Impact of infectious diseases on sustainability of cultured fisheries- understanding relationship between host, pathogen and environment

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ABSTRACT: Despite current advances in aquaculture practices, microbial diseases are still being increasingly incriminated as a significant constraint to regulate sustainable aquaculture production, trade and economic development of this sector. A systematic research to identify various infections which induce morbidity and mortality among trout and carps was undertaken in Himachal Pradesh, a northern state of India lying in the foothills of Himalayas. In total, 415 samples comprising of 275 carps and 140 trouts; either dead, dying, moribund or apparently healthy were microbiologically processed. The bacterial and mycobiotic involvement was revealed in 381 and 118 of these samples, respectively. Altogether, 549 isolates of microbes were identified. Histopathological and pathogenicity studies were also carried out. Among bacteria, *Aeromonas* spp. predominated whilst in mycobiota it was *Aspergillus spp.* followed by *Cladosporium spp.* Oxytetracycline, tetracycline, chlortetracycline, gentamicin and norfloxacin were adjudged best *in vitro,* whereas ampicillin, erythromycin and penicillin-G were least effective. The findings of this investigation are co-related with those of several other workers related to the impact of environmental stress and ecological disturbances on aquabiota and fish diseases. Likewise for mycobiotic isolates malachite green and sodium chloride were best, whereas, potassium permanganate was least effective. Comparative to previous year's studies the increased incidence of microbial involvement is being suggestive of host of factors like deteriorating environmental factors and poor water quality resulting from increased effluent discharges, movement of aquatic animals, rapid proliferation of farms and their inadequate management. This study emphasized the need for, *inter alia,* broader ecosystem management approaches to control environmental deterioration and to take preventative measures against the introduction of pathogens to aquatic system as suggested by (ADB/NACA, 1991) and Phillips (1996).

KEY WORDS: Trout, Carp, Bacteria, Fungus, Environment, Stress

INTRODUCTION

Himalayan region is one of the ecologically rich yet fragile ecosystems of the world. The same holds true for its water bodies which harbour various edible fish, the trouts and the carps being one of the favoured ones. Himachal Pradesh, a northern state of India which lies in the western Himalayas between latitudes $30^{o}22'$ and $33^{o}10'$ N and longitudes $70^{o}46'$ and $79^{o}00'$ E is the hot spot of fish fauna diversity and is much suited for cold water fisheries. During the last two decades there has been a tremendous stress on the indigenous fish genetic resources of the state in the wake of over all development and over exploitation of the aquatic ecosystem owing to the ever increasing demand for fish. As a result, some of the species have already been declared as endangered or threatened and a few species are at the verge of extinction. Commercially, important indigenous species are declining day by day from the area. Intensive fish production is usually accompanied by increased disease problems as several physical and environmental stresses like low dissolved oxygen, excess of nitrogen or ammonia, chemical toxicities, handling stress and high water temperatures more often are predisposing causes to infectious diseases.

It is well established fact that for similar levels of invasiveness and environmental conditions, the incidences of diseases and mortalities seem to be quite comparable (Wilhelm, 1986).There is a vast range of fish diseases and the losses ascribed to these are enormous under different set of climatic and managemental conditions. Despite the promising potential of fisheries, the fish diseases, diagnosis and research had not taken even a start in this state. Therefore, there was urgent and time pressed need to undertake a systematic study to have information and understanding so as to resolve some of these problems. In the following text, some of the important research findings will be discussed particularly in relation to trouts and carps and their habitat, which were carried out with the following objectives:

1. Prevalence and distribution of diseases in fish being reared in different types of managemental conditions in cold water zones.
2. Isolation of bacterial and mycobiotic agents.

3. Characterisation of the isolates on the basis of cultural, morphological and biochemical behaviour.
4. Histopathological confirmation of the association of pathogen(s) from some of the representative morbid materials.
5. Testing drug sensitivity profiles of these isolates.
6. Pathogenicity trials
7. Investigations on epidemiological aspects with respect to habitat, and other factors on prevalent diseases in order to work out a plan to contain them.

MATERIALS AND METHODS

In the present study, 415 samples of fish fauna were collected from different parts of Himachal Pradesh which included 275 specimens of carps and 140 specimens of trouts. The fish specimens selected for study included overtly diseased fish to almost healthy specimens. They were either collected fresh, moribund or freshly killed specimens. They were brought to the diagnostic laboratory at the earliest possible on ice for microbiological processing. After collection and transportation to the laboratory, the external examination together with the degree of fat deposition, degree of freshness was adjudged and then the necessary bacteriological and mycobiotic exploration was carried out.

Isolation and Biochemical characterisation of microbial isolates:

The inocula were initially streaked on 6 % sheep blood agar, Sabouraud Dextroxe Agar (SDA) and Dermophyte Test Medium (DTM) to obtain isolated colonies. Selective media were used to purify different microbes as per requirement. The fungal observations were also made on direct 10% KOH mounts. Each microbial isolate was tested for its different biochemical potentialities by using standard protocols as described by Buchanon and Gibbons (1974) and Cruickshank et al. (1975).

Histopathological studies:

The pieces of outgrowths, gills, and internal organs like liver, heart, kidneys, spleen, and muscle etc. showing gross lesions were preserved in 10 per cent neutral buffered formalin. The tissues were washed, dehydrated, cleared and impregnated in wax. Finally, four to five micron thick sections were cut and spread onto a slide. Staining was carried out with routine Hematoxylin and Eosin staining (H & E).

