Review of Literature

The epizootic ulcerative syndrome (EUS) is one of the dreaded diseases of fish of all kinds, like fresh water, brakish water, esturine water, farmed, wild, sports and ornamental fishes. Ever since the emergence of EUS no single aetiological agent has been conclusively identified as causative agent of the disease. Disease results from a complex interaction between host, pathogen and environment. Studies on various aspects of biology of the host and the pathogen and interactions between them help to understand the disease process. The main purpose of this review is to present briefly the important observations of the previous workers as far as possible to highlight the present state of understanding of epizootic ulcerative syndrome. The different aspects of this review are:

- History of the disease.
- Fish species affected.
- Socio-economic impact of the disease
- Aetiology of the disease
- Environmental factors.
- Clinical signs
- Histopathology
- Haematological studies.
- Control measures

History of the disease

It is more than thirty five years different fish, cultured and wild have been affected by an ulcerative disease in different parts of Asia and Asia – Pacific region. The disease has been given various names viz. mycotic granulomatosis (MG) in Japan, red spot disease (RSD) in Australia and epizootic ulcerative syndrome (EUS) in Southeast and South Asia.

The above mentioned diseases have been described separately as distinct conditions in the past but the recent findings have shown that the some pathogenic *Aphanomyces* fungus is involved in each case .Epizootic

ulcerative syndrome is now recognized to be synonymous with mycotic granulomatosis and red spot disease (Chinabut and Roberts ,1999).

Egusa and Masuda (1971) reported an ulcerative conditon in farmed in ayu (*Plaecoglossus altivellis)* in Japan in the year 1971. The disease was named mycotic granulomatosis (Myazaki and Egusa, 1972). Various types of fish such as ayu , goldfish, bluegill and some wild fishes were affected by the disease (Miyazaki and Egusa, 1972, 1973 a. b. c). An epizootic characterized by shallow hemorrhagic ulcers named red spot disease (RSD) broke out affecting estuarine fish particularly grey mullet in Queens land , Australia in the year 1972 (Mckenzie and Hall, 1976) with recurrence in subsequent years (Rodgers and Burke, 1977;1981). The disease afterwards affected fresh water and estuarine fish in coastal rivers in New South Wales (Callinan *et al.,* 1989), Northern Territory (Pearce, 1990) and western Australia (Callinan, 1994a). After the out break of mycotic granulomatosis and red spot disease the fish disease characterized by demal ulcer with large scale mortalities was reported in fresh water and estuarine fishes in different countries of the Asia-Pacific region. The disease is called epizootic ulcerative syndrome, EUS (FAO, 1986). Papua New Guinea witnessed a disease similar to red spot disease characterised by dermal ulcers in the rivers of South during 1975-76 (Haines, 1983) and North during 1982-83 (Coates *et. al.* 1984). Sulawesi and Kalimantan Jothy (1981) reported the out break of the ulcerative disease in December, 1980 in rice-field fish in northern Malaysia . The affected fish had red or necrotic areas of ulcers all over their bodies and was called "Weback kudes". Shariff and Law (1980) reported high mortality rates in fish in southern peninsular Malaysia in 1979. Shariff and Saldin (1994) described the status of the epizootic ulcerative syndrome after 1986 in Thailand. The EUS outbreak occurred for the first time in 1981and the second (1982-83) and third (1983-84) outbreaks affected the intensive fish culture systems of Thailand causing devastating effects (Ulcerative fish Disease committee 1983; Tanguthai 1985).

The disease entered Myanmar, Lao PDR and Combodia during 1983-84 via Malaysia and Thailand (Roberts *et al.*, 1986; Lilley *et al.*, 1992) A severe out break of EUS in December, 1985 was reported from Laguna de Bay in the Philippines affecting snakeheads, gobies, gouramies, cat fish, crucian carp etc . (Llobrera and Gacutan 1987). In Philippines the disease later extended to whole country affecting wild fish in Lakes, rice-fields, swamps and pond cultured fish (Bondad-Reantaso, 1992; Bondad-Reantaso *et al.*, 1994).

Reports about the outbreak of EUS also came from China (Lian, 1990; Guizhen, 1990). The first occurance of EUS in Vietnam came from Mekong delta in 1983 (Xuan, 1990). Wilson and Lo (1992) reported EUS on snakeheads (*Channa maculata*) in late summer in Hong Kong since 1988. The outbreak of EUS was first reported in fresh water and estuarine fish in western Srilanka in December, 1987 (Costa and Wijeyaratne, 1989). It was suspected that the spread of the disease was from infected ornamental angel fish (*Pterophyllum scalare)* imported from south-east Asia (Balasuriya ,1994). In February, 1988 the disease extended across Myanmar into Chandpur district of Bangladesh (Kar and Dey , 1990; Roberts *et al*, 1992; Hossain *et el.*, 1992 Ahmed and Rab 1995; Ahmed and Hoque, 1998). Rahim *et al.*, (1985) reported ulcerative disease in five species of brackish water fish of Bangladesh prior to the out break of EUS in February , 1988.

Epizootic ulcerative syndrome broke out for the first time in May, 1988 in some North eastern states of India such as Tripura, Maghalaya and Assam (Das 1988). In Assam severe outbreak of EUS was reported from Borak Valley districts of Cachar and Karimganj. The disease had also been reported frim Nalbari, Jorhat, Kamrup, Naogao and Lakhkimpur of Assam. The villages adjacent to Bangladesh border were seriously affected. The northern, southern and western part of Tripura were seriously affected by EUS. In Meghalaya the ulcerative disease was reported from the districts of West Garo Hills, East and West Khasi Hills, Jaintia Hills and also from Borak Valley districts of Jowai (Jhingran and Das, 1990, Kumar *et al.*, 1991). In October,

