Literature Review

Fish disease occurs as a result of interaction between the environment, the host and the etiological agent. Stress also plays a role in making the fish more susceptible to infection. To understand disease is to understand various aspects of biology of the host and the pathogen and their interaction. This includes the understanding of biochemical interactions between the host and the pathogen, histopathological changes and change in haematological parameters of the host and an effective and economical method for restraining the infection chain. The main purpose of this review is to present briefly the observations of previous workers in concord with the present line of investigation. Besides this, a brief history of the ulcerative disease and the epidemiology of the present outbreak has been included. The different aspects of this review are :

- History of the disease.
- Area of disease outbreak.
- Fish species affected.
- Socio-economic impact of EUS.
- Etilogical investigations.
- Environmental factors related with EUS outbreak.
- Pathology of EUS.
- Control measures.

History of the disease

The first appearance of the epizootic ulcerative syndrome (EUS) characterized by shallow haemorrhagic ulcers was reported in the year 1972 which occurred in the estuarine fish stock of central Queensland, Australia with recurrence in subsequent years (Rodgers and Burke, 1981). From the beginning of the last decade, severe disease outbreaks of a similar nature causing mass mortalities were reported regularly from all the countries of the Asia Pacific region. Some workers (Subasinghe *et al*, 1990, Balasuriya *et al*, 1990, Ram, 1992) initially referred it as the ulcerative disease syndrome (UDS). By 1985, the disease had affected Papua New Guinea, Malaysia, Thailand, Laos PDR and Myanmar (Tonguthai, 1985). In view of the importance of the problem, FAO consultation of experts meeting was held in Bangkok and the name epizootic

ulcerative syndrome was adopted. It was accepted that the condition was primarily an infectious disease of a mixed etiology. They recommended that because of the complexity of its etiology, further studies should take place into the virology, bacteriology and mycology associated with the different outbreaks (FAO, 1986). By the time of FAO meeting in 1986, outbreaks were beginning in Philippines, and by the year 1988, the disease affected Myanmar, Bangladesh and India.

Prior to the recent outbreak of epizootic ulcerative syndrome in India, several workers had reported ulcerative fish diseases occurring in Indian waters affecting mostly the Indian major carps. Gopalkrishnan (1963, 1964) reported many instances of *Aeromonas hydrophila* infections among carps in the state of West Bengal. Pal *et al* (1978) reported skin lesions in *Anabas testudineus*. Incidence of opercular ulcer disease in *Labeo rohita* at a private carp culture tank in the state of Tripura was reported by Lipton (1983). Other workers (Manohar *et al*, 1976; Pal, 1984) also reported occurrence of dermal ulcers in Indian major carps and economically important catfishes. An ulcerative form of *Aeromonas hydrophila* infection was investigated by Karunasagar *et al* (1986) which occurred in the state of Andhra Pradesh. Though on all such occasions *A. hydrophila* could be isolated from the lesions, several other bacterial forms were also found to be present as secondary invaders. In spite of several attempts, no bacteria could be isolated from the internal organs. However, there was no extensive spread of the disease and the disease condition was effectively controlled through chemical treatment. *Catla catla* had been found to be the most susceptible species to the disease. Bilateral ulcerations of the opercula and the head and sometimes deep ulcers penetrating the cranial and opercular bones were observed (Kumar and Dey, 1991).

Since the first appearance of the epizootic ulcerative syndrome in India, it was distinct by its destructive nature and capacity of affecting a wide variety of fish species in both wild and cultured waters. Conditions became so alarming that within two years of its outbreak all fishing activities came to a standstill causing tremendous concern to the fishery scientists and administrators. The disease spread alarmingly and it was accepted that no fish disease in India has

been as virulent and menacing as the recent outbreaks of the epizootic ulcerative syndrome (Das, 1997).

In the past 10 years several workers have carried out extensive research work mainly in the field of microbiology in order to define the etiological agent. Some workers (Roberts *et al,* 1993; Willoughby *et al*, 1995; Lilley and Roberts, 1997) are now convinced that the disease is caused by a typical species of the fungus *Aphanomyces* named as *Aphanomyces invaderis* and in the Regional Seminar on Epizootic Ulcerative Syndrome organized by ODA at AAHRI, Bangkok, the disease was defined to be characterized by the presence of invasive *Aphanomyces* infection. This has provided a link with other ulcerative disease caused by *Aphanomyces* spp. such as red spot disease in Australia, ulcerative mycosis of Menhaden in the south eastern coastal fisheries of the US and the mycotic granulomatosis of fresh water and estuarine fishes in Japan (Bondad-Reantaso *et al*, 1994). However, several other workers (Llobrera and Gacutan, 1987; Boonyaratpalin, 1989; Pal and Pradhan, 1990; Chakraborty and Dastidar, 1991; Torres *et al*, 1993; Leano *et al*, 1995) investigating on the bacteriology of the disease are constantly reporting findings which clearly establishes the fact that bacteria is definitely involved in the ulcerative condition of the disease. Hence, no firm conclusion could be reached and the etiology of this dreaded disease continue to baffle the fishery scientists.

Area of disease outbreak

After the initial outbreak of the EUS, the disease spread in an epizootic fashion, generally progressing from an infected area to an adjacent one. However, occasionally it was reported to spread through transportation of fish or infected materials. The pattern of spread was similar in each location. The disease breaks out with explosive synchrony in many fishes of different species from around November or after heavy rains and outbreaks continue to the end of February or March of the following year (Tonguthai, 1985). The present spread of the disease is shown in Fig 1.

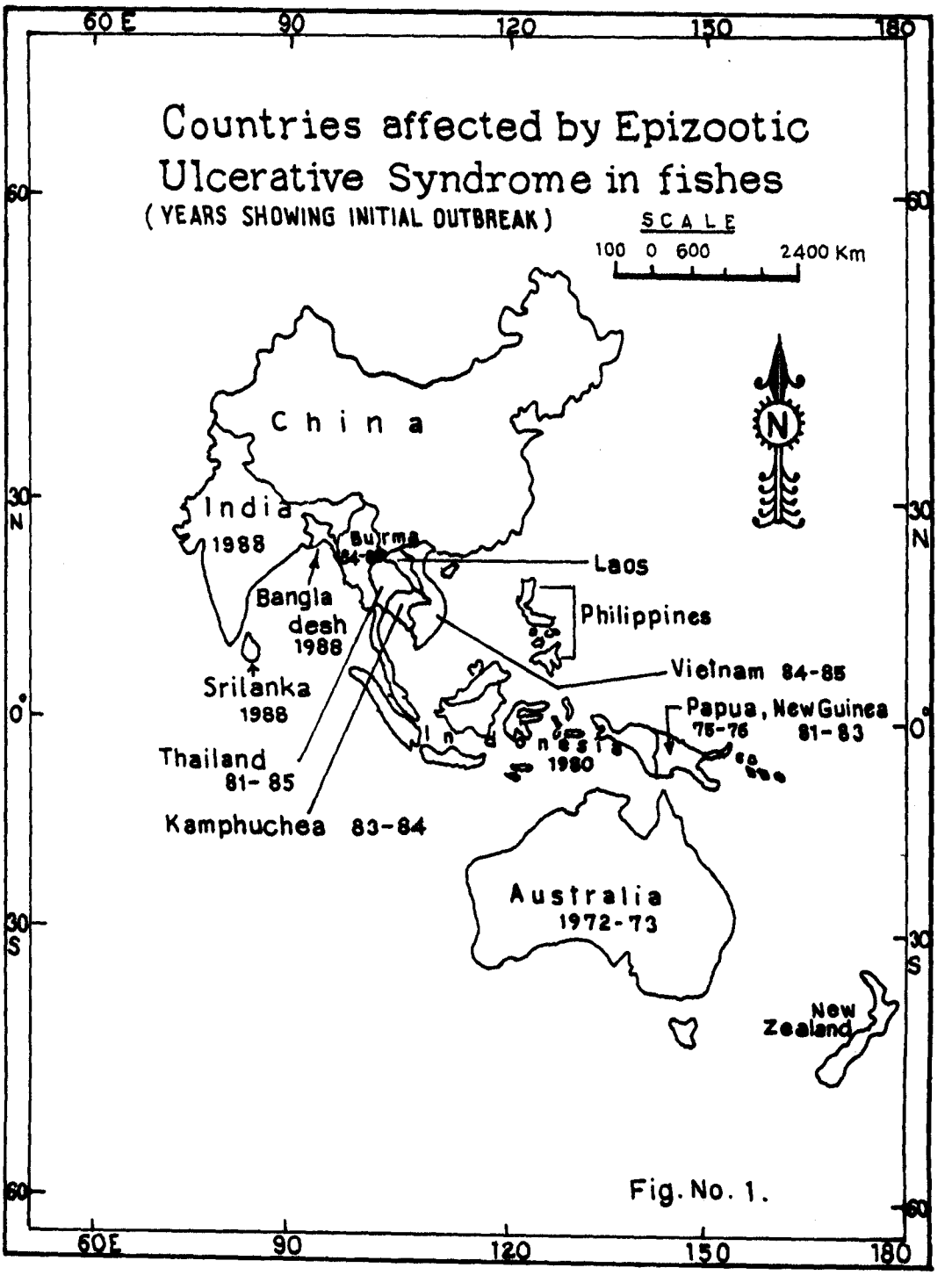
Countries affected by Epizootic Ulcerative Syndrome in fishes
(YEARS SHOWING INITIAL OUTBREAK)
SCALE
100 0 600 2400 Km
60 E
90
120
150
180
60
30 N
0°
30 S
China
India
1988
Burma
84-85
Laos
Philippines
Bangla desh
1988
Srilanka
1988
Vietnam 84-85
Papua, New Guinea
75-76 81-83
Thailand
81-85
1980
Kamphuchea 83-84
Australia
1972-73
New Zealand
N

Fig. No. 1.

