	R
SSA4B	S	S	R	S	I	R	S	I	R	R	S	I	R	R	S	R	R

Source of isolates; K-Kidney; L-Liver; S-Skin lesions. Antibiotics: AN: Amikacin, AZ: Azithromycin, CFC: Cefaclor, CH: Chloramphenicol, CLR: Clarithromycin, CL: Clindamycin, E: Erythromycin, G; Gentamycin, K: Kanamycin, NF: Nitrofurantoin, NR: Norfloxacin, OX: Ofloxacin, PP: Piperacillin, P: Penicillin G, SF: Sparfloxacin, T: Tetracycline, VV: Vancomycin. S: Susceptible; I: Intermediate; R: Resistant

The *Aeromonas* isolates from kidney (KSA¹S) of the diseased fish *C. carpio* showed susceptibility to 9 antibiotics, intermediate to 3 and resistant to 5 antibiotics out of 17 antibiotics tested. The other *Aeromonas* isolate from kidney (KSA²B) was susceptible to six antibiotics, intermediate to four and resistant to seven antibiotics. The isolate from liver (LSA¹B) was susceptible to five antibiotics, intermediate to two and resistant to the remaining antibiotics. The second isolate from the liver (LSP¹S) was resistant to nearly 50% of the antibiotics, i.e. eight, intermediate to three and susceptible to six antibiotics. The isolates obtained from four different parts of affected area (skin lesions) were susceptible to more than 50% of the antibiotics tested and were resistant to few and the remaining fell into category of intermediate in the susceptibility (Table 4). Among the 17 antibiotics tested, all the isolates were susceptible to erythromycin and nitrofurantoin and resistant to cefaclor and vancomycin. Occurrence of antibiotic-resistant strains of *A. hydrophila* from fish samples from integrated fish farms in a Southeast Asian country was also reported (Twiddy & Reilly 1994).

Conclusion

This study revealed the occurrence of six major diseases *viz.*, fin rot, saprolegniosis, gill rot, *Ichthyophthirius* (white spot), costiasis and argulosis. This is helpful to develop preventive measures, control and spread of the diseases

The study on physicochemical parameters of stocking and rearing ponds at Bomlai and Chiplima revealed variations between the ponds and the seasons. In both areas under study the physicochemical quality of the sediments in both the stocking and rearing ponds, the TDS is high (>10 mg.l⁻¹). The pH is within the optimum levels, i.e. 6.5-8.5. The bacteriological analyses of sediments collected from different points indicate occurrence of hygiene indicator bacteria at lower level. The trend is an increase along with increase in temperatures of pond from February to March in all bacterial groups wherever the occurrence is noted both in sediments and fish. The antibiotic susceptibility of *A. hydrophila* isolated from diseased *C. carpio* varied among and between the isolates from kidney, liver and skin lesions. This result will act as baseline data for future studies. However, more studies are required to understand changing pattern of antibiotic resistance in environmental strains.

