Review of Literature

Disease is a function of more than one factor which includes host, parasite and environment. To produce disease a pathogen must overcome host defenses. The host-parasitic interaction with fish, unlike that with homeothermic animals, is strongly influenced by the environment. As fish is an ectothermic aquatic animal, it is very much susceptible to environmental stresses compared to the homeothermic animals. Sublethal changes in water environment make fish more susceptible to pathogens and this is true in case of Epizootic ulcerative syndrome also. To understand any disease it is imperative to understand the different aspects of host biology which include histopathological changes, heamatological changes and immunological status.

The main purpose of this review is to assemble in brief the observations of previous workers in view of understanding the present state of epizootic ulcerative syndrome. The main features of this review are:-

- History of the disease.
- Fish species affected.
- Socio-economic impact.
- Etiological investigations.
- Environmental factors.
- Signs of the disease.
- Management of the disease.
- Lympho haemopoietic organs.
- Cytological studies.
- Haematological studies.
- Immunological studies.

History of the disease

Prior to 1971, there was no report of the out break of Epizootic Ulcerative Syndrome, in different parts of Asia and Asia-Pacific region. In 1971 from Japan an ulcerative condition of fish was first reported in farmed ayu (*Plecoglossus altivelis)* in

Oita prefecture and it was named mycotic granulomatosis (MG) (Egusa and Masuda, 1971). In 1972 a similar ulcerative disease in fish was reported from Central Queensland, Australia with recurrence in subsequent years and the disease was known as red spot disease (RSD) (Rodgers and Burke, 1981).

FAO consultation of experts meeting was organized in Bangkok where the name Epizootic ulcerative syndrome was adopted and it was also accepted that the condition was primarily an infectious disease of mixed etiology (FAO, 1986).

During 1975-76 the ulcerative disease spread to Papua New Guinea (Haines, 1983). Anon (1981) reported the outbreak of EUS from Java in 1980. Subsequently, the disease spread to Indonesian states like Sumatra, Swaweri and Kalimantan (Wadagdo, 1990) as well as in northern Malaysia (Jothy, 1981). Thailand first witnessed the outbreak of EUS in 1981 with subsequent recurrences (Ulcerative Fish Disease Committee, 1983; Chulalongkorn University, 1983, 1985, 1987; Tonguthai, 1985). During 1983-84 the disease entered Myanmar, Laos PDR and Cambodia from Thailand and Malaysia (Roberts et al, 1986; Lilley et al., 1992). A severe outbreak of EUS in December 1985 was reported from Laguna de Bay in the Philippines affecting snakeheads, gobies, gouramies, catfish, crucian carp etc. (Llobrera and Gacutan, 1987).

China (Lian, 1990; Guizhen, 1990), Vitenam (Xuan, 1990) and Hong Kong (Wilson and Lo, 1992) also witnessed the outbreak of EUS. Outbreak of EUS first took place in Sri Lanka in December 1987 (Costa and Wijeyaratne, 1989).The disease entered into Chandpur district of Bangladesh crossing the Myanmar in February 1988 (Kar and Dey, 1990; Roberts et al, 1992; Hossain et al, 1992; Ahmed and Rab, 1995; Ahmed and Hoque, 1998). According to Rahim et al. (1985), five species of brackish water fish of Bangladesh were affected with ulcerative disease prior to the outbreak of EUS in February 1988.

India first experienced the outbreak of epizootic ulcerative syndrome in the month of May 1988 in some states of northeastern India. Navgao, Karimganj, Kamrup, Cachar and Silchar areas of Assam were initially affected. Of the affected areas of Assam severe outbreak of EUS was reported from Borak valley which is situated in the districts of

Cachar and Karimganj. In the same year i; e in 1988 two adjoining states of Assam such as Tripura & Meghalaya were also affected.

After a few months, in October 1988 the disease spread to some northern districts of West-Bengal (Das, 1988; Pal and Pradhan, 1990). In the same year the disease extended to some southern districts of West-Bengal such as Nadia, Murshidabad and 24 Parganas and Midnapore (Pradhan and Pal, 1990). Slowly the outbreak of EUS spread to almost all the districts of West-Bengal except Purulia (Jain, 1990). From West-Bengal the disease first spread to the adjacent districts of Bihar like Katihar and Kishanganj but very quickly it spread to the other districts of Bihar (Prasad and Sinha, 1990). In Orissa also the disease spread the way it had spread in Bihar starting from adjacent districts of West-Bengal such as Balasore and Mayurbhanj to all the districts of Orissa (Prusty and Nayak, 1990).

From North-east India the disease gradually spread to Uttar Pradesh, Madhya Pradesh, Maharastra, Tamil Nadu, Andhra Pradesh, Kerala, Hariyana, Rajasthan and Karnataka. By 1993, the disease spread to almost all the states of India except Gujarat, Punjab, and Jammu and Kashmir (Das and Das, 1993). Though the disease first appeared in wild water of rivers and canals but in the long run it affected all types of water bodies like reservoirs and culture ponds.

In spite of declining trend of EUS in recent years the occurrence of the disease is taking place every year till today especially during the winter months in various areas of North-Bengal. Occurrence of the disease in every winter suggests that lowering of temperature during winter months may bring on some degree of changes in the immune status of the fish. So it is a crying need to explore the immune status of fish for providing better management of the disease.