After the first outbreak in Australia in the Central Queensland region in 1972, the disease progressed northwards and by 1975-'76 it affected the southern rivers of Papua New Guinea (Hains, 1983). A major outbreak was experienced in northern Papua New Guinea in 1982-'83 affecting mostly gudgeons and mullets. By this time neighbouring Indonesia also reported a severe outbreak in Bogor in 1980 (Anon, 1981) which subsequently spread to west, central and eastern Java. Occurrences of the disease in Malaysia was first reported during 1981-'83. In early 1984, the condition first manifested itself in the fishing areas of Kampuchea and southern and central parts of Laos. It recurred every year during 1980-'85 in different water bodies of Thailand (Tonguthai, 1985). EUS first occurred in the Philippines in the Laguna de Bay in December 1985 to February 1986. Between October 1988 to February 1989, EUS recurred in an epizootic form affecting wild freshwater fishes of the Laguna Lake and the Naujan Lake in Mindoro province. Paddy fields and swamps in at least 11 provinces, south and north of Luzon island was affected. During December, 1990, estuarine fishes from Buguey lagoon, Cagayan province in northern Luzon was affected (Reantaso, 1991). On the Richmond river, New South Wales, Australia, EUS prevalence was highest in those parts of the river fed by tributaries draining acid sulphate soil areas (Callinan *et al*, 1993, Callinan *et al*, 1995a). Virgona (1992) reported EUS outbreaks in the Clearance river in Australia.

Meanwhile, the extension of the disease continued westwards and by 1984-'85, wild and cultured fish stocks in Burma were affected. Fish suffering from epizootic ulcerative syndrome was observed for the first time in Srilanka in December, 1987, affecting freshwater and estuarine region of the southwestern zone (Costa and Wijayaratne, 1989). This sudden occurrence of the disease in Srilanka many years before it progressed down the Indian subcontinent suggested that infection occurred through transportation of fish and infected material by which it jumped from one area to one far away (Chinabut, 1995).

In Bangladesh, the disease was first detected around March, 1988 in the small and large water bodies of the Chandpur town in the south east. It quickly spread upstream through the Meghna river to Manikgonj and downstream to Bhola. It further extended to the west affecting the major belts like the Padma,

the Meghna and the Jamuna (Kumar *et al*, 1991, Hossain *et al*, 1992). Ahmed and Rab (1995) reported that among 257 cooperating fish farmer's ponds located in several clustered villages of Kapsia thana in the Gazipur district, 46% were affected by EUS either fully (18%) or partially (28%) during the 1991-'92 production cycle. The affected ponds occupied nearly 8.8 hectares (43%) of the total water bodies under fish culture. Rahim *et al* (1985) reported ulcerative disease in five species of brackishwater fish of Bangladesh prior to the outbreak of EUS in March, 1988.

Epizootic ulcerative syndrome was first detected in India in the month of May 1988 in some areas of northeastern India. The worst affected districts in the state of Assam were Lakhimpur, Naugao, Karimganj, Kamrup, Cachar and Silchar. Two other adjoining states namely Tripura and Meghalaya were also affected by the same time. In the month of October, 1988, outbreaks were recorded in the northern districts of West Bengal (Das, 1988; Pal and Pradhan, 1990). Gradually it spread throughout West Bengal covering all the districts except Purulia (Jain, 1990). In Bihar, the north east region adjacent to West Bengal were initially affected but it soon spread to the other districts (Prasad and Sinha, 1990). In Orissa also the disease spread to the northern districts adjacent to West Bengal namely Balasore and Mayurbhanj (Prusty and Nayak, 1990).

In India, the disease gradually spread to the states of Uttar Pradesh, Madhya Pradesh, Maharashtra, Andhra Pradesh Tamil Nadu, Kerala, Haryana, Rajasthan and Karnataka and by 1993, the disease spread all over India except Gujarat, Punjab and Jammu and Kashmir affecting various types of water bodies namely rivers, lakes, canals, reservoirs and culture ponds (Das and Das, 1993). Initially the epizootic was reported from wild waters, rivers and canals. With the entry of flood waters, the infestation covered almost the entire flood plain including low lying water logged areas, beels, submerged ponds, paddy fields, streams and ditches (Kumar *et al*, 1991). The estuaries in some areas were also found to be affected mainly in the monsoon months when the salinity is very low (<0.5 ppm) (Mohan and Shankar, 1994). In India the average percentage of incidence ranges from 4 to 15% in case of rivers and 10 to 55% in

case of confined waters (Das and Das, 1993). The details of the present spread in India is shown in Fig 2. By 1990, epizootic ulcerative syndrome was also detected in the neighbouring country of Nepal (Srestha, 1990).

EUS is endemic in many countries and is still extending its geographical range even into subtropical, subtemperate and temperate climates (Das, 1997). At present a declining trend has been observed mainly due to excessive application of various chemotherapeutic agents by cautious farmers. This has checked the severity of the disease outbreak but recurrences are reported every year. Occurrence of the disease is reported every year till today from various regions of North Bengal. Moderate to severe outbreaks are being reported from so far unaffected areas. Thus the primary source of the pathogen or the transmission factors are not yet controlled. Therefore, identification of the exact pathogen is of utmost importance to curb this dreaded disease.

Fish species affected

Freshwater as well as brackishwater species of fishes of both wild and cultured waters have been recorded to be seriously affected by the outbreak of epizootic ulcerative syndrome causing severe dermal ulceration and large scale mortality. It is unusual among fish diseases in that, when it first occurs in an area, it produces high levels of mortality in fishes of all ages over a very short time scale and it affects a wide range of species at the same time. The disease affects many species but losses occur more frequently in the snakeheads, *Channa* spp., *Puntius* spp. and in Indian major carps (Roberts *et al*, 1986).

Das and Das (1993) reported that in India, 30 species of fishes have been recorded to be affected by EUS out of which 4 species are exotic and the rest indigenous. They found that the incidence percentage was highest in the genera *Channa* (5-100%) and *Puntius* (20-100%). The other highly susceptible genera were *Mystus, Mastocembelus, Glossogobius, Anabas, Clarias* and *Heteropneustes*. The percentage of incidence was low in case of carps. An epidemiological analysis of EUS in the state of Karnataka India conducted by Mohan and Shankar (1994) revealed that the disease first affected the bottom

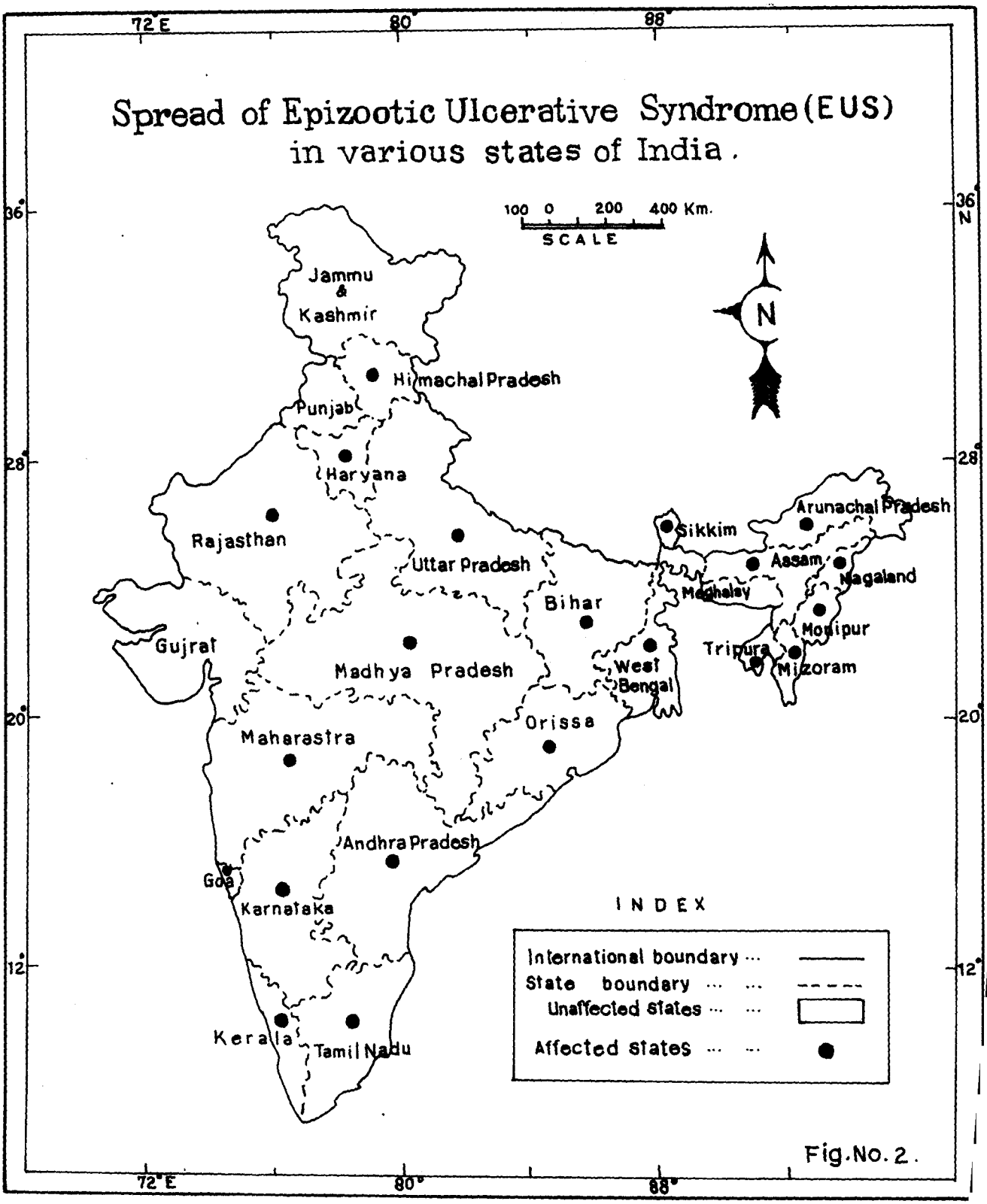
Spread of Epizootic Ulcerative Syndrome (EUS)
in various states of India.
72°E
80°
88°
36°
28°
20°
12°
36° N
100 0 200 400 Km.
SCALE
N
Jammu & Kashmir
Himachal Pradesh
Punjab
Haryana
Rajasthan
Uttar Pradesh
Bihar
Sikkim
Arunachal Pradesh
Assam
Nagaland
Meghalay
Manipur
Tripura
Mizoram
West Bengal
Gujrat
Madhya Pradesh
Orissa
Maharastra
Andhra Pradesh
Goa
Karnataka
Kerala
Tamil Nadu
INDEX
International boundary ...
State boundary ... ...
Unaffected states ... ...
Affected states ... ...