Chemotherapeutic sensitivity profile of microbial isolates:

The antimicrobial susceptibility pattern of different bacterial and fungal isolates was tested on Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (Hi-Media), respectively. Each isolate was tested for sensitivity against different drugs employing disc diffusion test as per the technique described by Bauer et al. (1966).

Pathogenicity tests of isolates:

Pathogenic potential of some of the important pathogens as well as commensals were tested. The pathogenicity of bacterial isolates was tested in common carps; and the pathogenic potential of isolates of fungal species were tested separately on the lines of Scott and O'Warren (1964).

RESULTS AND DISCUSSION

In the present study, out of the total number of 415 samples processed 381 samples comprising of 250 carps and 131 trouts showed bacterial involvement. Mycobiotic association was observed in 118 of the total fish samples whereas 309 were bereft of any mycobiotic etiology. Interestingly, the concurrent bacterial and mycobiotic association was revealed in all the 118 samples of fishes. The 34 samples did not harbour any bacterial or mycobiotic pathogen that comprised of 25 samples from carps and nine from trouts.

Bacterial and mycobiotic isolations from carps and trouts:

The overall bacterial isolation has been divided into four groups which are (i) Gram (+) isolates, (ii) Fermentative, oxidase (-) Gram (-) isolates, (iii) Fermentative, oxidase (+) Gram (-) isolates and (iv) Gram (-) Non fermenters as depicted in Table 1.

TABLE 1: IMPORTANT BACTERIAL AND MYCOBIOTIC ISOLATIONS

1	**Gram + isolates:**	*Bacillus mycoides, B. megaterium, B. coagulans, Bacillus spp. Staphylococcus saprophyticus, Staphylococcus aureus, Staphylococcus spp., Streptococcus spp., Micrococcus roseus, Micrococcus varians, Lactobacillus spp., Nocardia spp., Listeria spp.*
2.	**Fermentative, oxidase(-), Gram(-) isolates**	*Escherichia intermedium, Edwardsiella icataluri, Hafnia alvei Enterobacter agglomerans, Citrobacter freundii, Citrobacter diversus, Proteus vulgaris, Proteus rettgeri, Providencia rettgeri, Shigella spp., Serratia liquefaciens,*
3.	**Fermentative, oxidase(+), Gram (-) isolates**	*Aeromonas caviae, Aeromonas sobria, Aeromonas hydrophila, Aeromonas salmonicida, Yersinia spp., Vibrio anguillarum, Vibrio ordalii, Vibrio alginolyticus, Vibrio carchariae, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio fischeri, Vibrio spp., Plesiomonas shigelloides, Pseudomonas fluorescens*
4.	**Gram(-) Non fermenters**	*Shewanella putrefaciens, Moraxella lacunata,Moraxella nonliquifaciens, Moraxella phenylpyruvica, Flavobacterium spp., Acinetobacter baumani, Acinetobacter spp., Alcaligenes faecalis, Xanthomonas maltoplila*
5.	**Fungal isolates**:	*Aspergillus fumigatus, A. flavus, A. niger, Aspergillus spp., Acremonium spp., Alternaria spp., Fusarium spp., Cryptococcus spp., Candida stillatoidea, Chrysosporium spp., Candida krusei, Chromoblastomyces spp., Cladosporium spp., Curvularia spp., Paecilomyces spp., Phycomyces spp., Penicillium spp., Rhizopus spp., Saprolegnia spp., Saccharomyces spp., Scopulariopsis spp., Torulopsis glabrata, Trichosporon cutaneum,*

The major disease conditions that were identified during the present study were motile aeromonad septicaemia manifested as clinical dropsy caused by *Aeromonas hydrophila,* saprolegniosis due to *Saprolegnia spp.* , bacterial gill disease due to *Flavobacterium*, pseumonad septicaemia caused by *Pseudomonas fluorescens*, enteric septicaemia due to *Edwardsiella ictaluri,* fin rot caused by *Aspergillus niger*, generalized haemorrhages and septicaemic condition due to *Aeromonas salmonicida*, and in some cases the lesions were associated with multiple bacterial and fungal etiology.

This study recorded isolates of *Flavobacterium* spp. from gills of diseased fish. *Flavobacterium* is associated with the bacterial gill disease (BGD) and is a primary fish pathogen responsible for massive mortalities and colossal fiscal losses. BGD may well be a condition resulting from the complex interaction of adverse environmental conditions and bacteria of variable pathogenicity. Small fish in crowded conditions, poor water quality, over feeding of a fine ration and presence of the ubiquitous bacterium can lead to this kind of infection. Affected fish become lethargic and anorexic. They tend to remain near the surface or inlet or may be observed flaring their opercula and coughing. Their respiratory rate is elevated and mucus secretion may increase to the point where strands are trailing from the gills (Ferguson et al. 1991). Bullock (1972) has suggested that adverse environmental conditions are essential for the initiation of BGD.

Isolates of *Pseudomonas fluorescens* were recovered from the morbid samples processed. Pseudomonad septicaemia is a haemorrhagic condition of fish usually associated with stress or improper management. Probably all species of fish are vulnerable to pseudomonad septicaemia under adverse environmental conditions or when compromised by other factors.