Table 1. Fish species affected by EUS in India

Freshwater

Cultured	Wild
Cirrhinus mrigala	*Anabas testudineus*
Catla catla	*Heteropneustes fossilis*
Labeo rohita	*Clarias batrachus*
L. calbasu	*Channa punctatus, C. striatus, C. gachua*
Cyprinus carpio	*Chanda chanda*
Puntius javanicus.	*Mastocembelus* sp.
Ctenopharyngodon idella	*Callichrous pabda*
Hypophthalmichthys molitrix	*Mystus* sp.
	Nandus nandus
	Colisa fasciata
	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.

Fish species affected

A large number fish species, freshwater as well as brackish water has so far been confirmed by histological diagnosis to be affected by EUS. Surprisingly, it not only produces high mortality in fishes of all ages within a very short period, but also it attacks a huge number of species of fish of both wild and cultured water.

More than 100 fish species have so far been found to be affected by EUS (Lilley et al., 1992). Das and Das (1993) reported that in India, more than 30 species were affected by EUS. But among those 30 species, 26 are indigenous fish species and remaining 4 species are exotic. Along with very susceptible fish species under the genera *Channa, Puntius* and *Osphronemus,* the other susceptible genera are *Mystus, Mastocembelus, Glossogobius, Anabas, Clarius* and *Heteropneustis.* Indian major carps are also affected frequently by EUS. Among the Indian major carps, *Cirrhinus mrigala, Catla catla* and *Labeo rohita* are the most common victims of EUS (Jhingran and Das, 1990; Pal and Pradhan, 1990; Kumar et al., 1991; Abdul Hameed, 1996; Mukherjee, 1996; Das, 1997). However, Saha (1998) reported that at least 46 fish species were found to be affected by EUS in India.

In Philippines the fish species which were affected badly in a large scale, were snakehead (*Ophiocephalus streatus),* catfish (*Clarias barouches)* gorily (*Trichogoster pectoralis),* goby (*Glossogobius giurus),* crucian carp (*Carassius carassius),* Manila sea catfish (*Arius manilensis)* and silvery theraponid (*Therapon plumbius)* (Llobrera, 1987). Brackish water fish species or estuarine fish species affected during the outbreak of December 1990, in Brgueey lagoon, Cagayan Province, northern Philippines, were mullet (*Mugil* sp.), flatfishes (*Platyceptralus* sp. and *Psethodes* sp.), goat fish (*Upeneus bensan),* Croaker (*Johnius* sp.) and spad fish (*Scaptophogus* sp.).

In Australia yellow fin bream (*Acanthopagrus austtralis)* and striped mullet (*Mugil cepholus*) were the most susceptible fish species (Calinan et al., 1995a). The affected fish species in EUS outbreak in Thailand included snakehead fish (*Ophiocephalus strialies*), serpaint fish (*Channa micropeltis),* sand goby (*Oxydeotus marmoratus),* three spotted gourami (*Trichogaster trichopterus),* striped croaking gourami (*Trichopsis vittatus),*

Siamese fighting fish (*Betta splendens)* and wrestling half beak (*Dermogenns pustillus)* (Sailendri et al., 1986).

In Sri Lanka, Subhasinghe et al. (1990) found 19 affected fish species which included *Ophiocephalus striatus, Ophiocephalus punctatus, Heteropneustes fossillis* and *Mastacembelus armatus* etc. Ahmed and Rab (1995) from Bangladesh reported that Thai Silver barb, *Puntius gonionolus* was the most susceptible fish species.

Most surprisingly, three culture species of fish such as Nile tilapia, milkfish and Chinese carp have been shown to be resistant to EUS.

Socio-economic impact

There is hardly any confusion about the huge social damage and economic loss caused due to outbreak of Epizootic ulcerative syndrome or EUS. Estimations of situations in five districts of West-Bengai gave an indication of the actual depth of damage, both social and economic, done by Epizootic ulcerative syndrome or EUS. It showed that 73% of aquaculture units were badly affected by EUS and decline in fish consumption rate by 28.7%, 23.3% and 20.5% in urban, suburban and rural sectors respectively resulting a sharp decline in fish trade. More or less 70% aquaculturists suffered a huge economic loss, 50% of them incurred a loss amounting a range of rupees 1001 to 5000 and remaining 20% culturists suffered a greater loss amounting a range of Rs. 5001 to Rs 10,000. A section of fish farmers searched for alternate job because from fish farming they could no longer manage their livelihood. Almost 90% fish traders were affected and suffered economic loss to some extent during affected period (Bhowmik et al., 1991). Among the southern states of India, Kerala was the worst affected where outbreak of EUS almost paralyzed the inland fish markets. Consequently, a huge number of fishermen became jobless. The women fish vendors in particular were the worst sufferers (Sanjeevaghosh, 1992). A report from Abdul Hameed (1996) stated that during February, 1994 EUS caused mortality of 25 tones of fish valued at approximately Rs 5.00 lakhs in about 20 tanks in the Dhanwad district in the state of Karnataka. The report also

informed the large scale mortality of murrels in the rivers, pond etc. In Goa farmers incurred a loss of 20 metric tones fish which valued Rs 8.00 lakhs in 1993. Fish losses in the same state i.e. in Goa were 30 metric tones amounting to as 12.0 lakh and 15 metric tones amounting Rs 6.0 lakh in 1994 and 1995 respectively (Sardesai, 1996). Study in Assam, one of the north-eastern states of India, showed that a total loss of fish owing to EUS had been estimated at 10,625 metric tones affecting 81,400 numbers of fish farmers (Das, 1996). Fish trade in Bihar experienced the loss of fish which valued about Rs 48.00 lakhs during 1989-90 when initial outbreak took place (Prasad and Sinha, 1990). Orissa also suffered a loss of Rs 30.0 lakhs during 1989-91 (Das, 1994). Epizootic ulcerative syndrome or EUS has not only caused a huge damage in fish farming in India, but it has also affected fish farming in other South-eastern countries.