Fig.No.2.

dwelling snakeheads (*Channa* spp.). Next to be affected were the catfishes (*Mystus* spp. and *Wallago* sp.), minor carps (*Puntius* spp.), featherbacks (*Notopterus* sp.) etc. In the estuarine region bottom dwelling mullets were found to be highly susceptible to the disease followed by other species. The Indian major carps were mostly unaffected in the state of Karnataka. Other major fish species to be affected in India are *Ctenoparygodon idella, Hypophthalmichthys molitrix, Nandus nandus, Notopterus* sp. *Rasbora* sp. *Wallago* sp. *Ompak* sp. etc. (Abdul Hameed, 1996; Mukherjee, 1996) (Table 1).

In the Philippines the affected species in the Laguna Lake were snakehead (*Ophiocephalus striatus*), catfish (*Clarias batrachus*), gouramy (*Trichogaster pectoralis*), goby (*Glossogobius giurus*), crucian carp (*Carassius carassius*), Manila sea catfish (*Arius manilensis*) and silvery theraponid (*Therapon plumbius*) (Llobrera, 1987). During the outbreak of December, 1990, estuarine fish species from Buguey lagoon, Cagayan province, northern Philippines, such as mullet (*Mugil* sp.), flatfishes (*Platycephalus* sp. and *Psethodes* sp.), goatfish (*Upeneus bensasi*), croaker (*Johnius* sp.) and spadefish (*scaptophagus* sp.) were affected (Reantaso, 1991). In Australia, yellowfin bream (*Acanthopagrus australis*) and striped mullet (*Mugil cephalus*) were among the affected species (Callinan *et al*, 1995a).

In Thailand, affected fish species include snakehead fish (*Ophiocephalus striatus*), serpent fish (*Channa micropeltis*), sand goby (*Oxyeleotris marmoratus*), three spot gourami (*Trichogaster trichopterus*), striped croaking gourami (*Trichopsis vittatus*), Siamese fighting fish (*Betta splendens*) and wrestling half beak (*Dermogenus pustillus*) (Saitanu *et al*, 1986).

In a study on the ulcerative disease in Srilanka, Subhasinghe *et al* (1990) examined 19 affected fish species including *Ophiocephalus striatus, Ophiocephalus punctatus, Heteropneustes fossilis and Mastacembelus armatus.* An investigation by Ahmed and Rab (1995) showed that Thai silver barb, *Puntius gonionotus* (Bleeker) was among the most susceptible species in Bangladesh. The carps were less susceptible and the Nile tilapia (*Oriochromis niloticus* (L) was resistant to epizootic ulcerative syndrome.

Table 1 : Fish species affected by the recent outbreak (1988-1998) of the epizootic ulcerative syndrome in India.

Freshwater

Cultured	Wild
Catla catla	*Anabus testudineus*
Labeo rohita	*Heteropneustes fossilis*
L. calbasu	*Clarias batrachus*
Cyprinus carpio	*Channa punctatus, C. striatus, C. gachua*
Cirrhinus mrigala	*Chanda chanda*
Puntius javanicus	*Mastocembelus* sp.
Ctenopharyngodon idella	*Callichrous pabda*
Hypophthalmichthys molitrix	*Mystus* sp.
	Nandus nandus
	Colisa fasciata
	Puntius sophore
	Amblypharyngodon mola
	Ambassis ranga
	Glossogobius giuris
	Gadusia chapra
	Macrognathus aculeatus
	Notopterus sp.
	Mastocembelus armatus
	Rhinomugil corsula
	Trichogaster sp.
	Acrossocheilus hexagonolepsis
	Wallago sp.
	Salmostomo bacaila
	Monopterus cuchia
	Amphipnous cuchia
	Ailia coila
	Ompak sp.
	Rasbora sp.

Brackishwater

Mugil parsia	*Scatophagus* sp.
Mugil cephalus	*Epinephelus* sp.
Mugil subviridis	*Platycephalus* sp
Sillago sp.	*Etroplus* sp.

Socio-economic impact of EUS

Though there are only very few accurate estimates of losses incurred from EUS, the potential social and economic impacts of the disease are obviously great. After the first outbreak in India, investigations carried out in 5 districts of West Bengal revealed that 73% aquaculture units were adversely affected by EUS. The outbreak of the disease lowered the fish consumption rate by 28.7%, 23.3% and 20.5% in urban, suburban and rural sectors respectively. Consequently the fish trade was also affected seriously. The pecuniary loss faced by 50% aquaculturists was in the range of Rs. 1001 to Rs. 5000, while 19.73% culturists suffered a greater loss ranging from Rs. 5001 to Rs. 10,000. A section of the farmers was forced to search for alternate jobs and 88.9% fish traders also suffered losses to some extent during the affected period (Bhowmik *et al*, 1991).

In the state of Kerala, southern India, a study showed that the spread of EUS completely paralyzed the inland fish markets and threw the fishermen out of their occupation. The women fish vendors in particular were subjected to severe hardship. They had to seek alternate employment as agricultural labourers, head-load and quarry workers etc. without much success (Sanjeevaghosh, 1992).

Another study in the state of Assam situated in north east India revealed that the total loss of fish from this area had been estimated at 10,625 metric tones affecting 81,400 number of fish farmers as well as members of the fishing community (Das, 1996). Abdul Hameed (1996) reported that during February, 1994 EUS caused mortality of about 25 tones of fish valued at approximately Rs. 5.00 Lakhs in about 20 tanks in the Dharwad district in the state of Karnataka. In the rivers and irrigation tanks, large scale mortality of murrels, which is one of the highly priced fish had an adverse impact on the livelihood of the fishermen.

Though there is a lack of systematic quantification of losses owing to epizootic ulcerative syndrome, the misery of the fish farmers and the apathy of the general people towards infected fish is quite apparent. In the initial stages, a

panic was created in the affected areas and the farmers were afraid to bring the catch in the market (Sardesai, 1996).

The occurrence of the disease in India had caused a decrease in the demand even for healthy fishes. There was a false but wide spread apprehension of the disease being transmitted to human being (Jhingran, 1990).

Both the fisheries department and the health department of severely affected states took up special publicity programs through different media in order to enlighten the public about the disease. The farmers were advised to apply lime in precise doses in their ponds which would act as water purifiers. In this connection lime was distributed free of cost to the farmers (Prusty and Nayak, 1990). Among the media of communications, radio ranked first as a source of information for the farmers followed by extension functionaries of the state fisheries department (Bhowmik *et al* , 1990).

In the Philippines, the first outbreak of EUS affected 15,000 lakeshore families in Laguna lake with a 30% decrease in average daily income of fishermen (Llobrera, 1987). In Buguey lagoon, Cagayan province, EUS outbreak among estuarine fishes in December, 1990 affected 50% of the total catch, and prices of affected fish decreased to 40-60%.

During the initial outbreak, fish farmers were very hesitant to give any information about its occurrence because of the fear that this will further affect the marketability of what was left of the catch. They had resorted to slicing off affected portion of the fish and tried to market the uninfected parts. Before the 1990 outbreak among estuarine fishes, further confusion among consuming public was complicated by rumors that marine species were affected as well. On the part of the fish farmers, there was a growing fear concerning the eventual decline of the natural fish population with continual annual recurrences of EUS (Bondad-Reantaso *et al*, 1994).

In Philippines, the panic that was created mainly by the press gradually subsided with the improvement of the situation through government's education programme and the farmers learned to accept the occurrence of EUS at particular times of the year (Bondad-Reantaso *et al*, 1994).

With the outbreak of epizootic ulcerative syndrome, the problem of fish disease has drawn attention of the governing bodies and fish health

management has been noted as a major thrust area in sustainable aquaculture. Closer links between the fisheries department and the fish farmers has been established which has been beneficial not only for the problem of EUS but also for controlling fish diseases in general.

The outbreak of EUS has also necessitated research in this area. Scientists worldwide are engaged in finding out the etiology and ultimately an effective control measure to curb this dreaded disease. At present, almost the entire population in the worst affected parts of India is aware of the disease. Though they have accepted yearly occurrences, every one is waiting for favourable results of the research being undertaken through various government projects in the entire affected region.