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References

- Al Harbi, A.H. 2003. Faecal coliforms in pond water, sediments and hybrid tilapia *Oreochromis niloticus* x *Oreochromis aureus* in Saudi Arabia. Aquaculture Research 34 (7):517-524.
- Anon. 2002. Anonymous. Annual Report, Central Institute of Fisheries Technology, Matsyapuri, P.O., Kochi. 44-45 pp.
- Baur, A.W., W.M.M. Kirby, J.C. Sherris and M. Truck. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical pathology 36:493-496.
- Bennet, R.W. 1984. Bacteriological analytical manual, 6thed. Association of Official Analytical Chemists, Arlington. Chapters 3-7 and 13-14.
- Boyd, C.E. 1995. Bottom soils, sediment, and pond aquaculture. Chapman and Hall, New York. 348 pp.
- Boyd, C.E. 1997. Practical aspects of chemistry in pond aquaculture. The Progressive fish culturist 59 (2):85-93.
- FAO. 2004. The state of world fisheries and aquaculture. FAO Fisheries Department, Rome. 153 pp.
- Garcia, M.J.A., F.A.N. Gonzalez, O.C. Pineda, R.H.A. Rodriguez and M.R.E. Hernandez. 2003. Microbiological quality of the culture of rainbow trout (*Oncorhynchus mykiss*) and water quality in the northwestern region of Chihuahua, Mexico. Hidrobiologica (Iztapalapa) 13:111-118.
- Golas, I., I. Zmyslowska, M. Harnisz, M. Teodorowicz, D. Lewandowsca and J. Szarek. 2004. Isolation of heterotrophic bacteria in the water, fish feed and fish skin in Poland, during the pond rearing of European catfish (*Silurus glanis L.*) fry. Bull Sea Fish Institute, Gdynia 161:3-14.
- Havelaar, A.H., M. During and J.F.M. Versteegh. 1987. Ampicillin dextrin agar medium for the enumeration of Aeromonas species in water by membrane filtration. Journal of Applied Bacteriology 62:279-287.
- Hussenot, M. and J.L.M. Martin. 1995. Assessment of the quality of pond sediment in aquaculture using simple, rapid techniques. Aquaculture International 3 (2): 123-133.
- Karunasagar, I. and I. Karunasagar. 1994. Bacteriological studies on EUS in India. Proceedings of ODA Regional seminar on EUS (ed. R.J. Roberts and I.H. Mac Rae), pp 158-170. Aquatic animal Health Research Institute, Bangkok.
- Karunasagar, I., G.M. Rosalind, I. Karunasagar and G.K. Rao. 1989. Aeromonas hydrophila septicemia of Indian major carps in rohu commercial fish farms of West Godavari District, Andhra Pradesh. Current Science 58:1044-1045.
- Karunasagar, I., I. Karunasagar and S.K. Otta. 2003. Disease problems affecting fish in tropical environments. Journal of Applied Aquaculture 13 (3/4):231-249.
- Karunasagar, I., P.K.M.M. Ali, G. Jeyasekaran and I. Karunasagar. 1986. Ulcerative form of Aeromonas Hydrophila infection in Catla catla. Current Science 55:1194-1195.
- Kasai, H., N. Nakamura and M. Yoshimizu. 2002. Observations on bacteria collected in the Shibetsu, Ichani and Kotanuka rivers in Eastern Hokkaido, Japan. Bulletin of Fisheries Sciences, Hokkaido University 53 (2):75-82.
- Khatri, S. 2004. Prevalence of fish diseases in Sambalpur, Orissa. M.Sc., Dissertation. G.M. College, Sambalpur. 35 pp.
- Lio-po, G.D., L.J. Albright and E.V. Alapide-Tendencia.1991. *Aeromonas hydrophila* in Epizootic Ulcerative syndrome (EUS) snake head (*Ophiocephalus striatus*) & Catfish (*Clarias batrachus*). Quantitative estimation of natural infection & experimental induction of dermonecrotic lesions. In: Diseases in aquaculture 1. (ed. M. Shariff, R.D. Subasinghe and J.D. Arthur). Asian Fisheries Society, Manila.
- Markosova, R. and J. Jezek. 1994. Indicator bacteria and limnological parameters in fish ponds. Water Research 28 (12):2477-2485.
- Niewolak, S. and S. Tucholski. 2000. Evaluation of bacteria identified on broth agar in 20 and 37 degree C, coli forms, fecal coli forms, fecal streptococci, anaerobic sulfite-reducing *Clostridium perfringens*, *Pseudomonas aeruginosa*, *Aeromonas* sp., and Salmonella sp. in the muscles, skin, and digestive tract content of common carp (*Cyprinus carpio*). Archiwum rybactwa polskiego/Arch Polish Fish. Olsztyn 8:63-74.

- Ogbulie, J.N. and I.O. Cobiajuru. 2003. The impact of seasonal changes on the bacteriological water quality and substrate specificity of isolates in a *Clarias lazera* culture system at Obirikom, Rivers State, Nigeria. Journal of Aquaculture Science 18:105-112.
- Prasad, M.M., C.C.P. Rao and P.K. Surendran. 1998. Motile aeromonads associated with Epizootic Ulcerative Syndrome affected *Channa striatus*. In: Advance and priorities in fisheries technology (ed. K.K. Balachandran, T.S.G. Iyer, P. Madhavan, J. Joseph, P.A. Perigreen, M.R. Raghunath and M.D Varghese), pp 374-377. Society of Fisheries Technologists of India, Kochi.
- Shankar, K.M. and C.V. Mohan. 2002. Fish and shellfish health management. UNESCO, New Delhi. 104 pp.
- Shaw, K.L. and S.C. Tyagi. 1986. An eye disease in silver carp *Hypothalamichthys molitrix* held in tropical parts associated with its bacterium *Staphylococcus aureus*. Aquaculture 55:1-4.
- Shireman, J.V. and C.E. Cichra. 1994. Evaluation of aquaculture effluents. Aquaculture 123: 53-68.
- Sivakami, R., G. Premkishore and M.R. Chandran. 1996. Occurrence and distribution of potentially pathogenic Enterobacteriaceae in carps and pond water in Tamil Nadu, India. Aquaculture Research 27 (5): 375-378.
- Twiddy, D.R. and P.J.A. Reilly. 1994. The occurrence of antibiotic-resistant human pathogenic bacteria in integrated fish farms in a Southeast Asian country. Research contributions presented at the 9th Session of the Indo-Pacific Fisheries Commission Working party on fish technology and marketing, Cochin, and Kerala, India. FAO Technical papers 514:23-37.
- Wedemeyer, G.A., F. Meyer and L. Smith. 1999. Environmental stress and fish diseases. Narendra Publishing House, New Delhi. 181 pp.