Different species of *Aeromonas* viz. *Aeromonas caviae, A. sobria, A. hydrophila* and *A. salmonicida*, were isolated from morbid samples. *Aeromonas salmonicida* were generally isolated from diseased fish showing generalized haemorrhages, abdominal swelling and necrotic swelling in the muscles. *Aeromonas salmonicida* is the causitive agent of furunculosis of salmonids and related diseases of other species and characterized by septicaemia and development of furuncles. Outbreaks are invariably stress associated and mortalities may be very high, posing a real threat, in particular to the salmon farming. *Aeromonas hydrophila* causing motile aeromonad septicaemia is usually associated with stress from factors such as crowding, elevated or changing water temperatures, reproduction, and spawning and water quality.

The isolation of *Edwardsiella ictaluri* from common carp (*Cyprinus carpio*) in the present study is in concurrence with the observation of Plumb and Sanchez (1983). The culture of *Edwardsiella ictaluri* was highly haemolytic and caused β- haemolysis of sheep red blood cells in blood agar plate at 25°C. This observation is in agreement with the findings of Waltman et al. (1986) but differs with Janda et al. (1991) as they reported a lack of haemolytic activity in microtitre assay. Fish-eating birds have been incriminated as potential vectors of *E. ictaluri* (Taylor, 1992). That perhaps would have been the cause of *E. ictaluri* infection in common carps

collected from such fish farms. Furthermore, the gastrointestinal tract of cold blooded animals act as the natural reservoir of *Edwardsiella spp.* and some of these species have been incriminated to cause occasional gastrointestinal infections among human beings (Ewing et al.,1965). The stressors associated with *Edwardsiella* septicemia infection are high level of organics, over crowding and temperature greater than $20^{O}C$ in case of salmonids and otherwise temperature higher than $30^{O}C$.

From the samples processed, *Vibrio* isolates of different species were recovered. Whenever fishes are stressed or traumatized, either under farming conditions or in the wild, vibrios tend to produce infections which take the form of focal haemorrhagic ulcers on the mouth and skin surface, or focal necrotic lesions in the muscle, the orbit or along the edge of the fins (Hjeltnes and Roberts, 1993). Such lesions may be associated with strains of *V. anguillarum* and are also characteristic of the pathology associated with the other vibrios.

The members of genus *Yersinia* are recognized as potential fish pathogens. During the present study, isolates of *Y. enterocolitica* which is a human enteric pathogen were identified from the intestines of carps and trouts. No isolation of Y. *ruckeri* could be possible from the processed samples. However, Sharma et al. (1995) have isolated *Y. ruckeri* from rainbow trout from two outbreaks in Himachal Pradesh for the first time in India. It is widely accepted that Enteric Red Mouth (ERM) outbreaks occur due to poor environmental conditions and stress suffered by fish, i.e. low oxygen level, high water temperature, poor water quality, transport or handling stress (Rucker,1966 ; Roberts,1983). Similar poor environmental conditions and stress were prevalent at both the fish farms where ERM outbreaks were observed. Since the fish farms of both the places were open and birds had free access to them it is possible that the fish might have picked up the infection from the droppings of aquatic birds, aquatic invertebrates and wild fish as reported by Willumsen (1989).

The present study also recorded the isolates of *Serratia liquefaciens*. *Serratia* is an opportunistic pathogen affecting fishes under environmental stress. These findings are supported by Austin and Mc Intosh (1991). They also forecasted *S. liquefaciens* as emerging future pathogens of aquaculture. There have been isolations of *Citrobacter diversus* which is an enteric pathogen and mostly present in soil and water. It is known to cause neonate meningitis. However, Dhar, (1998) also isolated *C. diversus* from pooled organs of two carps without any conspicuous disease symptoms. The present study enlists unspeciated isolates of *Shigella* recovered from the haemorrhagic intestines of carps. *Shigella* is an important bacterial pathogen known to cause dysentery in humans and animals. The major source for spread of these bacteria is contaminated water and feed. This study also records the isolates of *Hafnia alvie* that emanated from the kidney of carps and trouts. Although *Hafnia alvei* is known as an inhabitant of the aquatic environment but it is suspected that this organism may cause opportunistic infection in fishes as a result of stress. Teshima (1992) reported kidney disease due to *Hafnia alvei,* where the diseased fish revealed a dark body surface and furuncles like eminencies were present on the ventral side of kidney.

Most of the fish farms are dependent on natural stream as a source of water supply. Of late, many human habitations have mushroomed closely to the vicinity of the water bodies and there is constant, unchecked seepage of human and animal excreta, adding many resistant strains of bacteria into the aquatic environment. The isolation of different members of the family Enterobacteriaceae from fish samples from such farms points towards the potential health hazard that it can pose for human and animals as some of them are important pathogens of animals and humans.

The large scale use of pesticides and herbcides in agriculture and horticulture in this region has also led to deterioration and contamination of water bodies, rivers, reservoirs and also other fish farms. Dunier and Siwicki (1993) have reported the toxic effects of pesticides on the immune system of fish that can range from decreased humoral response (DDT), atrophy of lymhoid organs (endosulfan), elevation in serum cortisol which is a stress marker (aldrin,etc.). Many industrial wastes like phenols, heavy metal ions, polychlorinated biphenyls, ammonia and nitrite in aqua environment affect the immune status of the fish, eg. if fish is raised in water having phenol, such fish has a inhibited antibody immune response to *Y. ruckeri* (Anderson et al. 1984). In the same manner, fish exposed to toxic level of $ZnSO_4$ are prone to get easily infected by *Proteus vulgaris* (Sarot and Perlmutter, 1976). Salmonids are particularly sensitive to stress, detected by an increase in the plasma cortisol level, which affects glucocorticoids (Kaattari, 1991). The consequence is a selective suppression of essential interleukins, a modification in the sensitivity of lymphoid organs and of the affinity of leukocyte receptors. Kaattari and Tripp (1987) reported that cortisol decreases specific B cell precursor frequency in fish and this also accounts for a decreased number of specific antibody secreting cells following pollutant exposure. That could explain why stress has been reported to lower the antibody response or the disease resistance (Peters et al. 1991).