Etiological investigations

It has been accepted that epizootic ulcerative syndrome is primarily an infectious disease, and that it is a complex condition involving certainly fungal and bacterial elements in its later stages and probably one or more viruses (Chinabut, 1995). It is also suspected that certain physico-chemical parameters of water are responsible for creating stress to the fish. The investigations carried out so far by various workers on the different probable causative agents are discussed in the following pages under the following headings : i) virus, ii) bacteria, iii) fungus and iv) animal parasites.

i) Virus

Since the spread of epizootic ulcerative syndrome is consistent with that of virus diseases, viral ulceration or other viral inhibition of defense mechanisms allowing secondary invasion by other microorganisms, it has for sometime been hypothesized virus as a possible basis for the disease (Roberts *et al*, 1993). A rhabdovirus was first isolated by Freirichs *et al* (1986) from a number of snakeheads (*Channa striata*) affected by the disease in Srilanka and suggested that this could be the initiating factor in the outbreak of the disease. Though this was an attractive proposal, virus was not isolated from more than 5% of the

diseased fishes examined and could not been shown experimentally to induce the disease in healthy fishes.

A probable new virus, designated as snakehead fish virus (SHV) was isolated by Saitanu *et al* (1986) from various species of infected fishes *e.g.* snakehead fish (*Ophiocephalus striatus*), serpent fish (*Channa micropeltes*), sand goby (*Oxyeleotris marmoratus*), three spot gourami (*Trichogaster trichopterus*), striped croaking gourami (*Trichopsis vittatus*), Siamese fighting fish (*Beta spendens*) and wrestling half beak (*Dermogenus pustillus*). The virus was not affected by ether or chloroform and was resistant at 60^0C for 30 minutes. SHV produced cytopathic effects, rounded cells and complete destruction of cell sheet on BB, BF_2 and FHM cells. Experimental infectivity studies using snakehead fish showed that SHV elicited lesions similar to those seen in naturally affected fishes. The ulcerative disease rhabdovirus isolates exhibited bullet shaped morphology and a vesiculovirus-type protein profile (Kasornchandra, 1992). Hedrick *et al* (1986) reported the isolation of a birna virus from cultured sand goby (*Oxyeleotris marmoratus*). Ahne *et al* (1988) also isolated a rhabdovirus from snakehead (*Ophiocephalus striatus*) in Thailand showing ulcerative syndrome. The virus did not exhibit any serological relationship to VHSV, IHNV, RVC, PERV or EVX.

In India, Siddhi (1989) found no cytopathic effect on snakehead cell line upto 14 days after exposure to tissue extracts of EUS affected fishes from the states of Assam, Tripura and West Bengal. Electron microscopic studies also failed to detect any viral agents. However, electron microscopic studies conducted by Kar *et al* (1990) revealed the presence of viruses in the muscle and gills of ulcerative disease affected fishes in Assam. Kumar *et al* (1991) reported that inoculum from affected *Channa* sp., *Puntius* sp. and *Mastocembelus* sp. when injected in confluent cultures of BB, FHH, EPC cell lines showed cytopathic effect within 7 days in culture. Microscopic studies showed spherical virus particles.

Freirichs *et al* (1991) reported the isolation of a retro virus which was capable of inducing cytopathological effects (CPE) in a wide variety of tissue cultures. Thus in the past decade, a number of birna viruses, rhabdo viruses

and a retro virus had been isolated from occasional fish affected with the EUS. Because of this heterogeneous nature of these isolates together with a low and inconsistent level of recovery from diseased specimens, these viruses may only represent adventitious infections unrelated to outbreaks of EUS. Moreover, experimental induction of the diseased condition in fish by any of the isolated viruses had not been achieved so far (Freirichs, 1995).

ii) Bacteria

Bacterial pathogens have been claimed to play a major role in the disease outbreak by scientists working both in India and abroad. Studies on affected fishes in different countries recorded a wide range of pathogenic bacteria isolated from the ulcerated area and the internal organs such as kidney, liver, intestine and gills of affected fishes. Das (1997) gave a brief account of the variety of bacteria isolated from diseased fishes in different states of India. Though there are differences in the bacterial species isolated from different areas, *Aeromonas* sp. is the most predominant bacterial pathogen isolated not only from India but also from other EUS affected countries.

Llobrera and Gacutan (1987) reported the consistent association of *Aeromonas hydrophila* with necrotic ulcers and lesions in snakehead (*Ophiocephalus striatus*), Thai catfish (*Clarias batrachus*), crucian carp (*Carassius carassius*) and goby (*Glossogobius giurus*) in Laguna de Bay, Philippines, from December, '85 through February '86. The bacteria were isolated from body lesions and ulcers of all fishes examined and rarely from the kidney and liver of carp and catfish.

Boonyaratpalin (1989) reported that the epizootic ulcerative syndrome involving both wild and cultured fish in Burma, Indonesia, Lao Peoples' Democratic Republic, Malaysia, Singapore and Thailand was associated with bacterial pathogens, primarily *Aeromonas hydrophila* and occasionally *Pseudomonas* sp. *Aeromonas hydrophila* was also reported to be associated with EUS affected fishes in Srilanka (Costa and Wijeyaratne, 1989). Subasinghe *et al* (1990) examined 19 species of fish from Srilanka including *Ophiocephalus striatus, Ophiocephalus punctatus, Heteropneustes fossilis*, and *Mastacembelus*

armatus and during these examinations, the consistent association of *Aeromonas hydrophila* with the haemorrhagic lesions and open necrotic ulcers on the body surface was revealed. The occasional occurrence of *Pseudomonas fluorescens* and some unidentified gliding bacteria were also found.

Various workers in India have isolated pathogenic bacteria and induced disease symptoms by inoculating healthy fishes by the pure isolates. Jhingran and Das (1990) isolated and characterized *Micrococcus* sp. from the lesions and other haematopoietic tissues of the affected fishes. They tested the transmission of the isolated bacteria *in vitro* on healthy murrels and found that manifestation of ulcers took place within 72 hours, both through inoculation and when kept in association with the bacteria. Kar *et al* (1990) found *Pseudomonas aeruginosa* in the surface muscle lesions and gill tissues.

Pal and Pradhan (1990) isolated four types of bacteria, two fluorescent Pseudomonads (R1 and R2), one Aeromonad (R3) and one *Micrococcus* sp. (C) from the skin lesions of air breathing fishes. R1 resembled *Pseudomonas fluorescens*, R2 resembled *Pseudomonas aeruginosa* and R3 was found to be *Aeromonas caviae* (Pradhan, 1992). The bacterial cultures in mixed condition induced severe ulcers in healthy *Anabas testudineus*. The pure cultures of the fluorescent Pseudomonads and the Aeromonad induced only superficial ulcers and *Micrococcus* sp. did not induce ulcer at all. Pradhan *et al* (1991) again isolated two Pseudomonads (R4 and R5) which resembled *Pseudomonas fluorescens*, one Aeromonad (R6) and another coccus (C1) from the Indian carp *Cirrhinus mrigala*.

Chattopadhyay *et al* (1990) opined that in India, an atypical *Aeromonas hydrophila* was the causative agent. They investigated on 23 fishes of various species with typical ulcers in their body. Samples from skin, ulcers, heart, blood and kidney were processed for microbiological studies using conventional techniques. *A. hydrophila* was isolated from large number of ulcerated fishes only and not from healthy fishes.

Mc Garey *et al* (1991) studied the role of motile Aeromonads in the fish disease ulcerative disease syndrome. The disease was characterized by the presence of severe open dermal ulcers on the head, midbody and dorsal

regions of the fish. *Aeromonas hydrophila* and *Aeromonas sobria* were recovered more often from the diseased fish than other bacteria and these Aeromonads were thought to play an important role in this degenerative disease.

Ali and Tamuli (1991) isolated three genera of bacteria namely *Vibrio*, *Aeromonas* and *Micrococcus* from the ulcers of 4 species of fishes (*Labeo rohita, Clarias batrachus, Channa punctatus* and *Anabas testudineus*) from Assam, India. No fungal or protozoan parasites were observed. On reinfection tests, pure cultures of *Vibrio* produced similar disease symptoms, *Aeromonas* induced only mild infection and *Micrococcus* failed to induce any disease symptom. Incidence of *Vibrio* was found to be 100% in experimentally infected fishes. Mukherjee *et al* (1991) could isolate 5 distinct strains of *Aeromonas hydrophila* from 182 samples of various species of freshwater and brackishwater fishes showing ulcerative lesions on their body surface.

Chakraborty and Dastidar (1991) reported the presence of Chemoautotrophic nocardioform (CAN) bacteria regularly and isolated the same repeatedly from different types of skin lesions of fish affected with EUS as the major or only pathogenic agent.

Aeromonas hydrophila was reported to be associated with EUS by Lio-Po *et al* (1992). They isolated several strains of *Aeromonas hydrophila* and showed that EUS like lesions could be induced on snakehead (*Ophiocephalus striatus*) and catfish *Clarias batrachus* using at least 10^6 c.f.u. / ml at 21-25^{0}C in 24 to 96 hours.

Torres *et al* (1993) conducted numerical taxonomic analysis and virulence screening of 54 *Aeromonas* isolates. Among these strains, the highly pathogenic and serologically homogeneous *A. hydrophila* came from fish exhibiting epizootic ulcerative syndrome. Two host species, *Channa striata* and *Clarias* sp., from which the greatest diversity of motile Aeromonads were recovered, were heavily affected by EUS. A virulent strain of *A. hydrophila* associated with EUS was used by Cartwright *et al* (1994) to produce monoclonal antibodies that identified virulent strains of A. *hydrophila*. Antibodies from a clone designated as F26P5C8, were found to identify the *A. hydrophila* serotype

I isolates associated with EUS fish, and which were found to be virulent after subsequent inoculation studies.