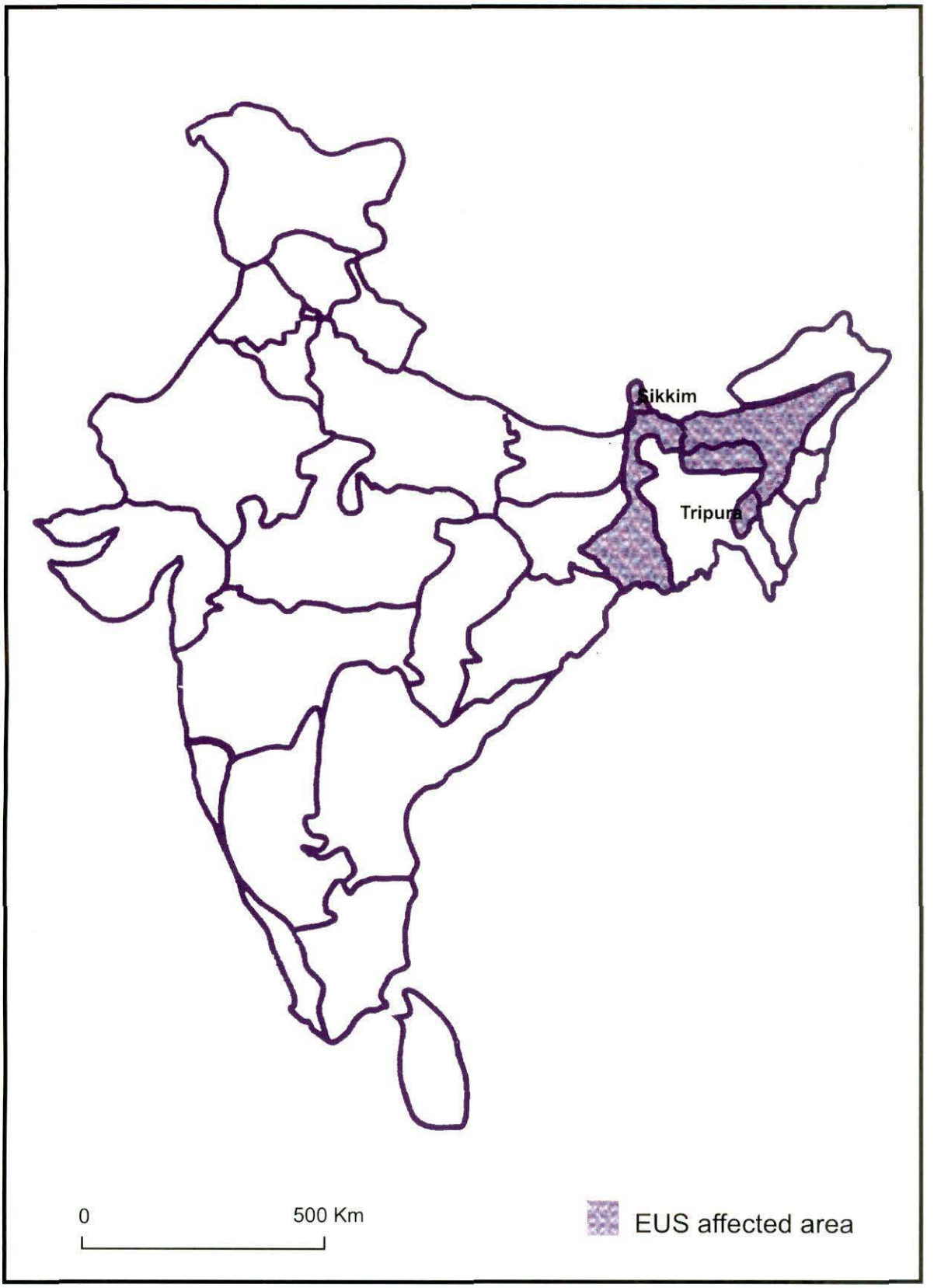

Fig. 1: Spread of EUS in India during 1988

Fig. 2: Spread of EUS in India during 1993

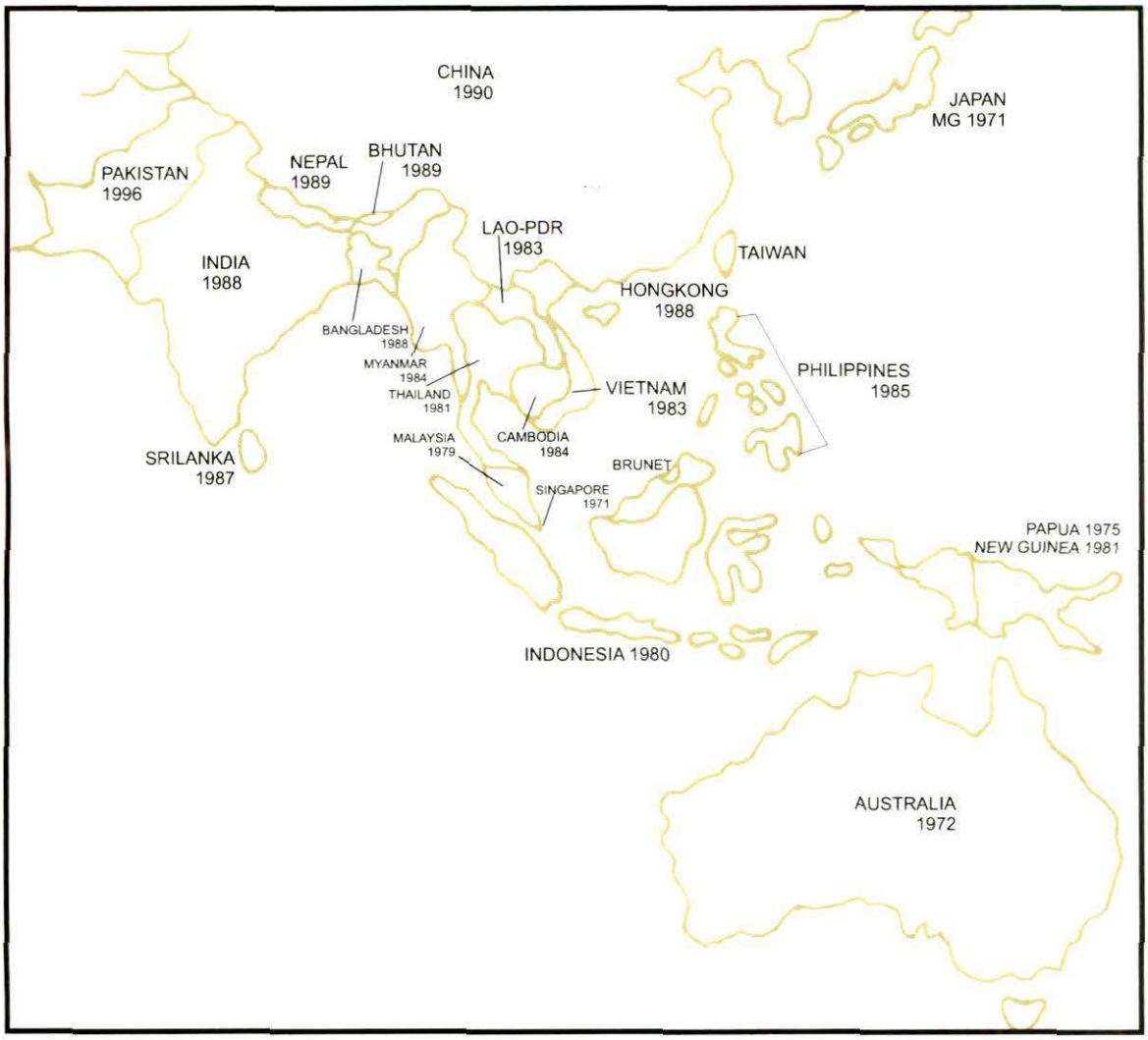

Fig. 3: Map showing the countries affected by EUS across the Asia-Pacific region.

1988 severe out breaks of the ulcerative fish disease occurred in northern districts of West Bengal such as Coochbehar, Jalpaiguri, West Dinajpur and plains of Derjeeling District (Pal and Pradhan, 1990). In the same year the disease spread to some southern Districts of West Bengal such as Murshidabad ,Nadia and 24-parganas and Midnapore (Pradhan and Pal, 1990) (Fig.1). By the year 1989, the disease had spread to almost all the districts of West Bengal except Purulia (Jain, 1990). In Bihar, Katihar and Kisanganj districts boardering the West Bengal was affected during April and May ,1989 (Prasad and Sinha, 1990; Kumar *et al.,* 1991). In Orissa the disease spread to the northern districts adjoining to West Bengal namely Balasore and Mayurbhanj (Prusty and Nayak, 1990). The disease also spread to other states of India in 1989 viz. Mizoram, Arunachal Pradesh, Manipur, Uttar Pradesh and within 1993 almost all the states of India were affected except Jammu & Kashmir , Punjab, Himachal Predesh and Gujarat (Das and Das, 1993) (Fig.2). Sardesai (1996) reported that the disease first broke out in Goa during August, 1993. EUS first broke out in the Cauvery river and its tributary Kabini river in T. Narasipura Taluk of Mysore district of Karnatak in August . 1991 and then spread to other part of the state (Abdul Hamed, 1996).

Bhutan and the eastern Terai of Nepal were first affected in 1989 and by 1993 it spread to Himalayan Valley regions (Phillips , 1989; Shresta , 1990;1994).EUS entered Pakistan in 1996 where EUS was confirmed in snakeheads from Punjab province in April 1996 and in *Cirrhinus mrigala* from Sindh province in January , 1998 (DFID, 1998) (Fig.3).

At present a declining trend of the outbreak of EUS is observed in different states of India. In the districts of North Bengal the disease has been reported every year since its first outbreak in 1988 (Pal and Pradhan, 1990; Pradhan and Pal, 1990; Pradhan, 1992; Pal 1996, 1997; Saha, 1999; Saha and Pal, 2000; Roy and Pal, 2005).

Fish species affected