During the present study, *Streptococcus spp.* were also isolated from carps and trouts. *Streptococci* may occur as saprophytes, commensals and pathogens in men and animals. The streptococcal septicaemia is the most grave disease in fish during high water temperature and other stressors. Minami (1979) and Bragg and Broere (1986) documented a disease caused by *Streptococcus spp.* that resulted in high mortality in rainbow trout farms during

summer months. Symptoms included extreme exophthalmia along with haemorrhages in the eye chamber, darkening of skin, spleenomegaly, intestinal haemorrhages and yellowish fluid in intestine. Only the adult fishes were affected. Craig et al. (2001) reported that *Streptococcus iniae,* originally isolated from a fresh water Amazon dolphin, has gained attention as a pathogen of fish and the apparent association with streptococcal infection in humans.

A major portion of bacterial isolates in this investigation were classified as genus *Bacillus, i.e. Bacillus mycoides, Bacillus coagulans, Bacillus megaterium* and unspeciated *Bacillus spp*. Though *Bacillus* are generally considered as normal inhabitants of soil and water and their role as opportunistic or potential fish pathogens is not firmly established, but their role as primary pathogens in humans and animals gives importance to their isolation in large numbers in fish. This investigation revealed the isolation of *Micrococcus varians* and *Micrococcus roseus* from the gills and fins of carps. Undoubtedly, *Micrococci* are seen on the mammalian skin, soil and water and are ubiquitous in distribution. They are usually present in water and have been associated with diseases in stress and immunocompromised hosts. Olayemi et al. (1990) have reported *Micrococcus* to be associated with fresh water fishes.

Flexibacter spp. though not isolated during our investigation, yet its infections are too stress related. *Flexibacter psychrophilus* syn *Cytophaga psychrophila* is the causative agent of bacterial cold water disease which is a serious septicaemic infection of hatchery-reared salmonids. Infected fish may exhibit typical lesions of the peduncle muscle, jaw, snout or have no external lesions (Holt et al., 1993). They may also be anaemic with haemorrhagic gills. It has been found that C*ytophaga psychrophila* losses drop as temperature rises and is compounded by crowding, organics and high flow rate. Columnaris disease caused by *Flexibacter columnaris* (not isolated in this study) has been reported to be highly virulent. The environmental factors associated with this disease are elevated temperature, usually > 15^{O}C and severity increases with temperature, high pH, hard water, organic matter, crowding and uncleanliness. The first indication of the infection is generally the appearance of a white spot on some part of the head, gills, fins or body. This is usually surrounded by a zone with a distinct reddish tinge, leading to under-running of adjacent skin. Lesions are covered with a yellowish white mucus consisting largely of swarms of *F.columnaris.*

Renibacterium salmoninarum (not isolated during this study) causing bacterial kidney disease, is a serious pathogen of cultured and feral salmonids. The infected fish may or may not show external lesions which include pale gills, exophthalmia, abdominal distension, skin blisters, shallow ulcers and haemorrhages. Internally there may be fluid in the abdominal and pericardial cavities, haemorrhages on the walls of abdominal cavity, membranous layer on visceral organs and creamy white lesions in the kidneys (Evelyn,1993).The genus *Mycobacterium* consists of major fish pathogens like *M. fortuitum, M. marinum, M. chelonae*. Mycobacteriosis is piscine tuberculosis and affects a wide range of fresh water and marine species but particularly aquarium fishes. The infected fish show anorexia, emaciation, dyspnoea, exophthalmia, skin discolouration (Frerichs, 1993). The external signs of disease range from scale loss to nodules, ulcers and fin necrosis as signs of advancing infection (Parisot, 1958). This investigation did not encounter any *Mycobacterium spp.*

Fungi are present everywhere in saltwater or fresh water, in cool or warm temperatures. In most cases, fungi serve a valuable ecological function by processing dead organic debris. However, fungi can become a problem if fish are stressed by disease, by poor environmental conditions, receive poor nutrition or are injured. If these factors weaken the fish or damage its tissue, fungus can infest the fish. Fungi can also prevent successful hatching when it invades fish eggs. In the present study, mycobiotic genera like *Saprolegnia*, *Pencillium, Aspergillus, Chrysosporium, Rhizopus, Candida* and many others have been isolated from tissue samples of carps and trouts (Table 1). Okaeme and Olufemi (1997) described the prevalence of fungi, associated with tilapia culture ponds in Nigeria. The most frequent mycobiotic agents isolated with all culture systems were *Pythium, Saprolegnia, Cryptococcus neoformans, Mucor, Aspergillus, Microsporum, Trichophyton* and *Chrysosporium* while *Rhizopus, Aspergillus*, and *Candida* were also prevalent in animal manure. Mycobiotic agents like *Pythium, Cryptococcus neoformans, Mucor, Microsporum, Branchiomyces* and *Icthyophonus* were absent from the present investigation which might be probably due to various factors such as environment, nature of ponds and water bodies in this state. Saprolegniasis was first noticed by observing fluffy tufts of white coloured cotton like material on skin, fins, gills or other parts of the body. The disease can attack an existing injury on the fish and can spread to healthy tissue due to poor water quality (like water with low circulation, low dissolved oxygen or high ammonia) and high organic loads, including the presence of dead eggs.