Llobrera (1987) estimated the huge economic and social impact owing to outbreak of EUS in Philippines. Lake shore peoples were the worst sufferers. Near about 15,000 lakes shore families in Laguna Lake were badly affected resulting a 30% decline in average daily income of fishermen. The situation was worse around 5000 lakes in Margabol Swamp in Pangasian province of Philippines where at least 75,000 people were affected incurring a loss over 50% and 40% during 1989 and 1990 outbreaks of EUS respectively. Not only the huge economic loss faced by fishermen and lake shore people, even a panic spread far and wide among the consumers and farmers in Philippines.

Outbreak of EUS in Sri Lanka also compelled the fishermen and fish traders to incurr a heavy economic loss which amounted to Rs 1 million during 1988-89 (ADB/NACA, 1991) and increased upto Rs 20-40 million in Sri Lankan currency (Balasuriya, 1994).

Spread of EUS had more than one fold negative influence in societies of different south-east Asian countries. It created a huge panic and unprecedented fear of disease transmission from fish to human among common people resulting a sharp decline in the demand of fish. It had heavily affected the socio-economic status of fish farmers and fish traders in Bangladesh (Rahaman et al., 1988). Consequently, fall of price of fish upto

75% led to economic loss of about 118 million and 88.2 million taka in Bangladesh in the year 1988 and 1989 respectively (Barua, 1990).

During 1982-83 in Thailand the economic loss due to EUS was upto 200 million in Thailand currency (Tonguthai, 1985) and over ten years i.e. from 1983-93 the loss was about 3600 million in Thailand currency (Chinabut, 1994).

Our neighbouring countries like Nepal and Pakistan were also no exceptions. During 1989-90 the total economic loss in eastern Nepal only was about Rs 30 million (ADB/NACA, 1991). In Pakistan the fishermen and fish traders were also badly affected. In 1996 the total economic loss incurred was about Rs 15 million (AAHRI, ACIAR, 10A and NACA 1997).

Etiological Investigations

Opinions differ regarding the exact etiology of epizootic ulcerative syndrome and it has been accepted that epizootic ulcerative syndrome is a complex condition involving certainly fungal and bacterial elements in its later stages and probably one or more viruses (Chinabut, 1995). Some environmental factors also play the role of predisposing factors of EUS. Naturally, etiological studies have been the subject of major interest for ichthyologists in the countries where the outbreak of EUS has occurred. A lot of works have been carried out by different researchers on the role of various etiological agents, such as viruses, bacteria, fungi and animal parasites on the outbreaks of the EUS.

Virus

Virus like particles was detected in different tissues of affected fish during 1982-83 outbreaks in Thailand (Rattanaphani et al., 1983; Wattanavijarn et al., 1983 a, b, 1984). Rhabdovirus was isolated from diseased fish in some south-east and south Asian countries by Frerichs et al. (1986, 1989). They suggested that it could be the initiating factor in the outbreak of EUS. But Frerichs et al. (1986, 1989) could isolate the virus from not more than 5% fish examined and the virus could not induce the disease in

healthy fish experimentally. Saitanu et al. (1986) isolated a new virus, snakehead fish virus (SHV) from infected *O. striatus, C. micropeltes, Oxyeleotris marmoratics, T. trichopterus, T. vittatus* etc. This virus was not affected by ether or chloroform and was found resistant at 60°C for 30 min. SHV also produced cytopathic effects, rounded cells and complete destruction of cell sheet on BB, BF_2 and FHM cells. Hedrick et al. (1986) from cultured sand goby (*Oxyeleotris marmoratus)* and Subramaniam et al. (1993) in Singapore from infected fish isolated birna virus. Ahne et al. (1988) also isolated a rhabdovirus from snakehead (*O. striatus)* in Thailand. He also showed that this rhabdovirus was serologically different from VHSV, IHNV, RVC, PERV or EVX. Rhabdoviruses were also isolated from the diseased fish collected from Thailand, Myanmar, Australia (Roberts et al., 1989; Roberts et al., 1994; Lilby and Frerichs, 1994) and the viruses were named as ulcerative disease rhabdovirus (UDRV).

Siddhi (1989) carried out virological studies on EUS affected fish species in Assam, West-Bengal and Tripura and found no relationship of any virus with EUS. Electron microscopic studies by Kar et al. (1990) showed the presence of viruses in muscles and gills of ulcerative disease affected fish in Assam.

Bacteria

The constant and relentless search for pathogens responsible for outbreak of EUS by different scientists from different affected species of fish has led the isolation of a number of bacteria from the ulcers as well as the internal organs such as kidney, liver, spleen etc. Though the bacteria isolated from infected fishes by different workers differ, yet it has been established that *Aeromonas sp.* is the most predominant pathogenic bacterium.

Llobrera and Gacutan (1987) showed the consistent association of *Aeromonas hydrophila* with necrotic ulcers and lesions in snakehead (*Ophiocephalus striatus)*, Thai catfish (*Clarias batrachus)*, crucian carp (*Carassius carasius)* and goby (*Glossogobius giurus)* in Laguna de Bay, Philippines, from December, 1985 to February, 1986. They also isolated *Aeromonas hydrophila* from body lesions as well as from internal organs from affected fish. Later Boonyaratpalin (1989) found the association of primarily

Aeromonas hydrophila and occasionally *Pseudomonas sp.* with the outbreak of EUS in Burma, Indonesia, Lao Peoples Democratic Republic, Malaysia, Singapore and Thailand. Association of specially *Aeromonas hydrophila* was also established with EUS affected fishes in Sri Lanka (Costa and Wijeyaratne, 1989). During the extensive examinations of 19 species of EUS affected fishes such as *Ophiocephalus striatus, Ophiocephalus punctatus, Heteropneustes fossillis* and *Mastacembelus armatus* etc. in Sri Lanka. Subashinghe et al. (1990) showed the consistent association of *Aeromonas hydrophila* and occasional association of both *Aeromonas sp.* and *Pseudomonas sp.* with haemorrhagic lesions and open necrotic ulcers on the body.