The epizootic ulcerative syndrome affected more than 100 species of both fresh water and brackish water fish (Lilley *et al.*, 1992). Das and Das (1993) reported that the occurrence of EUS was highest in the genera *Channa* (5-100%) and *Puntius* (20-100%). Besides these *Mystus, Mastocembelus ,Glossogobius, Anabas, Clarias* and *Heteropneustes* were highly susceptible to EUS. The percentage of incidence was low in case of carps. Mohan and Shankar (1994) reported that EUS first attacked the bottom dwelling snakeheads (*Channa* sp.), and than attacked the catfish (*Mystus* sp. and *Wallago* sp.) minor carps (*Puntius* sp.) featherbacks (*Notopterus* sp.) etc. The Indian major carps were mostly unaffected in the state of Kamataka. The other major fish species affected in India are *Ctenopharyngodon idella, Hypophthalmichthys molitrix, Nandus nandus, Rasbora* sp. *Ompak* sp. *Mugil cephalus, Mugil parsia, Sactophagus* sp. *Epinephelus* sp. *Catla catta. Labeo rohita, Labeo calbasu, Cirrhinus mrigala* (Jhingran and Das, 1990; Pal and Pradhan, 1990; Kumar *et al.*, 1991; Abdul Hameed, 1996; Mukherjee, 1996; 1997) In India about 46 fish species are affected (Table 1).

In Thailand snakehead fish (*Ophicephalus striatus*), serpent fish (*Channa micropeltis)*, sand goby (*Oxyeleotris marmoratus*), three spoted gourami (*Trichogaster trichopterus*), striped croaking gourami (*Trichopsis vittatus*) Siamese fighting fish (*Betta splendens*) and wrestling half beak (*Dermogenus pustillus*) were the most affected fishes (Saitanu *et al.*, 1996).

In philppines the affected fish species were snakehead (*O. striatus),* catfish (*C.batrachus*) gourami (*Trichogaster pectoralis)* goby (*Glossogobius girus*), crucian carp (*Carassius carassius*), Manila sea catfish (*Anus manilensis*) and silvery theraponid (*Therapon plumbius)* (Llobrera,1987). During the out break of 1990,the estuarine fish species such as mullet (*Mugil sp*) flatfishes (*Platycephalus* sp.and *Psethodes* sp.*),* goat fish (*Vpeneus bensasi)* croaker (*Johnius* sp.) and spadefish (*Scaptophagus* sp.) were affected (Bondad-Reantaso, 1991).

Table 1: Fish species affected by EUS in India

FRESH WATER	
Wild	**Cultured**
Anabas testudines	*Catla catla*
	Cyprinus carpio
Acrossocheilus hexagonolepsis	
Ambasis ranga	*Cirrhinus mrigala*
Amblypharyngodon mola	*Ctenopharyngodon idelle*
Amphipnous cuchia	*Hypophthalmichthys molirix*
Ailia coila	*Labeo rohita*
Clarias batrachus	*L bata*
Channa punctatus	*L calbasu*
C. gachua	*Puntus javanicus*
C.striatus	*P..sarana*
Callichrous pabda	
Chanda chanda	
Colisa fasciata	
Gadusia chapra	
Glossogobius giuris	
Heteropneustes fossils	
Mystus sp .	
Macrognathus aculeatus	
Mastocambelus sp.	
Monopterus cuchia	
Nondus nandus	
Mystus cabasius	
Notopterus sp	
Ompak sp	
Puntius sophore	
Rhinomugil corsula	
Rasbora sp	
Salmostoma bacila	
Trichogaster sp	
Wallago sp. '	
BRACKISH WATER	
Epinephelus sp.	*Mugil parsia*
Etroplus sp.	*M. cephalus*
Platycephalus sp	*M. subviridis*
Scatophagus sp	*Sillago* sp.