Other important fungal isolates like *Branchiomyces spp.* and *Icthyophonus hoferi* could not be recovered from either diseased or healthy fish. *Branchiomyces sanguinis* and *B. demigrams* cause branchiomycosis in fish suffering from an environmental stress, such as low pH (5.8-6.5), low dissolved oxygen or a high algal bloom. *Icthyophonus* disease caused by *Icthyophonus hoferi* grows in fresh and saltwater but is restricted to cool temperatures. The disease is spread by fungal cysts which are released in the faeces and by cannibalism of infected fish.

There are reports that microbial flora in the water bodies can be used as an environmental indicator of the status of the environment. Burkholderia (*Pseudomonas) cepacia* which is a common inhabitant of streams and soils, does not thrive in polluted waters thus its absence indicates pollution. (Janakiraman and Leff, 1999; Lemke and Leff, 1999). There is widespread *Aeromonas spp*. in nutrient rich waters and sewage, its increase in concentration indicates fecal contamination in surface waters and the presence of *Vibrio* indicates salinity (Brion et al. 2000). Moreover, Janakiraman and Leff (1999) have reported that *Bacillus thruingiensis* is a biological insecticide and its presence in environment indicates widespread insecticide use. It has been reported by Lemke et al. (1997) and Lemke and Leff (1999), that *Acinetobacter calcoaceticus* is commonly found in soil and water and thrives very well in polluted areas. From the samples processed, isolates of *Acinetobacter baumani* and unspeciated *Acinetobacter spp.* were recovered. It has been reported by Holt et al. (1994), that *Proteus vulgaris* is an indicator of faecal material. In the present investigation, no isolation of *Proteus vulgaris* could be made but some of the samples revealed *Proteus rettgeri.* During the isolations from fish, a major portion consisted of different *spp*. of *Aeromonas* and *Vibrio* among the environmental indicators.

Histopathological findings:

The histopathological findings were corroborative of infectious etiology. In some of the tissue and organ sections the microbes could also be demonstrated. The histopathological alterations were suggestive of chronic myositis, along with presence of fibrous tissue in the muscles and degenerative changes as well as inflammatory reaction. The gills showing gross lesions, on histopathology revealed degeneration of necrosis and sloughing of secondary lamellae cell infiltration. The histopathological alteration of fins and tails discerned haemorrhages and necrosis with mixed lymphocytic infiltration. In kidneys, the important histopathological changes that were conspicuous were extensive accumulation of melanomacrophages around the renal tubules along with atrophy of renal tubules. Scattered focal areas of melanomacrophages were observed in some cases with atrophic renal tubes and showing interstitial nephritis.

Antibacterial sensitivity profile of microbial isolates:

The antimicrobials that are used to treat fish pathogens should be used judiously as indiscriminate use of these can lead to spread of antibiotic resistance amongst the fish population and their consumers. Any antimicrobial that is used to treat fish should be subjected to detailed pharmokinetic and toxicity studies that establishes patterns of both the parent treatment compound, any excipient and their metabolites before using them for treatment of fish and for human consumption. All the isolates of bacteria in the present study were tested against 15 antimicrobials. The oxytetracycline, chlorotetracycline, gentamicin, tetracycline and norfloxacin respectively, were best in this order on *in vitro* testing. Moderate sensitivity was observed for enrofloxacine and amoxycillin. Higher resistance was observed towards chloramphenicol, ciprofloxacin and streptomycin, whereas, ampicillin, erythromycin, penicillin-G, potassium permanganate, malachite green were least sensitive towards bacterial isolates of fish origin. The sensitivity pattern of the antifungal agents in descending order was 97.32 % for malachite green, 75.36 % for sodium chloride and 63.68 % for potassium permanganate.

Due to rapid increase in demand of fish food, intensive fish farming is in practice because of which fish fauna is stressed and susceptible to many diseases and as a result the use of antimicrobial agents has also increased significantly. Also, due to intensive aqua cultural management practices involving high stocking densities, artificial based diets containing antibiotics and frequent chemical treatments in the ponds have considerable effects on the antibiotic resistance. During this study it was found that antimicrobial resistance has increased in recent years, which may be due to any one of the above discussed reasons. *Bacillus mycoides* showed resistance to potassium permanganate in earlier years of findings but in recent years it was found resistant to penicillin-G as well as malachite green. *Micrococcus spp*. were found resistant to streptomycin, penicillin-G, amoxicillin, malachite green and potassium permanganate. Initially, *Hafnia alvei* was found resistant to malachite green and potassium permanganate but in last two years it was also found resistant to gentamycin, penicillin-G, malachite green and potassium permanganate. However, Dhar (1998), had reported resistance of *H. alvei* only to malachite green and potassium permanganate.