Bacteriological examinations conducted by Qureshi *et al* (1995b) on EUS affected fishes in the water bodies of Bhopal, India, revealed the presence of 9 types of bacteria; three Pseudomonads (*Pseudomonas fluorescens, P. aeruginosa* and *Pseudomonas* sp.), two Aeromonads (*Aeromonas hydrophila* and *Aeromonas* sp.) one Cytophagan (*Cytophaga* sp.) and three cocci (*Micrococcus variance*, *Streptococcus* sp. and *Staphylococcus* sp.). Out of the bacterial isolates, Aeromonads and Pseudomonads were found to be highly pathogenic while Micrococcans and Cytophagan were less pathogenic. Rest of the bacteria were found to be nonpathogenic.

Karunasagar *et al* (1995) reported the isolation of *A. sobria* and *A. hydrophila* in a typical outbreak of epizootic ulcerative syndrome during December '92 to February '93 affecting *Puntius* spp. in Karnataka, India. Both organism could be isolated from the external ulcers as well as from the internal organs and enzymatic activities indicate that they have a role in the syndrome and might contribute to fish mortality.

iii) Fungus

Since the first outbreak of the epizootic ulcerative syndrome, fungal species were consistently isolated from the lesions of affected fishes. Initially the species most frequently isolated was *Achlya* sp., *Saprolegnia* sp. (Das *et al*, 1990) and *Aspergillus* sp. (Kumar *et al*, 1991). However, attempts to show a causal relationship between the fungi isolated from such lesions and the disease failed at that time. In Philippines, *Aphanomyces* sp. was isolated from lesions of EUS affected freshwater and estuarine fishes (Reantaso, 1992; Callinan *et al*, 1993). Roberts *et al* (1993) emphasized that the very delicate and culturally demanding *Aphanomyces* sp. was the pathogen which causes so much tissue damage in this disease. They observed that this particular fungus having a typical morphology was consistently present within the lesion of fishes affected with epizootic ulcerative syndrome in countries through south and south east Asia. When a mycelium from these strains was placed below the dermis of

healthy fish, it caused inflammatory response and continued to migrate down into the tissues of the fish, inducing severe myonecrosis with chronic epithelial reaction. However, the authors did not claim *Aphanomyces* sp. to be the primary pathogen in its own right.

Willoughby and Roberts (1994) formulated a scheme for the isolation of this slow growing pathogenic strain of *Aphanomyces* from epizootic ulcerative syndrome affected fishes. Willoughby *et al* (1995) described *Aphanomyces invaderis* sp. nov. as the fungal pathogen of freshwater tropical fish affected by epizootic ulcerative syndrome. Callinan *et al* (1995b) suggested that the causative agent behind the red spot disease (RSD) of Australia was the same strain of *Aphanomyces* sp. and was responsible for massive outbreaks of epizootic ulcerative syndrome in some Asian countries.

Aphanomyces sp. is in fact a well known fish pathogen causing ulcerative mycosis of Atlantic menhaden. A salt resistant isolate was recovered and thought to be the causative agent of this disease (Hearth and Padget, 1990). Miyazaki and Egusa (1972) reported mycotic granulomatosis in gold fish and successfully isolated the specific muscle penetrating fungus from the lesions. Mycotic granulomatosis in freshwater fishes of Japan was also suggested to be caused by *Aphanomyces* sp. (Levine *et al*, 1990). In India, *Aphanomyces* infection affecting *C. mrigala* was recorded in the state of Uttar Pradesh in 1974 (Srivastava, 1979).

The investigations conducted by Chinabut *et al* (1995) on the ability of infected fungal spores of the pathogenic *Aphanomyces* sp. isolated from EUS affected fishes to induce ulcers at winter and summer temperatures showed that once the water temperature was above 25^{0}C, the fungus was rapidly eliminated by the host's inflammatory response.

Lilly and Roberts (1997) conducted experiments on the pathogenicity and culture studies of *Aphanomyces* strains isolated from EUS affected fish, saprophytic *Aphanomyces*, *Achlya* and *Saprolegnia* spp. from infected waters and further Saprolegniaceous fungi involved in other diseases of aquatic animals. Zoospores from 58 fungal isolates were given intramuscular injection to snakehead fish, *Channa striata.* The *Aphanomyces* strains isolated from fish affected by EUS, Australian red spot disease and mycotic granulomatosis were

able to grow invasively through the fish muscle and induce typical EUS lesions. The EUS *Aphanomyces* was shown to be unable to infect noble crayfish, *Astacus astacus* L. The snakehead pathogenic strains of *Aphanomyces* were distinguished from all other fungi under comparison by either characteristic temperature growth profile and inability to grow on certain fungal media like SDA, CMA and MEA.

Mohanta and Patra (1992) detected *Saprolegnia parasitica* in the infected specimens of *Anabas testudineus* in India. In another study by Karunasagar *et al* (1994) on the mycological aspects of epizootic ulcerative syndrome in India, fungi was observed in deep musculature underneath the ulcers in freshwater and estuarine fishes. However, in early stages before the development of ulcer, fungi was not observed in the area of the lesions. *Aphanomyces* and *Saprolegnia* were the most common fungi associated with the ulcerative condition. The fungal isolates could not infect uninjured EUS susceptible fishes in the laboratory. However, when placed under the scales or skin by damaging the intact skin, lesion developed which subsequently healed without treatment. They suggested that EUS in the natural environment is of complex etiology involving more than one pathogen.

Qureshi *et al* (1995a) conducted mycological examination of affected fish *Mystus cavasius* collected from Bhopal, India and observed the presence of aseptate hyphae within and around the lesions. In most of the specimens, only the hyphae of different species of fungi were found while in some cases zoosporangia of *Saprolegnia* were also seen. They isolated seven species of fungi belonging to the genera *Saprolegnia*, *Achlya* and *Aphanomyces* from the lesions of EUS affected fishes.

Histopathological studies of freshwater and brackishwater fishes affected with epizootic ulcerative syndrome from different ecosystems of Karnataka, India was conducted by Mohan and Shankar (1995). They observed numerous non septate, highly invasive fungal hyphae associated with massive tissue necrosis and therefore suggested that fungus is one of the etiological agents of epizootic ulcerative syndrome.

Presence of different types of fungi for e.g. *Saprolegnia* sp., *Dictyuchus* sp. and *Aspergillus* sp. were detected within the ulcer of EUS affected *Channa punctatus*, *Anabas testudineus* and *Catla catla* by Pal (1996, 1997). He also reported presence of a highly branched and aseptate slender fungus, most probably *Aphanomyces* sp. on the operculum of an infected *A. testudineus* and no fungus was detected at the initial phase of the ulcer formation.

iv) Animal parasites

Jhingran (1990) reported that the commonly found animal parasites associated with the ulcerative disease are *Palesintis* sp., *Trianchoratus* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., *Trichodina* sp. and *Epistylis* sp., *Tripartiella*, and several myxozoans. However, he opined that since most of the parasitic infections found on sampled fish were at a very low intensity, these parasites could not be attributed as the primary cause of ulceration.

Kumar *et al* (1991) detected Myxozoan parasites in the skin and kidney of *Catla catla* and the kidney and liver of *Clarias* sp. Mandal *et al* (1990) reported the presence of flagellated protozoan *Costia necatrix* Henneguy in association with epizootic ulcerative syndrome in fishes of West Bengal, India. In the state of Haryana, myxozoans, *Myxobolus* sp. and *Thelohanellus* sp. were reported to be associated with EUS outbreak (Ram, 1992).

Subasinghe (1993) studied the possible relationship between *Trichodina* sp. infection and induction of EUS in naive *C. striatus* by feeding with infected fish and by direct contact with water from infected environments. He observed that heavy primary infection of *Trichodina* sp. at >400 parasites per gill filament level, accelerates the appearance of EUS like lesion and ulcers when induced by feeding and cohabitation with EUS infected *E. dandicus*. He suggested that heavy *Trichodina* infection suppresses the natural defence mechanism *of C. striatus*, causing acceleration of the clinical signs of EUS. Thus secondary infections are an important contributory factor in causing mortality of EUS infected fishes.

Environmental factors related with EUS outbreak

Fish pathology is often explained in the light of sudden changes in the subtle reaction between fish, their environment and the potential pathogenic organism present in the environment. The role of environment is considered of prime importance by many authors (Snieszko, 1974, 1983; Wedemeyer *et al*, 1977, Walters and Plumb, 1980; Csaba *et al*, 1981; Ahne *et al*, 1982; Schäperclaus, 1986). But though it is not very difficult to obtain a detail knowledge of fish population and of characteristic fish pathogenic organisms, it is more difficult to clarify the combination of factors which interact mutually and are influencing these organisms.

Like other fish diseases variations in the environmental parameters are suspected to act as predisposing factors in the outbreak of the epizootic ulcerative syndrome. Roberts *et al* (1986) opined that EUS outbreaks occur in a cyclic manner when the temperature falls especially after a heavy rainfall. Lilley *et al* (1992) observed that EUS outbreak is correlated with the lowering of temperature, together with periods of heavy rainfall, low alkalinity and pH fluctuations.

In India, an extensive survey of the environmental factors in the affected states showed that incidence of disease outbreak was as high as 65% in waters of low alkalinity (13-30 ppm) and hardness (6-45 ppm) in comparison to 20% to 30% in water bodies having higher alkalinity (76-200 ppm) and hardness (62-190 ppm) (Jhingran, 1990).

Zachariah (1992) studied the effect of some physicochemical factors on EUS in the Vembanad lake in the state of Kerala, India. He opined that the significant changes noted in the water quality parameters of the EUS zone may cause the stress leading to the outbreak of EUS.

Saha *et al* (1992) reported that in the Sunderbans after a devastating cyclone in 1988, EUS was detected in many confined waters polluted by carcass of domestic animals and rotten leaves of plants.