In Australia yellow fin bream (*Acanthopagrus australis*) and striped mullet (*Mugil cephalus)* were among the affected species (*Callinan et al.,* 1995a). Thai silver barb, *Puntius gonionotus (*Bleeker) was among the most susceptible species in Bangladesh (Ahmed and Rab, 1995). Different snakeheads and *Cirrhinus mrigala* were reported to be affected by EUS in Pakistan (*Callinan et al.,* 1997; DFID, 1998). In Srilanka, *O. striatus, O. punctatus*, *H. fossilis* were the commonly affected fish (Subhasinghe *et al.,* 1990).

However, some fishes are unaffected e.g., milk fish (*Chamos chamos*), Nile telapia (*Oreochromis niloticus*), striped cat fish (*Pangasius sutchi*) and big head carp (*Aristichthys nobilis*), (Flores, 1986).

Socio –economic impact

The social and economic impact of epizootic ulcerative syndrome are obviously severe. Scientists of the different countries have tried to estimate the economic values of the fish losses due to EUS but there are very few accurate estimate of losses.

Tonguthai (1985) reported the economic loss of Thailand during 1982-83 was of 200 million Thailand currency and the loss was of about 3600 million Thailand currency within 1983-93 (Chinabut, 1994).

It was reported that the first outbreak of EUS in the Philippines affected 15,000 lakeshore families in Laguna Lake with a 30% decrease in average daily income of fishermen (Llobrera, 1987). In Pangasina Province of Philippines 75,000 people dependent on the Mangabol swamp of 5000 ha suffered over 50 and 40% loses during the 1989 and 1990 outbreaks respectively (Bondad-Reantaso *et. al.,* 1994). They also reported about great panic created among the consumers and farmers in the Philippines. In 1988, in some communities, a wide spread but unfounded fear of disease transmission among consumers led to a drastic decrease in market demand for all food fish in Bangladesh. The concurrent deaths of ducks, cattle and

other animals related to paddy fields increased the fear more. Though, there was no scientific evidence that the disease itself caused any human or animal illness, but it has badly affected the socio-economic status of the fish farmers and fish traders (Rahaman *et al.*, 1988). In Bangladesh fish traders suffered heavy losses during 1988 and 1989 due to price reduction of fish upto 75%.The total economic loss were about 118million and 88.2 million Taka in Bangladesh in the year 1988 and 1989 respectively (Barua, 1990). In Srilanka the fisherman and fish traders faced a heavy economic loss due to EUS outbreak. During 1988-89 the economic loss was of Rs 1 million (ADB /NACA, 1991) but upto 1993 the economic loss increased to 20-40 million Srilankan Rupees (Balasuriya 1994). About 15-20% of total fish production was lost in Napal during initial outbreaks. During 1989-90 the total economic loss in eastern Napal was about of 30 million (ADB/NACA, 1991). Pakistan also faced a heavy economic loss during the first outbreak of EUS in 1996, the total economic loss in Pakistan was about of Rs 15 million (AAHRI,ACIAR, IOA and NACA,1997).

In India economic loss due to EUS outbreak , was not estimated accurately from different parts of the country. The assumption that economic loss was obviously great during the initial outbreaks of EUS in different states of India. Panic was created among the people of the affected areas and fish consumption declined sharply (Das, 1988, 1997; Pal, 1996; Sardesai ,1996).

After the initial outbreak investigations carried out in five districts of West Bengal showed 73% aquaculture units were adversely affected by EUS, The fish consumption rate went down by 28.7%, 23.3% and 20.5%in urban, sub-urban and rural areas respectively. (Bhowmick *et, al.,* 1991). It was also reported that 73% of the culture ponds of West Bengal were affected by EUS outbreaks during 1988-89 and 30-40% of the stocked fishes were lost. The loss of fish costing about Rs 48.0 lakhs was reported during initial outbreaks of 1989-90 in Bihar (Prasad and Sinha, 1990). Orissa suffered loss of Rs 30.0 lakshs during 1989-91 (Das, 1994). EUS completely paralised the fish market in Kerala and the fisherman (both, men and women) had to seek

alternative employment to live (Sanjeevaghosh, 1992). Only during 1991-92 the economic loss of Kerala was about Rs 20 millilon (Das 1994). Sardesai (1996) reported that in Goa farmers sustained losses 20 metric tones fish amounting to Rs 8.0 laks in 1993. The fish losses were 30 metric tones mounting to Rs 12.0 lakh and 15 metric tones amounting to Rs 6.0 lakh in 1994 and 1995 respectively. Abdul Hameed (1996) reported that about 25 tones of fishes valued at approximately Rs 5.00 lakhs in about 20 tanks in three taluks in Dharwad district of Karnataka during February, 1994 was destroyed by EUS. The demand even for healthy fish also declined. Study in Assam showed that the total loss of fish due to EUS had been estimated 10,625 metric tones affecting 81,400 numbers of fish farmers (Das,1996). Das (1997) reported that about 42.19% of the aquaculturists suffered 31 to 40% loss of fish in their culture ponds , the pecuniary loss faced by 50% aquaculturists was in the range of Rs 1,001`to Rs 5,000while 19.73%aquaculturists suffered a greater loss ranging from Rs 5,001 to 10,000. A section of the farmers had to search for alternate jobs and 88.9% fish traders also suffered losses to some extent during the affected period.

Signs of the disease

After the initial outbreak of EUS in India Jhingran and Das (1990) reported that the symptoms and other characteristics of EUS were conspicuously different from the low level ulcerative conditions reported earlier. Fish in the river as well as in confined waters exhibited abnormal swimming behaviour. In the initial stage of the disease the infection usually occurred in the from of multiple inflammatory red spots on the body causing haemorrhage. In carps these appeared within scale pockets. In advanced stage of infection the ulcer spread to a larger area 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 stage 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 *Anabas*

testudineus,16 *Heterioneustes fossilis* and 11 *Clarias batrachus* from different affected areas of North Bengal. They observed that in case of fish without scales the symptoms of the disease first appeared as a red spot. Gradually the red spot increased in size and ulcer developed in the affected area. Ultimately the underlying muscle layer became affected and occasionally the ulcers became deep and necrotic. In scaly fish red spot appeared in some regions of the body. Ultimately the scales were sloughed and the ulcer became deep and necrotic. In both cases fins were also affected.