Previously, *Aeromonas hydrophila, A. caviae, A. sobria* were found resistant only to potassium permanganate but in past two years *A. caviae* was found resistant to streptomycin, penicillin-G, erythromycin*, A. hydrophila* and *A. sobria* to penicillin-G and malachite green in addition to potassium permanganate. Dhar (1998) found *Aeromonas spp.* resistant to potassium permanganate and sensitive to streptomycin, penicillin-G and malachite green. *Vibrio ordalli* was found resistant to ampicillin also in addition to penicillin-G, malachite green and potassium permanganate. *V. alginolyticus, V. vulnificus, V. anguillarum* were found resistant to amoxicillin, ampicillin, penicillin-G, malachite green and potassium permanganate. Similar findings were reported by Dhar (1998). *Edwardsiella ictaluri* was found resistant to penicillin-G, malachite green and potassium permanganate. In previous years as reported by Dhar (1998) *Edwardsiella spp*. was found resistant to potassium permanganate only. In the year 1998-2000 *Enterobacter agglomerans* were found resistant to malachite green and potassium

permanganate, but in 2000-2001 there has been increased resistance to gentamycin, chlorotetracycline, penicillin-G in addition to malachite green and potassium permanganate where as Dhar (1998) has reported resistance for penicillin-G, streptomycin, amoxicillin, ampicillin, malachite green and potassium permanganate.

Tanck et al. (1995) reported that the main metabolite of malachite green, leuco MG, was responsible for a decrease in haemotocrit value of rainbow trouts during first 12 hours after malachite green treatment and thus negative effect of malachite green on haematocrit value of rainbow trouts should be taken into account to treat both fungal and parasitic diseases of fish effectively. In this study, malachite green has been analysed to be the most effective against all the mycobiotic isolates recovered from the fish fauna. Acute toxicity was seen in case of malachite green in fishes if higher concentration than recommended dose in farms was used.

Pathogencity testing:

The pathogenicity of *Edwardsiella ictaluri*, *Vibrio anguillarum, Hafnia alvei, Serratia liquifaciens, Citrobacter diversus* were assessed and confirmed in common carps. Some of the isolates whose pathogenicity was tested produced lesions very much characteristics of the pathogens. In the pathogenicity trial of *E. ictaluri*, 22nd day post inoculation (DPI) the carps began to manifest symptoms like sluggish movements, reduced intake of feed etc. The visible lesions included sloughing of skin, haemorrhages, congestion and discolouration at the base of dorsal, pelvic and tail fins. The dying fish revealed typical "head up tail down" position. There was necrotic zone between the frontal bones of skull, posterior to the eyes. Post -mortem examination exhibited enlarged spleen and fibrinous deposits on the internal viscera coupled with mottled liver, engorged gall bladder, severe inflammation of kidneys, haemorrhages of adipose tissue and internal musculature. Pure culture of *E. ictaluri* was reisolated from kidneys, musculature, and pooled organs comprising of liver, heart and spleen of all the experimental fishes. The *Vibrio anguillarum* infection produced haemorrhagic and necrotic lesions in the muscles and also on the fin edges. The pathogenic potential of *E. ictaluri* and *Vibrio anguillarum* has been reported by several workers (Shotts et al. 1986; Ciembor et al. 1995). Other saprophytes tested for pathogenicity did not produce lesions. In the pathogenicity trial of mycobiotic isolates, the fungus isolated from the fish after 15th DPI was compared with the original fungus after cultivation on SDA plates. The Aspergillus and *Chrysopsporium* on pathogenicity assay produced minor disease with very less external lesions, where as *Saprolegnia* established very well when spores were introduced through injured parts in the dermis. Many of the commensals that are not otherwise directly associated with disease process, and were isolated in our study from diseased fishes were also screened for their pathogenicity in carps in fresh and clean water under controlled conditions. But we found that none of them could produce infection in these fishes which indicates that microbial diseases of fish are almost invariably stress related and establish only when host immune system or other natural barriers are disrupted.

CONCLUSION

A systematic research to identify various infections which induce morbidity and mortality among trouts and carps were undertaken by this department in the state of Himachal Pradesh, India. During this study several bacterial and mycotic diseases were identified and etiological agents characterized. The fish microflora is important not only to know the etiology of disease but it can also act as an indicator of change. The fishes have the ability to tolerate a range of conditions, but when change causes the conditions to stretch beyond the limits of tolerance, they decline or even disappear. Thus a study of hill stream fishes and their habitats and associated micro flora is a fascinating endeavor. This type of study can make us understand more about life in a habitat fraught with challenges.

In recent years, it has been observed that the microbial incidence of diseases in fish has increased due to several changes like the climatic changes, temperature which can act as stressors. Continued removal of trees, construction of roads and buildings, growing discharges of chemicals and changes in channels and flows, construction of dams, all increase stress and eventually lead to the emergence of disease in the aquatic system and also changes the habitat. During this study pathogenic micro flora which included the opportunistic pathogens were recorded. The negative findings of other important pathogens in this study do not rule out the presence of these organisms or the disease condition. This might have happened due to several reasons and it might have not been possible to isolate such organisms due to their fastidious nature or masking of their growth by other commensals or some pathogens might not have grown at the relatively low temperature specified for the fish pathogens. We have also encountered increasing number of fish pathogens revealing higher resistance to antimicrobials.

The environment plays a critical role in determining the degree to which the animal is susceptible to pathogens and occurrence of clinical disease (Flegel and Sriurairatana, 1993). Thus, proper appraisal of the relationship between host, pathogen and environment is important for understanding the cause prevention and treatment of

most aquatic animal diseases. The eradication of a disease, where it occurs, requires a programme that aims to remove diseased fishes, prevent reinfection, reduce stress and maintain optimal healthy conditions. Application of chemotherapy will provide only temporary relief from pathogens and if conditions are not improved, disease can again emerge due to stress or other reasons and fishes can again become susceptible to infections. Therefore, the best way to manage and avoid disease emergence in aquaculture is adoption of a broader approach which considers the management of the aquaculture system in a way which reduces the stress and the risk of the occurrence of disease. "Holistic" systems approach to aquatic animal health management which implies on-farm and off- farm management, within ecological limits as discussed by Phillips (1996) and (ADB/NACA, 1991) is likely to lead to sustainable solutions to aquatic disease problems.