Jhingran and Das (1990) had been able to induce the haemorrhagic ulcers inoculating pure bacterial isolates in healthy murrels within 72 hours after inoculation. Kar et al. (1990) also isolated *Pseudomonas aeruginosa* from the surface muscle lesions.

Four types of bacteria, two fluorescent Pseudomonads (R_1 and R_2), one Aeromonad (R_3) and one *Microceus sp.* (C) were isolated from skin lesions of air breathing fishes by Pal and Pradhan (1990) where R1, resembled *Pseudomonas fluorescens,* R2 resembled *Pseudomonas aeruginosa* and R3 showed strong resemblance with *Aeromonas caviae* (Pradhan, 1992). When a mixed culture of bacteria was inoculated, severe ulcers were produced but pure cultures of the fiuorescent Pseudomonads and Aeromonad induced only superficial ulcers while pure culture of *Micrococcus sp.* did not produce any ulcers. Pradhan et al. (1991) isolated two Pseudomonads (R_4 and R_5) which resembled *Pseudomonas fluorescens,* one Aeromonad (R_6) and another coccus (C_1) from the Indian freshwater major carp, *Cirrhinus mrigala. Aeromonas hydrophila* was only isolated from EUS affected fishes of more than 70 species by Chattopadhya et al. (1990). Several researchers in India and abroad reported associations of bacterial pathogens with EUS (Mc Garey et al., 1991; Ali and Timuli, 1991; Mukherjee et al., 1991; Lio-Po et al., 1992). Chakraborty and Dastidar (1991) repeatedly isolated chemoautotrophic nocardiofarm (CAN) bacteria from different types of skin lesions of EUS affected fishes.

Torres et al. (1993) performed virulence screening of 54 species of *Aeromonas* and found the *Aeromonas hydrophila* was the most pathogenic. Qureshi et al. (1995) also

performed the virulence test of eight bacterial isolates from EUS affected fishes and found *Aeromonad* and *Pseudomonad* were highly pathogenic while micrococcus and cytophogans were less pathogenic. Lio-Po et al. (1998) isolated four species of bacteria from EUS affected fishes from Philippines and Thailand and *A. hydrophila* was proved to be most pathogenic. Saha and Pal(2000) isolated sixteen (16) strains of bacteria belonging to the genus *Pseudomonas*, *Aeromonas*, *Micrococcus*, *Bacillus*, *Moraxella* and *Vibrio*. Out of these 16 strains belonged to Pseudomonad and Aeromonad were found to be pathogenic.

Fungus

Fungal species were consistently isolated from lesions of EUS affected fishes.

Different fungal species were isolated from the lesions of affected fishes, of which *Achlya sp.* and *Saprolegnia sp.* were the most common (Pichyangkura and Bodhalamik, 1983; Limusuwan and Chinabut, 1983).

Roberts et al. (1993) first isolated fungus, Aphanomyces from EUS affected fish of Thailand. An inflammatory response and severe myonecrosis were observed after the inoculation of a mycelium from this fungal strain below the dermis of healthy fishes. Chinabut et al. (1995) reported that at 19°C the fungal species induced more pathogenicity than 26°C and 31°C temperature.

Willoughby et al. (1995) first named the fungus *Aphanomyces invaderis*. Miyazaki and Egusa (1972, 1973a, 1973b and 1973c) were the first to isolate the fungus from affected fish in Japan. Their efforts did not draw the attention of wider community of scientists as their publications were entirely in Japanese (Chinabut, 1995). Hatai et al. (1977) isolated a fungus from fish *Plecoglossus altiveliss* from Shiga Prefecture, Japan. It was named *Aphanomyces piscicida* (Hatai, 1980).

Later involvement of *Aphanomyces sp.* was also reported from Australia, Philippines, Indonesia and Bangladesh (Fraser et al., 1992; Paclibare et al., 1994; Callinan et al., 1995a, b; Lilley and Roberts, 1997).

Analysis of protein banding profiles (Callinan et al., 1995b; Lilley et al., 1997b), growth characteristics (Lilley and Roberts, 1997) and chemical susceptibility (Lilley and Inglis, 1997) of *Aphanomyces sp.* isolated from EUS affected fishes in different countries showed that the same *Aphanomyces sp.* was involved in each case and finally the species was named as *Aphanomyces invadans* (David and Kirk, 1997).

In India also workers most frequently isolated *Saprolegnia sp.* and *Aspergillus sp.* from EUS affected fishes (Das et al., 1990; Kumar et al., 1991; Patra, 1992).

Karunasagar et al. (1994) traced out the existence of fungi deep into the musculature below the EUS affected ulcers in both freshwater and estuarine fish, but no existence of fungi was detected in early stages of lesions prior to development of ulcers.

Histopathological studies of EUS affected freshwater and estuarine fishes showed the presence of numerous non-septate, highly invasive fungal hyphae (Mohan and Shankar, 1995).