Kumar et al (1991) found that the distribution of severe ulcerative skin lesion varied species to species. In murrels the ulceration were more pronounced and occurred mostly in the head and caudal areas. In severe cases the tail lesion could affect the 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 hemorrhagic but superficial ulcer on the body side. In Indian major carps, long striped haemorrhagic lesions were found in the region of the caudal peduncle. Pradhan et , al (1991) reported infection of different stages of development in *Catla catla, Lebeo rohita and Cirrhinus mrigala.* In some fishes infections were at the primary stages with single or multiple haemorrhagic red sopts on the body.. Some fishes showed abnormal swimming behaviour and occasional jumping out the water in a pond.

Pradhan (1992) noticed that in severe infection the lesion eroded the total peduncle portion of a *Clarias batrachus.* Das and Das (1993) reported that in acute cases total loss of caudal region took place and in the head region the cranium was destroyed exposing the brain.

Viswanath et, al (1997) classified the lesions into three distinct type. 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 red spot were of normal colour and there was no discolouration of the skin.

Type II lesions were large (2-4 c.m.)and appeared as a dark raised, circular, discoloured areas on the body surface. Skin and scales were relatively intact in these lesions.

Type III lesions occurred as a circular to oval open dermal ulcers extending in to skeletal musculature. These advanced lesions were characterised with haemorrhagic and necrotic open ulcers on the body surface and were devoid of epidermis and scales.

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

Type I. In this case the affected fishes had highly distinctive dark red to brown lesion on one or both flanks and float on the surface grasping for air some time before expiring.

Type II. This type of infections were with chronic and extensive lesions. This type were mostly found in the top predator air breathing fishes, such as the snakeheads.

Type III. This type of lesion depended on the infection and the size of the fish. In this case the ulcers were chronic and extensive and in earliest lesion a small red rosacea or ulcerated scale bed were found. This type was also founds in the snakeheads.

Type IV. The gray mullet (*Mugil cephalus*) showed this type of lesion. In this type the important characteristic feature was small necrotic erosions of one or more scale beds which gradually extend to produce a shallow ulcer with a red centrums, white rim and surrounding black edge.

Type V. This type was found in some very large carps. In this case small, often single, area of haemorrhagic ulceration on fin or back was found. This type was also found in some fishes, which were resistant to EUS as Tilapia.

Aetiology of the disease

Epizootic ulcerative syndrome has been accepted primarily as an infectious disease and it is a complex condition involving certainly fungal and bacterial elements in its later stages and probably one or more viruses (Chinabut, 1995). Aetiological studies have been the subject of major research in the affected countries. The FAO Consultation recommended, that further research should take place, because of the complexity of its aetiolog in different fields viz.. virology, bacteriology and mycology associated with different outbreaks. The investigations carried out by various workers on the aetiology of the desease will be discussed in the following headings:(i) fungus (ii) bacteria (iii) virus (iv) animal parasites.

(i) Fungus

Fungus was suspected to be involved in the aetiology of epizootic ulcerative syndrome when "severe chronic granulomatous mycosis" was found in histological sections of affected fishes in Thailand (Limsuwan and Chinabut, 1983). A wide range of saprolegniaceae, including *Achlya* sp. *Saprolegnia* sp. were identified on the surface of EUS lesions (Pichyangkura and Bodhalamik 1983; Limusuwan and Chinabut, 1983). These were afterwards recognized as secondary agents (Tonguthai, 1985). Roberts *et al.*, (1993) isolated a slow growing and thermo-labile fungi, *Aphanomyces* from the affected fishes of Thailand. When a mycelium from these strains was placed below the dermis of healthy fishes, it caused an inflammatory response and proceed to migrate down into the tissues of the fish, inducing severe myonecrosis with chronic epithelial reaction.

Chinabut *et al.* (1995) reported that spore suspension of the specific pathogenic *Aphanomyces* induced histopathological changes in the muscle of injected fishes kept at three different temperatures, 19, 26 and 31^{0}C. The mortalities and myonecrosis were considerably higher in the fish kept at 19^{0}C than in the fish kept at 26 and 31^{0}C Willoughby *et al.*, (1995) named the fungus *Aphanomyces invaderis*. Miyazaki and Egusa (1972, 1973a, 1973b,

1973c) were the first to isolate a specific fungus from mycotic granulomatosis affected fishes in Japan. As their publication were entirely in Japanese the efforts did not draw the attention of wider community of scientists (Chinabut, 1995). Hatai *et al.* (1977) reported isolation of a fungus from fish, *Plecoglossus altiveliss* in Sliga Prefecture, Japan and it was named *Aphanomyces piscicida* (Hatai, 1980). Association of *Aphanomyces* sp. was reported from RSD outbreask in Australia in 1989 (Fraser *et al.* 1992). Paclibare *et al.* (1994) reported isolation of fungus from EUS affected fish in Philippines. Callinan *et al.*, (1995a,b) reported isolation of *Aphanomyces* sp. from EUS in Philippines and RSD affected fish in Australia. *Aphanomyces* sps. were also isolated from EUS affected fish from the Philippines, Indonesia, Bangladesh (Lilley and Roberts, 1997).