REFERENCES

ADB / NACA., Fish Health management in Asia-Pacific, Report on a Regional Study and Workshop on Fish Disease and Fish Health management, ADB Agricult. *Dep. Rep. Ser. No.1 Network of Aquaculture Centres in Asia-Pacific (NACA)*, Bangkok, Thailand, 1991.

Anderson,D.P., Van Muiswinkel W.B., Roberson B.S., Effects of chemically induced immunomodulation on infectious diseases of fish, in *Chemical Regulation of Immunity in Veterinary Medicine* (Alan R. Liss, ed.), p.187-211,1984.

Austin, B. and Mc Intosh, D., New bacterial fish pathogens and their implications for fish farming, *Reviews-in Medical Microbiology*, 2:4, 230-236, 37 ref, 1991.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, Antibiotic susceptibility testing by a standardized single disc method. *America Journal of Clinical Pathology,* 45, 493-496. 1966.

Bragg, R.R. and Broere, J.S.E., Streptococcosis in rainbow trout in South Africa, *Bulletin-of-the-European-Association-of-Fish-Pathologists*, 6:3,89-91, 4 ref, 1986.

Brion, G.M., Mao, H. H. and Lingireddy, S., New approach to use of total coliform test for watershed management, *Water Sciences and Technology,* 42(1-2):65-69, 2000.

Buchanon, R.S. and Gibbons, N.E., *Bergey's Manual of Determinative Bacteriology*, 8th Ed. The Williams Wilkins Company, Baltimore, 1974.

Bullock, G.K., Studies on bacterial gill disease in hatchery reared salmonids, *Bureau of Sport Fisheries and Wild life*, Technical Paper, 60: 20-29, 1972.

Ciembor, P.G., Blazer, V.S., Dawe, D. and Shotts, E.B., Susceptibility of channel catfish to infection with *Edwardsiella ictaluri*; effect of exposure method. *Journal of Aquatic Animal Health,* 7 (2),132-140, 1995.

Craig, A., Shoemaker, Phillip, H., Klesius and Joyce, J. Evans., Prevalence of *Streptococcus iniae* in tilapia hybrid striped bass, and channel catfish on commercial fish farm in United States, *Amercian Journal of Veterinary Research,* 62(2), 174-77, 2001.

Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A., Medical Microbiology, Vol.2, *The practice of medical microbiology*, 12th edition, Chirchill Livingston, Edinburgh, 1070 pp, 1975.

Dhar, P., Prevalence of some bacterial associated diseases in the fish fauna of Himachal Pradesh, *M.V.Sc. Thesis*, submitted to Himachal Pradesh Krishi Vishvavidyalya, Palampur, India, 1998.

Dunier, M. and Siwicki, A.K., Effects of Environmental contaminants and chemotherapeutics on fish defense mechanism, *Fish disease diagnosis and preventions method, FAO-Project GCP/INT/526/JPN, International Workshop and Training Course in Poland, August 23-September 3, 1993.* ISBN83-901037-1-0, pg 21-51, 1993.

Evelyn, T.P.T., Bacterial kidney disease, in: *Bacterial diseases of fish*, Valerie Inglis, Ronald J. Roberts and Niall R. Bromage, eds., Blackwell Science Ltd., Cambridge, ISBN 0-632-03497-1,177-195,1993.

Ewing, W.H., McWhorter, A.C., Escobar, M.R. and Lubin, A.H., *Edwardsiella*, a new genus of Enterobacteriaceae based on a new species, *Edwardsiella tarda, International Bulletin of Bacteriological Nomenclature and Taxonomy*,15: 33.1965.

Ferguson, H.W., Ostland, V.E., Byrne, P. and Lumsden, J.S., Experimental production of bacterial gill diseases in trout by horizontal transmission and by bath challenge, *Journal of Aquatic Animal Health*, 3: 118-23, 1991.

Flegel, T.W., and Sriurairatana, S., Shrimp health management: an environmental approach, in *Diseases in Aquaculture: the Current Issues*, R.P. Subasinghe and M. Shariff , eds,, Malaysian Fish. Soc. Publ., No.8, p. 1-48, 1993.

Frerichs, G.N., Mycobacteriosis: Nocardiosis, in *Bacterial diseases of fish*, Valerie Inglis, Ronald J. Roberts and Niall R. Bromage eds. Blackwell Science Ltd., Cambridge, ISBN 0-632-03497-1, 219-234, 1993.

Hjeltnes, B. and Roberts, R.J., Vibriosis, in: *Bacterial diseases of fish*, Valerie Inglis, Ronald J. Roberts and Niall R. Bromage eds. Blackwell Science Ltd., Cambridge,ISBN 0-632-03497-1, 109-121, 1993.

Holt, R. A., Rohovec, J. S. and Fryer, J. L., Bacterial cold water disease, in: *Bacterial diseases of fish*, Valerie Inglis, Ronald J. Roberts and Niall R. Bromage eds. Blackwell Science Ltd., Cambridge,ISBN 0-632-03497-1, pp. 3-32, 1993.

Holt, John G., Noel R. Kreig, Peter H. A. Sneath, James T. Staley, and Stanley T, Williams, *Bergey's Manual of Determinative Bacteriology*, 9th ed., Williams and Wilkins, Baltimore, Md.1994.