Das (1996) mentioned that the EUS initially occurred during the summer season when the flood waters entered the pond, canal and ditches during the period of May to October, 1988 in Assam.

Abdul Hameed (1996) reported that the predominant reason of EUS outbreak during four continuous years from 1991 to 1994 in Karnataka was after major floods during monsoon from July to September.

Sardesai (1996) mentioned that the disease started occurring from last week of July to September, 1993 in Goa as salinity in estuarine water bodies at the outset of the monsoon was reaching to zero. He also reported that during 1994 and 1995 monsoon season, the disease spread in several parts of Goa affecting water bodies, in rivers, ponds etc.

Data collected by Das (1997) reveals that the selected disease prone areas in the severely affected states of India had low alkalinity and hardness but with lesser intensity. In the state of West Bengal, the disease outbreak occurs after the monsoons, at the stage of waning rainfall and onset of gradual stagnation from September and fall in winter temperatures (Das and Das, 1993).

Virgona (1992) studied the environmental factors influencing the prevalence of a cutaneous ulcerative disease (red spot) in sea mullet, *Mugil cephalus* L., in the Clarence river, New South Wales, Australia. He found significant correlation between weekly rainfall in the lower catchment and the prevalence of early stage lesion. Progress to the later stages of the disease occurred after heavy rainfall and high river flows.

Regular monitoring of water qualities by Bondad-Reantaso *et al* (1992) in the Philippines revealed that there were variations in temperature, chloride, rainfall and hardness of water at the time of outbreak. Based on experiments conducted between January 1988 to December, 1989 in Laguna Lake, Philippines, Palisoc and Aralar (1994) observed that the depth, secchi disc transparency, temperature (surface and bottom), chloride and alkalinity were significantly correlated with EUS outbreaks.

Callinan *et al* (1995a) observed that in both Philippines and Australia, EUS outbreaks in estuarine fish were reported only from estuaries having significant areas of acid sulphate soil in their catchments. They found that on the Richmond river, New South Wales, EUS prevalence was highest in those parts

of the river fed by tributaries draining acid sulphate soil areas. Localized EUS outbreaks were observed after a rainfall and was associated with pH values between 5 and 6.3. They suggested that run off water from acid sulphate soil areas may cause epidermal damage in exposed fishes, thus making the fishes susceptible to infection (Sammut *et al*, 1995).

Lumanlan-Mayo *et al* (1996) conducted field and laboratory experiments using rice field plots at the Freshwater Aquaculture Centre, Munoz, Nueva Ecija, Philippines. She suggested that low water temperature (<30^{0}C) plays a significant role in EUS outbreak. Data from initial experiments showed that rainfall and decrease in alkalinity and calcium and magnesium hardness were associated with the disease outbreak in striped snakehead, *Channa striata*, but at the onset of winter, as the temperature began to decrease, artificial maintenance of high levels of alkalinity and hardness failed to prevent the outbreak.

Influence of pesticides and other agro chemicals

High incidence of the disease in the rice field areas in India as in case of other countries especially in areas of indiscriminate pesticide application suggest that pesticides may have a role as a predisposing factor for the outbreak of the disease. Studies conducted by Kurup (1992) in the EUS struck regions of north eastern Kuttanad in Kerala, India reveal that indiscriminate pesticide application in the rice fields have aggravated water pollution problem. The concentration of pesticide in water was found to be above toxic levels and he suggested that this may create a stress condition for aquatic life and this may be a predisposing factor for EUS outbreak.

Analysis of pesticide residue in water, fish and plankton of some specific EUS affected water areas in India were carried out by Chowdhury *et al* (1994). Analysis of residues of organochlorine pesticides such as BHC, DDT and their metabolites and endosulphan, methyl parathion etc. in water bodies near rice field areas indicate that although occasionally higher concentrations of organochlorine and organophosphorous pesticides have been found in water

and fish samples, no correlation could be made with the presence of pesticide residue and disease outbreak.

Palisoc and Aralar (1995) monitored several environmental parameters in the two lakes Laguna and Naujan in Philippines for two years to detect the relationships between EUS and the environment in both lakes. Levels of pesticides (heptachloride, endosulphan, heptachlorepoxide) in sediment and water from Laguna Lake was found to be higher than those samples from Lake Naujan. Though they could not correlate the levels of pesticides with the EUS outbreak it was found that the prevalence of infection was higher in Laguna Lake than in Lake Naujan.

Pathology of EUS

Pathogenic organism bring about infections in susceptible fish, i.e., they infiltrate their tissues or cells, where they have the opportunity of continuous multiplication with the result that infection occurs and symptoms of the disease are produced.

The pathogenicity of microorganisms often involves a biochemical interaction between the pathogen and the host. Bacteria bring about pathogenic actions especially due to the effect of exotoxic substances such as haemolysin and other proteases. After infection and multiplication, the pathogens retreat to specific organs. A knowledge on the microscopic anatomy of fishes along with haematological parameters helps in understanding the pathological changes caused by the pathogen (Schaperclaus, 1986). The symptoms of the ulcerative disease and the pathology associated with the condition as observed by various workers are discussed below.

Symptoms of the disease

After the first outbreak in India, Jhingran and Das (1990) observed that the symptoms and other characteristics of the epizootic ulcerative syndrome were conspicuously different from the other low level ulcerative conditions reported earlier. It had some distinct manifestations : fishes in the rivers as well as in confined waters exhibited abnormal swimming behaviour with head

projected out of water. In the rivers abnormal swimming behaviour was observed with several fishes floating listlessly near the bank. In the initial stages of the disease, the infection usually commenced in the form of multiple inflammatory red spots on the body causing localized haemorrhage. In carps these appeared within scale pockets. In advanced stages of infection, the ulceration covered larger areas with sloughing of scales and degeneration of epidermal tissue. With further advancement of the disease, the ulcers became deep, haemorrhagic and necrotic often with a black melanistic rim. In advanced stages of the disease, large and deep ulcers were very commonly seen in all parts of the fish, especially in the head, abdomen and peduncle.

Pal and Pradhan (1990) collected 129 *A. testudineus*, 16 *H. fossilis* and 11 *Clarias batrachus* showing haemorrhagic ulcerative lesions on their body surface from the North Bengal region during the initial outbreak of the disease in the state of West Bengal, India. They observed that in case of fishes with scales, the symptoms of the disease first appeared as a red spot on the skin of the fish body. Gradually the red spot increased in size and an ulcer developed in the affected area. Ultimately the underlying muscle layer became affected and occasionally the ulcers became deep and necrotic. In scaly fishes, the mucous layer covering the scales was first affected. Red spots appeared in some regions of the body. In an advanced stage, the scales were sloughed and the ulcer became deep and necrotic. In both cases the fins were also affected.

In a study conducted by Kumar *et al* (1991) among different affected fish species throughout India, it was found that the distribution of severe ulcerative skin lesions varied between species. In murrels, the ulcerations were more pronounced and occurred mostly in the head region and the caudal areas. Sometimes the ulcerations were deeper enough exposing the cranium and resulting in greyish or red necrotic areas. In severe cases, the tail lesion could erode the affected area to such an extent that there was total loss of peduncle portion and sometimes even the erosion extended upto the posterior abdominal cavity. In *Puntius* sp. there had been usually a dark red haemorrhagic but superficial ulcer on the body side. In Indian major carps, long striped haemorrhagic lesions were found in the region of the caudal peduncle.

Different types of lesions associated with EUS in India were classified by Viswanath *et al* (1997). They used clinical and histological features to characterize three distinct type of EUS lesions among 300 EUS affected fish samples collected from affected freshwater and brackishwater bodies of the state of Karnataka, India. Type I lesion appeared as tiny red spots on the body surface with no noticeable haemorrhages and ulcerations. This lesions were of pin head size. Skin tissues around the tiny red spots were normal and there was no discolouration of the skin. Type II lesions were large (2-4 cm) and appeared as dark raised discoloured areas on the body surface. Skin and scales were relatively intact in these lesions. Type III lesions occurred as circular to oval open dermal ulcers extending into skeletal musculature. These advanced lesions were characterized with haemorrhagic and necrotic open ulcers on the body surface and were devoid of epidermis and scales.

After the first outbreak in the Philippines, in the Laguna de Bay, Llobrera and Gacutan (1987) observed that the disease was characterized by deep haemorrhagic lesions and open necrotic ulcers on the body of the fish, particularly the head, the mandible and the maxilla and the caudal peduncle regions. Erosion of the head bone tissues and the tails were observed in very severe cases.

In a study on the experimental transmission of epizootic ulcerative syndrome in snakehead, *Ophiocephalus striatus* by Cruz-Lacierda and Shariff (1995), 297 fishes of two different size groups were examined for gross pathology. The slightly affected fish (stage 1 of EUS) had small (9-400 mm^2) and flat haemorrhagic lesions with areas of scale loss and superficial erosion of the skin. Moderately affected fish (stage 2 of EUS) had small to medium sized (25-600 mm^2) lesion, and eroded skin and surface layer of the underlying muscle. Severely affected fish (stage 3 of EUS) had small to large (100-1400 mm^2) open, edematous, necrotic musculature with very foul odour. Some fish had totally eroded caudal peduncle. Most of the fish had only one lesion (43.1%) while the rest had 2 (32%), 3 (14.8%) or more than 3 lesions (10.1%). Although lesions were found on all parts of the body, they were more frequent on the head and posterior subdivisions of the body surface.