The *Aphanomyces* sp. from various countries have been compared and shown by means 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) to belong to the same species. Genetic fingerprinting techniques have also been used to show that the various isolates were all very similar (Lilley *et al.*, 1997). The pathogenic *Aphanomyces* sp. has been reported under various names such as *Aphanomyces piscicids* (Hatai, 1980), *Aphanomyces invadaris* (Willoughby *et al.*, 1994) and EUS related *Aphanomyces,* ERS (Lumanlan-Mayo *et al.*, 1997). *Aphanomyces invadaris* was renamed to *Aphanomyces invadaris* (David and Kirk, 1997). Willoughby (1999) suggested that *Aphanomyces invadans* could not produce zoospores on the fish itself, through which they can transmit from one fish to another, and the fish become infected from the spores produced in the environment. Shahen *et al.*, (1999) reported that the EUS like ulcerated disease was caused by *Aphanomyces* sp. in Egypt. Vogelbein *et al.*, (2001) suggested from histological evaluation of skin ulcers in over 200 wild menhaden from Virginia and Maryland portions of the Chesapeaks Bay and the Pamlico Estuary, North Carolina, that all ulcers harboured a deeply invasive, highly pathogenic fungus now known to be

Aphanomyces piscicidans. Kurata *et al.*, (2002) recently purified galactose-binding protein (GBP) from *Aphanomyces invadans* and the molecular weight of this GBP is 40KD. They observed that this 40 KD GBP is closely associated with *Aphanomyces* infections such as mycotic granulomatosis, epizootic ulcerative syndrome, red spot disease and ulcerative mycosis.

Mycosis due to *Saprolequia parasitica* was recorded in Indian major carps by Lal *et al.* (1990). Kumar *et al.* (1991) reported presence of *Aspergillus* sp. from EUS affected fishes. *Saprolegnia parasitica* was detected by Mohanta and Patra (1992) in the infected specimens of *Anabas testudineus* in India. Karunasagar *et al.*, (1994) observed the fungi in the deep musculature underneath the EUS effected ulcers in both fresh water and estuarine fishes. However, the fungi was not found in the early stages of the lesions before the development of ulcers. *Aphanomyces* and *Saprolegnia* were the common fungi associated with the ulcerative condition. The fungal isolates could not infect uninjured EUS susceptible fishes in the laboratory. But if placed under the scale 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.

Mohan and Shankar (1995) conducted histopathological studies of EUS affected fresh water and brakish water fishes of Karnataka and observed that numerous nonseptate, highly invasive fungal hyphae associated with massive tissue necrosis and therefore suggested that fungus is one of the etiological agent of EUS.

Qureshi *et al.*, (1995a) after conducting mycological examination of affected fish *Mystus cavasius* collected from Bhopal, suggested the presence of aseptate hyphae within and around the lesions. In most of the cases the hyphae of different species of fungi were found but 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.

Pal (1996, 1997) found different types of fungi within the ulcer of EUS affected fishes such as *Saprolegnia* sp. *Dictyuchus* sp. and *Aspergillus* sp. in *C. punctatus, A. testudineus* and *C. catla.* He also reported the presence of highly branched and aseptate slender fungus most probably *Aphanomyces* on the operculum of an infected *Anabas testudineus* and no fungus was detected at the initial stages of the ulcer formation. Viswanath *et al.*, (1998) hypothesized that EUS specific fungus capable of entering the fish, only following primary damage to the skin. Mohan *et al.*, (1999) has suggested that the invasive fungus *Aphanomyces invadans* associated with EUS is a primary pathogen.

(ii) Bacteria

Available evidences have suggested that bacterial pathogen have important roles in the outbreak of EUS. Various types of pathogenic bacteria were isolated by different workers from the ulcers as well as from internal organs of the EUS infected fishes. Though the bacteria isolated from different fishes varies from species to species but *Aeromonas* sp. were most predominant pathogenic bacteria. *Aeromonas hydrophila* was consistently isolated by Llobrera and Gacutan (1987) from the ulcers of *O. striatus. C. batrachus, G. giurus* in Laguna de Bay, Philippines from December, 1985 to February, 1986. Boonyaratpalin (1989) reported that primarily *A. hydrophila* and occassionaly *Pseudomonas* sp. were associated with the outbreak of EUS in Burma, Indonesia, Lao People Democratic Republic, Malaysia, Singapore and Thiland. Costa and Wijeyaratne (1989) reported association of *A. hydrophila* with EUS affected fish in Srilanka. Subasinghe *et. al.*, (1990) reported association of *A. hydrophila* with EUS affected 19 species of fish from Sri Lanka such as *O. striatus, O. punctatus, H. fossilis* and *M. armatus. P. fluorescens* and some unidentified bacteria were also found occasionally.

Jhingran and Das (1990) isolated *Micrococcus* sp. from the infected fishes. Kar *et. al.* (1990) isolated *Pseudomonas aeruginosa* from the 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 ulcer of air breathing fishes. R1 resembled with *Pseudomonas fluorescens,* R2 resembled with *P. aeruginosa* and R3 was found to be *Aeromonas caviae* (Pradhan, 1992). This was the only report of the isolation of *A. caviae* from the ulcer of EVS affected fishes. The bacterial culture in mixed condition induced severe ulcer in healthy *A. testudineus.* Pure culture of each of two pseudomonads and an aeromonad induced superficial ulcers. Pradhan *et. al.,* (1991) also isolated two Pseudomonads (R1, R2) which resembled with *Pseudomonas fluorescens, one Aeromonad* (R3) and another coccus (C1) from the Indian major carp *Cirrhinus mirgala.* Ali and Tamuli (1991) isolated *Vibrio* sp. *Aeromonas* sp. and *Micrococcus* sp. from the ulcers of *L. rohita, C. batrachus, C. punctatus* and *A. testudineus* from Assam. Mukherjee *et. al.,* (1991) isolated five distinct strains of *A. hydrophila* from 182 EUS affected fresh water and brackish water fishes. Chakraborty and Dastidar (1991) reported isolation of chemoautotropic nocardioform (CAN) bacteria from different types of skin lesion of EUS affected fish as the major or only pathogenic agent.

McGarey *et. al.,* (1991) isolated *A. hydrophila* and *A. sobria,* from the EUS affected fish. It was thought that these played an important role in this disease. Lio-Po *et. al.,* (1992) isolated *A. hydrophila* from the EUS affected fish and showed that EUS like lesions could be induced in *O. striatus* and *C. batrachus.* Torres *et al.* (1993) isolated 54 strians of *Aeromonas* sp. and among these strains *A. hydrophila* was highly pathogenic. Lio-Po *et al.* (1998) also isolated four types of bacteria such as *Aquaspirilum* sp. *Pseudomonas* sp. *Streptococcus,* sp. and *A. hydrophila* from the ulcers of EUS affected fishes of Philippines. After inoculation of those bacteria to healthy *C. batrachus* and *O. striatus* they observed that *A. hydrophila* was highly pathogenic and *Aquaspirillum* sp. and *Pseudomonas* sp. were slightly and *Streptococcus* sp. was moderately pathogenic to *C. batrachus. A. hydrophila* also induced severe ulcer in *O. straitus* and *Streptococcus* sp. induced slight ulcer which healed rapidly.