Qureshi et al. (1995) isolated seven species of fungi belonging to the genera *Saprolegnia, Aphanomyces, Achyla* from lesions of EUS affected fishes from Bhopal.

Pal (1996, 1997) also isolated three species of fungi but he also stated that no fungi were found in the primary stage of ulcer formation. Viswanath et al. (1998) assumed that EUS specific fungus can only enter into the fish after the primary damage to the skin Mohan et al. (1999) suggested that an invasive fungus *A. invadans* is the primary pathogen of EUS. Roy (2003) reported isolation of an aseptate fungus, *Aphanomyces* sp. from infected *C. mrigala* and experimentally fungal zoospores induced ulcer in healthy *C. punctatus*. Routh (2006) reported isolation of *Apanomyces* sp. from infected *C. striata*, *C. punctatus*, *L. rohita* and *L. bata*. Pathogenicity studies with the zoospores of fungus, *Aphanomyces* sp. (F_{CS1}) isolated from ulcer of *C. striata* induced ulcer at the site of injection and caused 44% mortality in experimental *C. punctatus*.

Animal Parasite

Reungprach et al. (1983) examined 273 EUS affected fishes during 1982-83 outbreaks in Thailand and found a number of metazoans (*Dactylogyrus sp.* and *Gyradactylus sp.*) and protozoans (*Chilodnella sp., Trichodena sp., Costia sp., Henneguya sp.* and *Folthyopthirus sp.)* associated with the ulcers. A considerable number of protozoans belonging to genera *Epistylis sp.* were also reported from fishes with tiny red spots on the skin before the outbreak of the EUS in Thailand (Tonguthai, 1985). Callinan et al. (1989) and Pearce (1990) reported the presence of protozoan and metazoan parasites on some affected fish in Australia.

Jhingran (1990) detected the presence of some common animal parasites such as *Tripartiella sp., Phlebitis sp., Trianchortus sp.* and *Dactylogyrus sp.* in and around the ulcers of EUS affected fishes in a very low intensity. As the animal parasites were found in a very low intensity, Jhingran (1990) did not consider them as primary cause of ulceration of EUS affected fishes. Mondal et al. (1990) reported the presence of *Costia necatrix* in the ulcer of EUS affected fish in West-Bengal, India. Kumar et al. (1991) noticed the existence of myxozoan parasites in the skin and internal organs of *Clarias sp.* Ram (1992) reported that myxozoans, *Myxobolus sp.* and *Thelohanellus sp.* were associated with EUS outbreak in Haryana, India.

Subasinghe (1993) performed experiments with an aim to know the relationship, if there any, between some animal parasite infection like *Trichodina sp.* infection and induction of EUS in *Clarias striata* by feeding with infected fish and by direct contact of water from infected environments. The results showed that heavy infection of animal parasites accelerated the formation of ulcers in EUS affected fishes.

Environmental factors associated with EUS outbreak

Fishes, being poikilothermic aquatic animals, are much more influenced in their physiology by the variation and changes in the environment than the homeothermic vertebrates. The role of environment is regarded of prime importance in the outbreak of

the fish epizootic by many authors (Sneirzko, 1974, 1983; Wedemeyer et al., 1977; Walters and Plumb, 1980; Csaba et al., 1981; Ahne et al., 1982; Schaperclaus, 1986). Epizootic ulcerative syndrome or EUS is not an exception. Tonguthai (1985) reported that EUS outbreaks are frequently predisposed by adverse environmental parameters.

Temperature

Rodgers and Burke (1981) observed that maximum EUS prevalence in estuarine fish was due to fall of temperature. Roberts et al., (1986) recorded that the occurrence of EUS outbreaks in cyclic manner coincided with fall of water temperature after heavy rainfall. Jhingran (1990) also reported that EUS outbreaks took place when water temperature fell significantly. Ahmed and Hoque (1998) reported that outbreaks of EUS were highly related with fall of water temperature. Many other workers also found the some relationship of water temperature and EUS outbreaks (Phillips and Keddie, 1990; Das et al., 1990; Lilley et al., 1992; Das and Das, 1993; Mohan and Shankar, 1994; Lumanlan-Mayo et al., 1997; Sanaullah et al., 2001; Roy and Pal, 2005).

Water quality variables

Jhingran (1990), Jhingran and Das (1990) found that EUS outbreaks were also related with lowering of alkalinity of water.

Bondad-Reantaso et al. (1992), Palisoc and Aralar (1995) and Sanaullah (2001) showed that EUS outbreaks took place when alkalinity and chloride were reduced in water. Mohan and Shankar (1994) reported that during rainy season of 1993 when the salinity was very low. EUS outbreaks took place in estuaries of Dakshina and Uttara Kanoda districts of Karnataka, India. It was also suggested by Callinan et al. (1995) that exposure of fishes to acidified runoff water in Australia was a causal factor for EUS outbreak in estuarine water.

Sardesai (1996) suggested that decrease in salinity of estuarine water due to heavy rainfall was on of the causal factors for EUS outbreaks in 1993 in Goa.

Pathiratne and Jayasinghe (2001) showed that low amount of dissolved oxygen helped in outbreaks of EUS. Roy and Pal (2003) mentioned that outbreak of EUS occurred in the ponds of three areas of North Bengal when dissolved oxygen content, hardness and total alkalinity of water remained low.

Heavy metals

An attempt to find out the relationship between the concentrations of heavy metals e.g. Fe, Zn, Cu, Cr, Cd, Pb and Hg in water and the outbreaks of EUS was made by Jhingran and Das (1990). But till today no significant relationship between concentrations of heavy metals in water and EUS outbreaks has been established.