Histopathology of the skin lesion and other principal tissues

After the first outbreak in India, Kumar *et al* (1991) conducted histopathological examinations of the ulcerated areas, kidney, liver and heart of *Puntius* sp., *Mastocembelus* sp. and *Channa* sp. They found that there was complete loss of the epidermis of the skin at the ulcerated areas where dermis and hypodermis showed characteristic cyst like or nodule like granulomatous formations in abundant numbers. A high degree of inflammatory reactions involving macrophage cells and lymphocytes around some of these granuloma formations were found. Granuloma formations were also seen in the muscle layers of the skin which were degenerated and necrosed in many areas. Most of the granuloma formations seemed to contain a highly basophilic material inside. Necrotic changes in the epithelium of gill lamellae of these specimens were also found. Kidney haematopoietic tissue showed proliferation of macrophage cells especially indicating inflammatory reactions. Almost all the renal tubules and glomeruli were found necrotic having necrotic material inside their lumen. In the liver, most of the sinusoidal spaces and blood vessels were congested (Hyperaemic) and wandering lymphocytes were plenty in the liver parenchyma. Das *et al* (1990) however did not find any significant changes in the liver except vacuolization in certain cases.

Pradhan (1992) observed that the diseased fishes showed various degrees of degeneration in the liver and vacuolation of hepatocytes. In the kidney, tubular degeneration, tubular breakage and vacuolation of tubular cells were the most frequent changes. Sections of spleen showed vacuolation and necrosis in some regions.

Prasad and Qureshi (1995) observed that the epidermis of the skin of infected *Clarias batrachus* were completely destroyed and wherever it existed it showed spongiosis. The dermis lost its original compactness due to necrosis. The hypodermis appeared in the form of a network of thin fibrils.

Mohan and Shankar (1995) observed that in the early lesions non septate fungal hyphae was associated with severe necrosis of the dermis and epidermis. Acute spongiosis of the epithelium accompanied an intense inflammatory response. In advanced ulcers, massive invasion of the skeletal

musculature by the fungal hyphae was consistent with myofibrillar necrosis. The host macrophage response was found to be well organized with fibroplasma and the macrophages appeared to play a vital role in the granulomatous response in all the fishes examined. Apart from the tip of the mycelium, the mycelium inside the granuloma appeared dead with autolysed cell contents.

Pal and Pradhan (1995) studied the histopathology of experimentally infected *Clarias batrachus* with different bacteria isolated from EUS positive fishes. Histopathological examinations revealed vacuolation and necrosis in the liver, and tubular vacuolation and necrosis in the kidney. In the spleen, vacuolation and necrosis were detected in fishes treated with bacteria in mixed condition only.

Histopathology of experimentally infected *Channa striatus* (Bloch) with epizootic ulcerative syndrome associated *Aphanomyces* sp. at three different temperatures, 19^0, 26^0 and 31^0C were studied by Chinabut *et al* (1995). In the early stages of the disease, degenerative changes were observed in all samples, but inflammatory infiltrate was much more marked in fish kept at 26^0 and 31^0C. By 8 days post injection, the mycotic granulomata in the 26^0C sample were extensive and had progressed into the para-vertebral muscles on the contra-lateral side of the spine to the injection site. In the fish at 31^0C, the hyphae were starting to be walled off in a whirling pattern of macrophages. Fibrosis and many new blood vessels were observed at the site of injection in the specimens kept at 26^0 and 31^0C. The specimens kept at 19^0C showed a more limited host response. Severe myonecrosis of large areas of the myotome developed in these specimens with haemorrhages and small inflammatory foci around the fungal hyphae. The hyphae continued to progress through the myotome and had invaded the peritoneal cavity by 14 days post injection. A limited macrophage response was observed at this stage. From 14 to 28 days post injection, the healing process became well established in the specimens kept at 26^0 and 31^0C. Hyphae were walled off by a whirling pattern of macrophages. Melanin containing cells were observed in increasing numbers in the fibrous stroma as healing progressed. In the late stages of infection, regenerating muscle fibres were observed replacing the fibrous tissue. Surviving fish kept at 26^0 and 31^0C appeared to have recovered completely by 28 days

post injection. There were no signs of any healing response at 21 days post injection in the specimens kept at 19^{0}C and subsequently all the fish died. Thus mortalities in the fish kept at 19^{0}C were considerably higher than in the groups of fish kept at 26^0 and 31^{0}C.

Cruz-Lacierda and Shariff (1996) studied the progressive development of EUS lesion through histopathology of EUS positive snakehead *Ophiocephalus striatus*. Classification of dermal lesion based on gross histopathological features resulted in a series of developmental stages. Histopathologically, all stages exhibited chronic, necrotic and mycotic granulomatous response. They reported association of a highly invasive, broad (upto 24.6 μm in diameter), branching aseptate fungal hyphae with all stages. The internal organs of fish with advanced stages of EUS also exhibited mycotic granulomatous response.

In a study on EUS affected fishes in the state of Karnataka, India, Viswanath *et al* (1997) found that initially there were inflammatory changes in the sections of the epidermis. Sections stained by Grocott's methanamine silver staining technique (GMS) revealed the presence of fungal hyphae in some of the nodular structures. The fungal presence and the associated inflammatory changes were restricted to the epidermis. The dermis and skeletal musculature were free of pathological changes at the early stage of the lesion. In a later stage, they observed mycotic granulomas in the epidermis, dermis and skeletal musculature associated with numerous non septate fungal hyphae. Necrotizing dermatitis and myostisis due to fungal invasion was significant. In most of the lesions of this stage, the scales and epidermis were not completely lost. In the advanced stage of the lesion, the epidermis and scales were completely lost with partial loss of dermis at the site of ulcer. These ulcers showed mycotic granulomatous response and massive necrotic changes associated with fungal invasion in the integument and skeletal musculature.

Haematological studies

A study on the haematological parameters of EUS affected fish by Das and Das (1993) showed higher counts of phagocytic cells and reflected initiation of defence phagocytosis in blood circulation. There was a decline in counts of

erythrocytes (RBC) followed by a drop in haemoglobin content and hematocrit values which indicated anaemic condition. Another study by Prasad and Qureshi (1995) showed that there were remarkable changes in the numbers of RBC, WBC, differential count of WBC and haemoglobin content.

Akela (1995) studied the behavioural patterns of leukocyte population in *Anabas testudineus* which badly suffered from ulcerative disease. He found that bacterial ulcer caused an abrupt fall in the number of leukocytes, mainly the lymphocytes, neutrophils and monocytes in different tissues to phagocytose the pathogen. Another study by Akela *et al* (1995) on biochemical constituents of blood in ulcerated fishes revealed that there was a significant fall in blood glucose, serum cholesterol and plasma protein levels, while the blood urea level increased. He attributed the decrease in blood glucose and plasma protein levels to least or no food consumption by the diseased fishes and the increase in blood urea to necrosis or decreased rate of renal filtration.

Pradhan and Pal (1995) observed erythrocyte count and haemoglobin content of healthy *Channa punctatus* for seven days after intraperitoneal injection of four bacteria, two fluorescent Pseudomonads (R1 and R2), one Aeromonad, *Aeromonas caviae* (R3) and one coccus, *Micrococcus varians* (C) isolated from air breathing fishes. The injected fishes showed significant decrease of TEC and Hb content while the TEC and Hb content of fishes treated with coccus (C) and the control fishes showed no significant change even after 168 hours of inoculation.

In another study, Pradhan and Pal (1992) observed that a mixed culture of R1, R2 and R3 produced significant decrease in Hb content and TEC in *Channa punctatus* after 24 and 48 hours of inoculation.

Pathiratne and Rajapakshe (1995) conducted a study on the haematological parameters of healthy and epizootic ulcerative syndrome positive fish *Etroplus suratensis* collected from Srilanka. They studied the total erythrocyte and leukocyte counts, hematocrit and haemoglobin content , mean corpuscular volume, mean corpuscular haemoglobin concentration and differential leukocyte count. Results demonstrate that EUS positive fish were anaemic through loss of blood and destruction of erythrocytes as shown by a

significant reduction in the TEC, hematocrit and Hb content. In addition, the total and differential counts of severely affected EUS positive fish indicated leukocytosis coupled with marginal increase in macrophage like cell population.

Control Measures

Bacterial infections in fishes are generally considered as problems relating to stress of intensitive aquaculture. Unfavourable conditions, including overcrowding of fish, bad water quality and care less handling tend to produce poor physiological conditions and increase bacterial infections. Stress interferes with both humoral and cellular responses thus making the fish more susceptible to infection. The importance of good environmental conditions in controlling disease is well understood but further preventive measures become often necessary in case of severe outbreakes. Although some attempt has been made on the development of a suitable vaccine (Ali *et al*, 1996), an effective product is not yet available. Antimicrobial chemotherapy is still essential in controlling epizootics and is likely to remain so in the immediate future (Alderman, 1988).

The use of antimicrobial chemotherapy to control fish diseases has a long history. It has been widely accepted by aquaculturists for its high success rate, prompt action and easy mode of application. Among the wide range of antibiotics available, the aquaculturists have identified some of them to be the most effective ones. Problems including solubility, palatability, toxicity, cost etc. have limited the available antibiotics to a select few, especially in food fish culture. The chemotherapeutants most widely used in fish farms were chloramphenicol, ampicillin, amoxicillin, nitrofuran derivatives, sulphonamides, the sulphonamide and trimethoprim complex and tetracycline derivatives. More recently the fluoroquinones and the third generation cephalosporins have become popular for its low minimum inhibitory concentration values and high efficacy against bacteria resistant to other well known antibiotics (Dixon, 1994).

But unfortunately in a bid to control the disease, unprescribed treatments with a wide range of chemicals has not only failed to help the problem but has also adversely affected the fishes and in many cases has

encouraged the development of resistance of the pathogen against antimicrobial agents. So, it is very important that a correct dose be prescribed in order to ensure the efficacy of treatment and safety of the aquatic environment.