Qureshi *et al.* (1995b) isolated nine types of bacteria, from EUS affected fishes, of which three are Pseudomonads (*P. fluorescens; P. aeruginosa* and *Pseudomonas* sp.), two Aeromonads *(A. hydrophila* and *Aeromonas* sp.)., one Cytophage sp. and three cocci (*Micrococcus varians, Streptococcus* sp. and *Staphylococcus* sp.) Out of these bacteria aeromonads and pseudomonads were highly pathogenic while micrococcans and cytophagans were less pathogenic. Rest of the bacteria were non-pathogenic Karunasagar *et al.* (1995) isolated *A. sobria* and *A. hydrophila* from the ulcer of *Puntius* sp. in Karnataka, India.

Saha and Pal (2000) isolated 16 strains of bacteria from *C. punctatus, Puntius* sp. and *Mystus* sp. belonging to the genus *Pseudomonas, Aeromonas, Micrococcus, Bacillus, Vibrio* and *Moraxella.* They showed that four Aeromonads out of sixteen strains induced ulcers in healthy *A. testudineus* when injected intramuscularly. (Saha and Pal, 2002).

(iii) Virus

Virus was considered as the causative agent of EUS when virus like particles were demonstrated in various tissues of affected fish during 1982-83 outbreaks in Thailand (Rattanaphani et al , 1983;Wattanavjam et al , 1983 a, b; 1984). Subsequently these workers isolated snakehead rhabdovirus (SHRV) and this virus was shown to be serologically distinct from other fish rhabdoviruses (Ahne *et al.,* 1988; Kasornchandra *et al.,* 1992). Isolation of birna virus from cultured sand goby (*Oxyeleotris marmoratus*) was reported by Hedrick *et al.,* (1986). Frerichs *et al.,* (1986,1989) isolated rhabdoviruses from diseased fishes in south-east and south Asia and suggested this could be the initiating factor in the outbreak of EUS. Though this virus was not isolated from more that 5% of the diseased fishes examined and could not be shown experimentally to induce the disease in healthy fish. Saitanu *et al.,* (1986) isolated a new virus named as snakehead fish virus (SHV) from various infected fish such as *O. striatus, C. micropeltes, O. marmoratus, T. trichopterus, T. vittatus, B. splendens* and *Dermogenus pustillus.* This virus was not affected by ether or chloroform and was resistant at 60^{0}C for

30minutes. SHV produced cytopathic effects, rounded cells and complete destruction of cell sheet on BB, BF2and FHM cells. Intraperitoneal injections of this virus resulted in scale damage in 80% of small snakeheads but not all in larger fish. In early 1990's one birna virus in Singapore (Subramaniam et al, 1993), one rhabdovirus and reovirus like agent in Thailand (Roberts et al, 1994) were isolated from diseased fish. Rhadoviruses were also isolated from the diseased fish collected from Thailand, Myanmar, Australia (Roberts et al , 1989,Roberts et al , 1994, Lilley and Frerichs, 1994) and these were named ulcerative disease rhabdovirus (UDRV).

No virus was isolated from India (Boonyaratpalin, 1989a), Pakistan (AAHRI,ACIAR, IOA and NACA, 1997), Bangladesh, Lao PDR, Malaysia, Indonesia. Siddhi (1989) conduced virological studies on EUS affected *C. idella* , *Colisa* sp, *P. javanicus* , *H. molitrix* and *P. sophore* from Assam, *C. catla* , *C. carpio* from Tripura, *C. punctatus, M. armatus, N. nandus, P. sophore* from West Bengal and showed no evidence of virus by inoculation of tissue extracts. Kar et al, (1990) revealed the presence of viruses by electron microscope in the muscle and gills of ulcerative disease affected fishes in Assam. Kumar et al. (1991) reported that inoculums from affected *Channa* sp. *Puntius* sp. and *Mastocembelus* sp. when injected in confluent cultures of BB, FHH, EPC, cell lines showed cytopathic effect within seven days in culture. Microscopic studies showed spherical virus like particle.

Frerichs *et al.,* (1991) reported isolation of a retrovirus which was capable of inducing cytopathological effects (CPE) in a wide variety of tissue culture. A reovirus was isolated from a diseased snakehead in 1992 (Frerichs, 1995). Thus a number of rhabdo viruses, a birnavirus and a retrovirus have been isolated from EUS affected fish. The heterogenicity of viral isolates and their low recovery rate led to the conclusion that these were adventitious agents (Frerichs, 1995).

(iv) Animal parasite

Association of several metazoans (*Dactylogyrus* sp. and *Gyrodactylus* sp.) and protozoans (*Chilodonella* sp., *Trichodena* sp., *Costia* sp. *Henneguya* sp. and *Ichthyophthirus* sp.) parasites from 273 EUS affected fish during 1982-83 outbreaks in Thiland was reported by Reungprach *et al.*, (1983). A large number of protozoans (*Epistylis* sp.) were reported from several fish with tiny red spots on the skin before the second outbreak in Thiland (Tonguthai, 1985). Callinan and Keep (1989) and Pearce (1990) observed protozoan and metazoan parasites on some affected fish in Australia.

Jhingran (1990) recorded some commonly found parasites, such as *Dactylogyrus* sp. *Tripartiella* sp. and several myxozoans from EUS affected fishes in India. He also reported that as these parasites were found at a very low intensity they could not be they primary cause of ulceration. Mandal *et al.*, (1990) reported the presence of *Costia necatrix* in the the ulcer of EUS affected fish in West Bengal, India. Myxozoan parasites in the skin and kidney of Catla catla and kidney and liver of *Clarias* sp. were detected by Kumar *et al.*, (1991). Ram (1992) reported that myxozoans, Myxobolus sp. and *Thelohanellus* sp. were associated with EUS outbreak in Haryana, India.

Subasinghe (1993) conducted experiments for possible relationship between *Trichodina* sp. infection and induction of EUS in *Channa striata* 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, accelerated the appearance of EUS like lesion and ulcers when induced by feeding and cohabition with EUS infected fish. He suggested that heavy *Trichodina* sp. infection suppresses the natural defence mechanism of *C. striata* causing acceleration of the clinical sign of EUS.