Pesticide and other agrochemical

Kurup (1992) performed an extensive study in the EUS affected regions of north-eastern Kuttanand in Kerala, India and found that indiscriminate application of pesticides have aggravated water pollution problem exposing the fishes to more stressful conditions which played a predisposing factor for EUS outbreak.

Chowdhury et al. (1994) analysed the pesticide residues in water, fish and plankton in some EUS affected areas and mentioned that though high concentrations of organochlorine pesticides such as BHC, DDT and their metabolites were occasionally detected in water yet no tangible correlation had been found between concentration of pesticides and EUS outbreaks.

Investigations to find out relationship between EUS outbreak and environment in two lakes Laguna and Naujan in Philippines for two years failed to establish a correlation between pesticide concentrations in water and EUS outbreak (Palvisoc and Arator, 1995).

Flooding

Saha et al. (1992) reported that in Sunderbans EUS was detected in many confined waters after a devastating flood. Barua (1994) reported that in Bangladesh a rapid outbreak of EUS took place after a severe flood conditions. Mohan and Shankar (1994) observed that in Karnataka in Couvery river system outbreak of EUS first appeared after

the flood during August-September, 1991. Abdul Hameed (1996) observed that outbreaks of EUS occurred in Karnataka after major flood for four consecutive years (1991-1994).

Signs of the disease

Jhingran and Das (1990) in India reported that the signs and other characteristics of the epizootic ulcerative syndrome were different from other ulcerative condition in fishes. Fishes in the river as well as in ponds exhibited abnormal swimming behavior with head projected out of water. In the primary stage of the disease, the infection generally started in the form of multiple inflammatory red spots on the body causing localized haemorrhage. In case of carps, the infection first took place within scale pockets and in due course the infection spread to a larger area with sloughing of scales with degeneration of epidermal tissue. In advanced stage the ulcers became deep, haemorrhagic and necrotic often with a black melanistic rim. In the final stages or in acute stages deep, haemorrhagic, necrotic ulcers were generally found in all parts of the body of the fish, especially in the head, abdomen and peduncle.

During the initial outbreak of the disease in the plains of North Bengal, Pal and Pradhan (1990) observed keenly a considerable number of EUS affected air-breathing fishes which included 129 *A. testidineus*, 16 *H. fossilis* and 11 *Clarias batrachus*. They reported that the disease first appeared as a red spot on the skin of the fish body. Later ulcers developed in affected areas damaging badly underlying muscle layer. But in scaly fishes, initial damage of mucous layer covering the scales were followed by appearance of red spots and finally sloughing of scale took place with development of ulcers.

Kumar et al. (1991) reported that distribution of severe ulcerative skin lesions varied from species to species. In murrels the ulcerations were mostly pronounced and developed in the head and caudal areas. In advanced stages the tail lesions could erode the affected areas to such an extent that there was total loss of peduncle portion. Sometimes the erosion progressed deep into the body exposing the abdominal cavity.

In *Puntius sp.* dark red haemorrhagic, superficial ulcers area found on either side of the body. But in Indian major carps long striped haemorrhagic lesions were found in the region of caudal peduncle. Pradhan et al. (1991) reported infection of different stages of development in Indian major carps like *Catla catla, Cirrhinus mrigala* and *Labeo rohita*

By using clinical and histological features, Viswanath et al. (1997) classified different types of lesions associated with EUS in India. They observed more than 300 EUS affected fish and characterized three distinct types of EUS lesions:

Type1 lesions were like tiny red spots on the body surface with no observable haemorrhages and ulcerations.

Type2 lesions were large (2-4cm.) and appeared as dark raised discoloured areas on the body surface. Scales and skin were not affected.

Type3 lesions appeared as circular to oval open dermal ulcers extending into skeletal musculature. At these advanced stages haemorrhagic and necrotic open ulcers devoid of epidermis and scales were also found.

Chinabut and Roberts (1999) classified the disease into five types:

Type I It is characterized by a highly distinctive dark red to brown lesion on one or both blanks. The affected fishes had floated the surface of water and grasped air before they expired.

Type II This type was mostly found in top order predator air breathing fish, such as the snakeheads. The infections were characterized with chronic and extensive lesions.

Type III In this case the ulcers were chronic and extensive and at early stage of infection a small red rosette or an ulcerated scale bed were found. It depends on the age and size of fish. This type is also found in snakeheads.

Type IV This type of lesions is found in the grey mullet (*M. cephalus).* It is characterized by small necrotic erosions of one or more scale beds which gradually

extended to produce a shallow ulcer with a red Centrum, white rim surrounded by black edge.

Type V This type is common in some large Indian carp. In this type a single, small, areas of haemorrhagic ulceration on finback was found.

Management of the disease

Conditions like over crowding of fish, poor water quality, sudden fall of temperature interfere with the normal physiological conditions of fishes and the fishes are exposed to stresses which make them more susceptible to infections (Jhingran, 1990). Management of EUS includes both prophylactic measures as well as therapeutic measures.

Potassium Permanganate

Jain (1990) prescribed that bath treatment of EUS affected fishes with potassium permanganate @ 5 ppm. Jhingran (1990) observed that potassium permanganate @ 0·5-2 ppm in water showed a good result in curing ulcers.

Lime

Use of lime in the pond of EUS affected area @ 200-600 kg per hectare was proved to be effective in either checking the outbreak of EUS or in curing the ulcers (Jhingran et al., 1990). Jain (1990) reported that better result was recorded when liming was followed by bath treatment of the infected fish in 5 ppm potassium permanganate solution or common salt at 3% to 4% solution. Jhingran et al. (1990) stated that liming in water was more effective because it raised the pH value of the water, precipitated suspended or soluble organic materials, promoted biological productivity by enhancing the breakdown of organic substances by bacteria and killed most of the undesirable microorganism due to its caustic action.