Kumar and Dey (1991) opined that selection of the therapeutic compound must be based on the following considerations : a) Effectiveness against the causative agent, b) Adverse effects if any upon the host, c) Possibility of penetrating the site of infection , d) Effectiveness under existing water quality parameters, e) Local availability, f) Cost considerations , g) Convenience of application. They emphasized that the success of therapy depended very much upon the correct diagnosis of the problem and selection and application of the correct antibiotic.

Oxytetracycline is one of the most widely used antibiotic in aquaculture throughout the world. It is a broad spectrum antibiotic with considerable activity against Gram positive bacteria. Structurally all the tetracyclines are octahydronapthacene derivatives. Oxytetracycline acts by inhibiting protein synthesis in the bacteria. It binds to the 30S subunit of the ribosome in the prokaryotic cell at several sites. It also binds to the eukaryotic ribosome but at a higher concentration. Thus eukaryotic cells are protected from oxytetracycline at the concentration which affects the bacterial cells. However, it is important that a recommended dose should be maintained in chemotherapy so as to prevent the antibiotic from affecting the host cells.

In the USA, oxytetracycline is one of the two available antibiotics approved by the United States Food and Drug Administration (FDA) for the treatement of the bacterial fish disease furunculosis (Stoffregen *et al*, 1993). Oxytetracycline and other tetracycline derivatives are among the most widely used chemotherapeutant in Japanese fish farms. Its extensive use and often abuse has led to the development of resistance of *Vibrio anguillarum* against these antibiotics (Aoki *et al*, 1987). Somsiri *et al* (1996) reported that oxytetracycline is the most commonly used antibiotic in catfish culture in Thailand. In Indonesia oxytetracycline is among the extensively used antibiotic for the treatement of bacterial fish diseases (Supriyadi and Rukyani, 1992). The tetracyclines are also widely used in the treatment of bacterial diseases in Norwegian aquaculture (Ingebrigtsen *et al*, 1985) . In Australia, oxytetracycline is

among the most commonly used antibiotic in marine finfish cage culture (Anderson, 1992). It is also among the most widely used antibiotic in the Philippines (Bacticados and Paclibare, 1992). Ackefors *et al* (1990) reported that to produce a tonne of farmed fish in 1988, Norway used 0.21Kg of oxytetracycline, Finland 0.115Kg and British Columbia, Canada 0.48 Kg.This drug is also commonly used for fish and prawn culture in Malaysia (Choo,1994).

In India, aquaculture is largely of extensive type and the available information on the use of chemotherapeutic in culture system is limited (Gopal Rao *et al*, 1992). However, with the recent emergence of large-scale, semi intensive culture of certain selected fishes in a few states, the need for proper disease diagnosis and subsequent therapy have assumed utmost priority. Severe losses are incurred annually due to disease induced mortality and growth loss. The recent outbreak of the dreaded epizootic ulcerative syndrome has added a new dimension.

According to Jhingran (1990), the Central Inland Capture Fisheries Research Institute conducted experiments employing two antimicrobial agents together such as nalidixic acid and erythromycin at the rate of 50mg per Kg body weight per day in a formulated micro encapsulated feed containing 30% protein and fortified with vitamin A and C. Trials with the pelleted feed on EUS positive fish *Puntius sophore* showed healing of ulcers. In subsequent batch of experiments, where the medicated feed was supplied for three days together with antibiotic bath (chloramphenicol @15 ppm), total recovery was observed.

Jain (1990) reported that the use of antibiotics like erythromycin @ 200 mg/Kg supplimentary feed had been observed to control UDS in government farm at Kalyani, West Bengal, India. He also reported that another medicine having the composition of trimethoprim and sulphamethoxazole was effective when given along with supplimentary feed to the fish in some ponds near Nimpith Ramkrishna Mission Krishi Bigyan Kendra in the district of South 24-Parganas. Purkait (1990) reported that antibiotics like terramycin @ 200 mg/ Kg feed had impede the disease within 1-2 weeks in some areas of Chanditala in Hooghly district, West Bengal.

Kumar *et al* (1991) however observed that terramycin at the rate of 100 to 135 mg/Kg/fish/day did not show any effect on the diseased condition of

epizootic ulcerative syndrome affected fishes. Mahapatra *et al* (1996) reported that antibiotic tablets (trimethoprim and sulphamethoxazole) were given to the infected fish mixed with the daily supplimentary feed @ 100 mg per Kg feed for 10 days which successfullu controlled EUS in fish. Das (1997) reported that in general, antibiotic either erythromycin or oxytetracycline at 60 to 100 mg per Kg of feed for seven days cured the ulcers of the diseased fishes.

The choice of oxytetracycline as an antibiotic for treating the diseased fishes may be justified by the fact that it has a very short half life in both fresh water and sea water. Choo (1994) studied the degradation of oxytetracycline hydrochloride under tropical conditions in fresh and sea water in plastic tanks placed in a hatchery. Reverse phase HPLC technique was used for the detection of oxytetracycline. The half-life of oxytetracycline hydrochloride in fresh water (average pH 7.9, average temp. 27^{0}C, under normal lighting) was found to be 298 hours. He opined that due to its short half-life in water and dilution from the aquatic environment, the danger posed by oxytetracycline in water is probably negligible. Oxytetracycline cannot be considered persistent in the aquatic environment as epimerisation and photolysis rapidly degrades oxytetracycline. However, though oxytetracycline does not persist in water; it has been found to persist in sediments and could pose adverse effect to environment (Jacobsen and Berglind, 1988; Samuelsen, 1989).

Björklund *et al* (1990) studied the residues and persistence of oxytetracycline in wild fish and sediments on two fish farms after chemotherapy of farmed fish. They found a wide difference among the two fish farms. The farm which was exposed to water currents showed low half-life (9 days), while the other farm where the condition were more or less stagnant showed much higher half-life (419 days). The drug was administered to fish through its diet. They suggested that much of the drug intended for curing the fish passed unchanged into the environment and bottom deposits of the fish farm. This was due to the fact when fish suffer from bacterial diseases, they usually show reduced feeding. Also oxytetracycline is shown to be very poorly absorbed from the intestinal tract of fish (Gravedi *et al* , 1987).

Elema *et al* (1996) investigated on the bioavailability of oxytetracycline hydrochloride in Atlantic salmon in sea water. They administered the antibiotic through intramuscular injection of 20 mg Kg^{-1} body weight and force feeding with medicated feed containing a dose of 50 mg Kg^{-1} body weight. They found that the oral bioavailability was only 2%.

Oxytetracycline residues in the tissues were measured by Xu and Rogers (1994) in stripped bass Morone saxatilis that had been injected intraperitoneally with 50 mg oxytetracycline/ Kg or had been fed a ration containing 75 mg oxytetracycline/Kg of fish daily for 10 days. Concentration of oxytetracycline in the muscle of fish fed medicated feed was below the quantitation limit in all fish after 16 days at 23^0C. The calculated terminal elimination rate constant (beta) was 0.20/day (range, 0.15-0.37/day), and the half-life was 2.7 days (range, 1.9-4.5/day). The concentration of oxytetracycline in the muscle after intraperitoneal injection was below the quantitation limit after 24 days at 23^0 C. The beta value was 0.34/day and half-life was 2.0 days in fish injected with oxytetracycline intraperitoneally.

Somsiri *et al* (1996) carried out investigations on the accumulation of oxytetracycline in hybrid catfish (*Clarius gariepinus* x *C. macrocephalus*) bathed in OTC at the concentrations of 1, 3 and 5 ppm. The other groups of experimental fish were fed with pellets mixed with OTC at the doses of 1, 3 and 5 mg / Kg. After a 13 weeks period, fish fed with 3 and 5 mg /Kg of feed had high concentration of oxytetracycline in serum, muscle, kidney, spleen and liver. No OTC was detected by bioassay technique in serum, muscle, kidney, spleen and liver in bath exposure groups and fish fed with 1mg /Kg of OTC in the feed. After oxytetracycline was withdrawn from the system for seven days, OTC was not detected in any experimental fish.

Oral administration of antibacterial agents incorporated into feed permits the treatment of large quantities of fish relatively easily with minor labour costs and has become the prime route of medication. However, diseased fish tends to show reduced appetite. This gives rise to a situation where only the healthy individuals within a population that are still feeding are likely to be protected by the antibacterial. Bath treatment offers an equal dose to both healthy and diseased fish but is restricted to tanks of limited size. Therefore,

bath treatment may be an alternative in treating small fish, and where the feed uptake is minimal because of infection or low temperature or in the early stages of development (Samuelsen *et al*, 1997). Moreover, the unused feed falls to the bottom and poses greater threats to the environment. Due to low bioavailability of oxytetracycline, the concentration of the antibiotic should be high enough in the feed so that the treatment is efficacious. Thus application and often uncontrolled application of oxytetracycline through the feed not only causes development of resistance against the antibiotic (Chowdhury and Inglis, 1995) but also poses threat to the immune system of the aquatic fauna (Rijkers *et al*, 1980; Grondel and Boesten, 1982).

Read (1985) found that salamandors with skin ulcers responded very rapidly to a bath of 50 mg per litre of oxytetracycline. Soltani *et al* (1995) determined the *in vitro* and *in vivo* efficacies of commonly used chemotherapeutants for *Cytophaga johnsonae, Cytophaga psychrophila, Flexibacter columnaris* and *Flexibacter maritimus*. He studied the efficacy by both bath and feed medication against the infections. The correlation of *in vitro* and *in vivo* results indicated that oxytetracycline was useful against *F. columnaris* and *C. psychrophila*. He further observed that the bath treatment gave higher survival (8%) than feed medication, because the infected fish either did not take the feed medication or became anorexic immediately post challenge.