Janakiraman, A. and Leff, L.G., Comparison of survival of different species of bacteria in freshwater microcosms, *Journal of Freshwater Ecology,* 14(2):233-240, 1999.

Janda, J.M., Abbott, S.L., Kroske-Bystrom, S., Cheung, W.K.W., Powers, C., Kokka, R.P. and Tamura, K., Pathogenic properties of *Edwardsiella* species, *Journal of Clinical Microbiology*, 29:1997-2001, 1991.

Kaattari S.L., Stress and immunity in fish, Lecture 7 - In *Proceedings of the First Nordic Sympodium on Fish Immunology*, Tromso, Norway, 1991.

Kaattari S.L. and Tripp R.A., Cellular mechanisms of glucocorticoid immunosuppression in salmon , *J. Fish Biol*., 31A, 129-132,1987.

Lemke, M.J. and Leff, L.G., Bactrerial Populations in an Anthropogenically Disturbed Stream: Comparsion of Different Streams, *Microbial Ecology,* 38:234-243, 1999.

Lemke, M.J., Brown,B.J. and Leff, L.G., The response of three bacterial populations to pollution in a stream, *Microbial Ecology,* 34:224-231,1997.

Minami, T., Nakamura, M., Ikeda, Y.,and Ozaki, H., A beta-hemolytic *streptococcus* isolated from cultured yellowtail, *Fish pathology*, 14:33-38, 1979.

Okaeme, A.N. and Olufemi, B.E., Fungi associated with tilapia culture ponds in Nigeria, *Journal-of-Aquaculture-in-the-tropics*, 12:4,267-274; 17 ref, 1997.

Olayemi, A.B., Adedayo, O. and Ojo, A.O., Microbiological studies on freshwater fishes from the Asa River, IIorin, Nigeria, Journal-*of-Aquaculture-in-the-Tropics*, 5:2, 135-139, 14 ref, 1990.

Parisot, T.J., Tuberculosis of fish, *Bacteriological Reviews,* 22: 240-5, 1958.

Peters G., Nubgen, A., Raabe, A., Mock, A., Socialstress induces structural and functional alterations of phagocytes in rainbow trout (Oncorhynchus mykiss), *Fish and Shellfish Immunology*.1, 17-31,1991.

Phillips, M.J., Better health management in the Asia-Pacific through system management, in Health Management in Asian Aquaculture. Proceedings of the regional Expert Consultation on Aquaculture Health management in Asia and the pacific, R.P. Subasinghe, J.R. Arthur and M. Shariff (eds) pHo. FAO. *Fisheries technical paper,* No.360. Rome, FAO, 142p. 1996.

Plumb, J.A., and Sanchez, D.J., Susceptibility of five species of fish to *Edwardsiella ictaluri, Journal of Fish Diseases,* 6:261-266. 1983.

Roberts, M.S., A report on enzootic in hatchery reared rainbow trout, *Salmo gairdneri* Richardson at an English trout farm, caused by *Yersinia ruckeri, Journal of Fish Diseases*, 6:551-552, 1983.

Rucker, R.R., Red mouth disease of rainbow trout (*Salmo gairdneri), Bull. de l'office Int. Epizootics*, 65: 825-830,1966.

Sarot D. A. and Perlmutter A., The toxicity of zinc to the immune response of the zebra fish Brachydaniorerio injected with viral and bacterial antigens,*Trans. Amer. fish Soc*.,105,456-460, 1976.

Scott, W.W. and O'Warren, C., Studies of the host range and chemical control of fungi associated with diseased tropical fish, *Bull. Agric. Exp. Sta.* 171:1-24, 1964.

Sharma Mandeep, Katoch, R.C., Nagal, K.B., Sambyal D.S. and Asrani, R.K., Isolation of *Yersinia ruckeri* from rainbow trout suffering from Enteric Red Mouth disease in Himachal Pradesh, India*, J. Aqua Trop*., 10: 73-77, 1995.

Shotts, E.B., Blazer, V.S. and Waltman, W.D., Pathogenesis of experimental *Edwardsiella ictaluri* infections in channel catfish *(Ictalurus punctatus*), *Canadian Journal of Fisheries and Aquatic Sciences*,43: 36-42,1986.

Tanck, M.W.T., Hajee, C.A.J., Olling, M., Haagsma, N. and Boom, J.H., Negative effect of malachite green on haematocrit of rainbow trout (*Onchorhynchus mykiss*). *Bulletin of the Fish Pathologists*. 15:4, 134-136, 4 th ref., 1995.

Taylor, P.W., Fish-eating birds as potential vectors of *Edwardsiella ictaluri*, *Journal of Aquatic Animal Health,*4: 4, 240-243.1992.

Teshima, C., Kudo, S. and Ohtani, Y., Kidney pathology from the bacterium *Hafnia alvei:* experimental evidence, *Transactions-of-the-American-Fisheries-Socitey*, 121: 5, 599-607, 6 ref, 1992.

Waltman, W.D., Shotts, E.B. and Hsu, T.C., Biochemical characteristic of *Edwardsiella ictaluri, Applied and Environmental Microbiology*, 51:101-104, 1986.

Wilhelm Sehaperclaus, *Fish Diseases*, Vol.1, Oxonanian Pres Pvt. Ltd., New Delhi pp 297, 1986.
Willumsen, B., Birds and wild fish as potential vector of *Yersinia ruckeri, J. Fish Diseases*, 12: 275-277, 1989.