Antibiotics

Erythromycin, Nalidixic acid, Oxytetracyclin, Terramycin are commonly used for the treatment of EUS affected fishes. The prescribed dose for effective result was @ 60-100 mg per kg of feed for 7 days (Jhingran, 1990).

CIFAX

Application of CIFAX, a drug formulated by CIFA in EUS affected captive water @ 1L/hectare metre of water showed encouraging results (Das & Das, 1993).

Lymphohaemopoietic organs

In fishes, especially in teleosts, spleen and thymus are the two most important lymphomyeloid tissues (Fanage, 1984). Kidney in fish acts as the bone marrow equivalent of vertebrates containing lympho haemopoietic tissue, which is a typical condition in vertebrates that have bone marrow (Zapata, 2001).

There was evidence that the thymus played a vital role in the ontogeny of immunologic competence in fish (Miller, 1961; Good et al., 1962; Cooper and Hildemann, 1965; Cooper, 1973). Beard (1894) suggested that the thymus was the source of lymphoid cells in elashmobranch. In teleosts the head-kidney played definite role of a lymphoid organ (Rasquin, 1951; Smiths et al., 1980).

Morphological and histological studies of lymphoid organs of a teleost, *Tilapia mossambica* showed that organized lymphoid tissues were present in thymus, head kidney and spleen (Sailendri and Muthukarruppan, 1975). They found that the thymus was encapsulated by thin strands of collagen fibers and divided into three regions outer, middle and inner. The head kidney was characterized by the presence of lymphoid follicles, sub capsular sinus, a hilus like area and lymphoid vessels. The spleen was clearly divided into white pulp and red pulp regions. White pulp region harboured only a reticular area without definite lymphoid centers and the red pulp contained predominantly erythrocytes.

Other workers reported that the spleen and head kidney were important in the immunological defense mechanisms and haematopoiesis in fish (Ellis et al., 1976; Ellis, 1980; Secombes and Manning, 1980; Tamer et al., 1984; Lamers, 1986).

Kumar et al. (1991) performed histological examination of kidney of EUS affected *Puntius sp.* along with some other organs and noticed haemotopoietic as well as immunologic role of kidney specially in anterior part (head kidney) where renal function had disappeared. He also noticed some necrotic changes in the kidney of EUS affected *Puntius sp.*

Pradhan (1992) also found a varying degree of degeneration in kidney of EUS affected *Clarias batrachus.* Histopathological studies by Pal and Pradhan (1995) of kidney of EUS affected *Clarias batrachus* also showed similar degenerative changes.

Histological studies of thymus in the dog-fish, *S. canicala* showed that it was covered completely by a connective tissue capsule, containing fibroblasts, macrophages and collagen fibres (Navasro, 1987; Lloyd-Evans, 1993). Collagen fibres projected into the thymic parenchyma by connective tissue trabeculae carrying large blood vessels and nerves and dividing the thymic lobes into several lobules. Its histological structure showed the presence of cell types almost similar to those described in the most vertebrates.

Ultra structural studies or cytological studies

Information on ultra structure and cytology of lymphoid organs of fish was previously reported by other authors (Good et al., 1966; Bielek, 1981; Fange, 1982; 1984, 1987; Zapata, 1981, 1982; Pulsford et al., 1982; Fshizebki et al., 1984; Hart et al., 1988; Powley et al., 1988). They showed the presence of varying numbers of lymphomyeloid and erythroid cells within but did not notice the presence of any trabeculae. Ultra structural and cytological studies of spleen and head kidney in striped Bass showed that different regions of the spleen contained varying numbers of erythrocytes or their precursors, dividing haemotoblasts, promonocytes, lymphoblasts, lymphocytes and

plasma cells etc. (Bodammer et al., 1990). They also observed the presence of reticular cells closely associated with reticular fibres. Head kidney contained almost all cells types found in spleen but appeared to have much more neutrophils and their precursors. The cells were loosely packed compared to the cells in the spleen and this loose packing of cells allowed examining the developmental stages of blood cells more perfectly.

Zapata et al. (1996) studied the ultra structure and cytology of the lymphoid organs in elasmobranches. They observed mainly the presence of lymphocytes, epithelial cells and macrophages in thymus. But alongwith those cells they also noticed the presence of myoid cells in thymus. Except myoid cells, all other cell types found in thymus were also noticed in the spleen.

Ultra structural and cytological studies on thymus of a gobiid fish (*Pscudopocryptes lanceolatus*) showed the presence of plasma cells, cystic cells and macrophages etc. (De and Pal 1998). Transmission electron microscopic study showed the presence of hypertrophied cystic structures in the thymus of Gobiid fish, *Pseudocryptes lanceolatus* (De and Pal, 2002). De and Pal (2004) reported a special type of secretory cell containing numerous membranes bound dense vesicles, granules, multivesicular bodies (MVBs) and nuclei with two polarized noucleoli within the thymus of Gobiid fish, *Pseudocryptes lanceolatus*.

Cytological studies on the developing thymus in a marine teleost, *Diplodus puntazzo* showed the presence of four types of epithelial cells like a) limiting cells, b) medullary and cortical reticular cells, c) nurse cells and d) Hassall-like corpuscles by Romano et al. (1998). Along with four types of epithelial cells, large blast likes lymphoid cells and small lymphocytes were also reported in medulla and cortex of thymus respectively. Further studies on ontogeny of the thymus in a teleost fish, *Cyprinus carpio L* revealed a huge existence of apoptotic cells within the macrophages of cortex of thymus, by Romano et al. (1999). Romano et al. (2002) also studied the cytological organization of head kidney of Antarctic fishes and found almost all cell types found in the thymic micro-environment.

The thymus of mandarin fish, *Siniperca chuasi* showed the presence of cell types containing thymic epithelial cells, limiting epithelial cells (LEC), macrophages, lymphocytes and three types of granulocytes (Xie et al.,2005).

Ultra structural organization of the thymus of juvenile turbot, *Scophthalmus maximus* contained different cells like limiling thymic epithelial cells, dark stellate (TEC) with an electron dense cytoplasm, pale TEC characterized with electron lucent cytoplasm, macrophages, pigment cells (melanophores) lymphocytes and rodlet cells etc.

Haematological studies

Nearly sixty six years ago Duthie (1939) reporting on the region, development and functions of blood cells in certain marine teleost remarked "..... The development from small lymphocytes to erythrocytes is most difficult to follow, though I feel sure that it occurs....."

Mahajan and Dheer (1979a, 1979b) described different types of blood cells in the peripheral blood of *C. punctatus* and found almost all types of mammalian counter parts in fish blood. Mahajan and Dheer (1980) also studied haemopoiesis in *Channa punctatus* using spleen and head kidney imprints. Lewis et al. (1978) working with certain catfish found two types of lymphocytes, small and large.

Mahajan and Dheer (1979) showed that the number of blood corpuscles of fish blood (*C. punctatus)* vary with change of season. Hamers (1994) was able to prove more convincingly the presence of different cell types in fish (*Cyprinus carpio L)* by cytochemical studies. A generalized increase in leucocytes in the gut of goldfish *Carassius auratus (L)* suffering from frunculosis was observed by Mawdesby-Thomas (1969). Pavlidis et al. (2007) reported that the numbers of different leucocyte cell types were not influenced by sex or maturity stage in six Mediterranean fish species.

Immunological studies

The nonspecific defenses available to the fish are essentially those available to higher mammals (Fletcher, 1982). Goblet cells continuously secrete mucus which not only offers mechanical protection and physically removes microorganisms, but also it contains nonspecific anti microbial activity in the form of lysozyme, C - reactive protein and complement and can also provide immune protection in form of secretory immunoglobin (Fletcher, 1973, 1982). In teleost fishes both alternative complement and classical complement pathways have been shown to generate bactericidal activity (Corbel, 1975; Munn et al., 1982). The presence of interferon has been demonstrated in trout following infection by viruses (de Kinkelin et al., 1982).

Macrophages are widespread in tissues of teleosts, including the gills and peritoneum, but are mainly found as reticulo-endothelial cells in the kidney, the spleen and in some fish, the atrium of the heart (Ellis, 1982). These fixed macrophages appear to be very efficient in clearing the bloodstream of bacteria (Munn et al., 1984).

The fish is also able to respond to microbial attack with an immune defense but it is especially less sophisticated in anti body-mediated defense because the fish is restricted to one class of immunoglobulin (Ig) while higher vertebrates have two to five (Ellis, 1982).

But humoral immunity is not the only arm of specific immunity in fish. The growth and multiplication of many pathogens which live and multiply in host cells, especially phagocytes, are stopped by the activation of macrophages and accumulation of phagocytes to infective loci where activation of macrophages and accumulation of phagocytes are made by lymphokines produced by T-lymphocytes (Sissons et al., 1985).

In teleost, especially in carp cell mediated immunity matures rapidly (Manning et al., 1982, 1985).

Lymphocytes from fish immunized against *A. salmonicida* do produce the lymphokine macrophages migration inhibition factor (Smith et al., 1980).

Baba and Imamura (1988) studied the mechanism of protection in carp, *Cyprinus carpio L.* against *Acromonas hydraphila* which is one of the aetiological agents of epizootic ulcerative syndrome (EUS) in fish. The results indicated that the protection shown by carp immunized by dipping in crude LPS or lipopolysaccliaride was dependent on cellular immunity regulated by T-like cell macrophages system.

They further showed that the protection in carp against *Aeromonas hydrophila* by vaccination is not dependent on humoral immunity.

Later Karunasagar et al. (1996) performed experiments to study the effect of thymectomy on the humoral immune response of *Labeo rohita* against *Aeromonas hydrophila.* The results of the experiments showed that antibody titres in thymectomized fingerlings of *Labeo rohita* Ham. were 2-4 times lower than in non-thymectomized control. Not only, were the antibody titres significantly less in thymectomized fingerlings of *Labeo rohita* Ham. compared to the non-thymectomized fingerlings, even in case of the thymectomized fingerlings the protection against *Aeromonas hydrophila* was partially compared to non-thymectomized fingerlings after immunization against *Aeromonas hydrophila.* The results suggested that T-helper cells might be involved in the production of antibody against *Aeromonas hydrophila.*

Thompson and Lilley et al. (1999) examined the immune responses against a fungus *Aphanomyces invadans* which is also one of the aetiological agents of epizootic ulcerative syndrome in fish and found development of nonspecific antibody. The macrophages were able to phagocytose spores in vitro.

Miles et al. (2001) showed that fishes treated with immunostimulants developed an enhanced immune status.

Recent studies by Rao et al. (2006) on effect of *Achyranthes aspera* on the immunity and survival of a major carp, *Labeo rohita* infected with *Aeromonas hydrophila* showed that *Achyranthes aspera* stimulated immunity and increased resistance to infection.