Environmental factors

Disease is not a simple result of contact between host, pathogen and environment (Snieszko, 1974). Many types of stress have been implicated in epizootics of fish (Wedemeyer, 1970; Snieszko, 1974; Plumb *et al.*,1976; Wedemeyer *et al.*, 1977; Walters and Plumb, 1980). Tonguthai (1985) reported that EUS outbreaks are frequently precipitated by adverse environmental conditions.

Temperature

Rodgers and Burke (1981) reported that maximum EUS prevalence in estuarine fish populated with seasonal aggregation of fish was due to stress by low or rapidly changing water temperatures and rapid or prolonged depression of salinity. Roberts *et al.* (1986) have suggested that EUS outbreaks occur in a cyclic manner when the temperature falls after heavy rainfall. There is pronounced similarity in the geo-climatic conditions of the affected countries, which generally have rainfall followed by dry season. There is noticeable decrease in temperature, accompanied by wide fluctuation in the diurnal temperature regime (Jhingran, 1990). Phillips and Keddie (1990) reported that in Bangladesh, China, India and Lao PDR during 1988 and 1989 outbreaks which occurred in months in which the mean daily temperature was below the annual mean daily temperature. They also reported outbreaks in warmer months in Philippines and Thailand. Lumanlan-Mayo *et al.*, (1997) conducted field and laboratory experiments in Philippines and suggested that low water temperatures ($<30^0$C) plays an important role in EUS outbreak. They also showed that rainfall and decrease in alkalinity and calcium and magnesium hardness were associated with the disease outbreak in *Channa striata,* but at the onset of winter, as the temperature began to decrease, artificial maintenance of high level of alkalinity and hardness failed to prevent the outbreaks.

Immunosuppression at low temperatures was suggested as a likely mechanism for seasonality of EUS (Chinabut *et al.*, 1995). Several other

workers also indicated that low temperature was an important factor for some EUS outbreaks (Das *et al.*, 1990; Lilley *et al.*,1992; Das and Das, 1993; Mohan and Shankar, 1994; Ahamed and Hoque, 1998; Sanaullah *et al.*, 2001).

Water quality variables

The intensity of disease outbreak in various states of India was high in water of low alkalinity and hardness i.e. waters closely related to acidic low calcium soil (Jhingran, 1990; Jhingran and Das, 1990). The disease was not solely located in such waters and it was linked to periods of heavy rainfall in more alkaline environments with consequent drop in pH and alkalinity. Bondad-Reantaso *et al.*, (1992) revealed that there were variations in temperature, chloride, rainfall and hardness of water at the time of EUS outbreak in Philippines. Mohan and Shankar (1994) reported that during monsoon months of 1993 when the salinity was very low (<0.5 ppt) EUS occurred in estuaries of Dakshina and Uttara Kanada districts of Karnataka, India. Palisoc and Aralar (1995) observed that the depth, secchi disc transparency, temperature (surface and bottom), chloride and alkalinity were significantly correlated with EUS outbreaks during January 1988 to December 1989 in Laguna lake, Phillippines and outbreak in lake Nujan were associated with temperature only. Callinan *et al.*, (1995) suggested that sub-lethal exposure of susceptible fish to acidified run-off water from acid sulphate soils in Australia was a causal factor for EUS outbreak in some estuarine settlings.

Sardesai (1996) mentioned that the disease started occurring last week of July of September, 1993 in Goa as salinity in estuarine water bodies at the onset 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.

Ahmed and Hoque (1998) reported that water quality parameters such as temperature alkalinity and hardness were reduced in December, January and February in comparison with other months. Clinically and histologially in

the colder months (December to February) all fish specimens were more affected by EUS in comparison with other months. Sanaullah *et al.* (2001) concluded that rapidly declining seasonal temperature and changing water quality, particularly lower chloride and alkalinity with respect to hardness might cause severe stress, depress immunity and reduce resistance to pathogen which ultimately may result to EUS.

Pathiratne and Jayasingha (2001) observed that declining dissolved oxygen concentration in water coincided with initiation of EUS outbreak in Bellancoila-Attidiya wetlands in Srilanka. Ray and Pal (2003) reported that EUS outbreak occurred when dissolved oxygen content, hardness and total alkalinity of pond water remained low.

Heavy metals

Jhingran and Das (1990) analysed the concentration of various metals, e.g. Fe, Zn, Cu, Cr. Cd, Pb and Hg in the affected areas. The values for zinc ranged from 21.0 to 26.8 mg/l in water and 9.13 to 21.6 mg/l in fish where as the respective values for copper ranged from 1.2 to 3.92 mg/l and 2.39 to 2.47 mg/l. The available information did not suggest any perceptible role of the heavy metal contamination in creating stress to the fishes leading to outbreak of the disease (Jhingran, 1990, Jhingran and Das, 1990, Das *et al.*, 1990).

Pesticides and other agrochemical

The incidence of the disease is quite high in rice field environment in all the affected countries. It has been suggested that pesticides may have a role as a predisposing factor for the outbreak of the disease. Jhingran (1990) reported presence of isomers of DDT and BHC not only in water of an affected site, Antpur, Hoogly, India, but also in the muscle of affected fishes. Choudhury *et al.* (1994) analysed pesticides residue in water, fish and plankton of some specific EUS affected water areas in India. They reported occasional higher concentrations of organochlorine and organophosphorus pesticide in water and fish samples, but did not find any correlation with the

presence of pesticide residue and disease outbreak. Palisoc and Aralar (1995) studied levels of pesticides (heptachloride, endosulphar, heptachlorepoxide) in sediment and water of Lake Laguna and Lake Naujan in Philippines but did not find any correlation between the levels of pesticides and EUS outbreak in those lakes.

Flooding

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. Barua (1994) suggested that floods in Bangladesh in 1988 resulted in the rapid spread of EUS in that country. Mohan and Shankar (1994) reported that EUS first appeared in Karnataka in the Cauvery river system during August-September, 1991, immediately after the flood.

Abdul Hameed (1996) reported that EUS outbreak in Karnataka occurred after major flood (from July to September) for four consecutive years (1991-1994).

Histopathology of EUS infected fishes

Kumar *et al.,* (1991) conducted histopathological examination of ulcer, kidney, liver and heart of *Puntius* sp. *Mastocembelus* sp. and *Channa* sp. and found that complete loss of the epidermis of the skin at the ulcer region where dermis and hypodermis showed cyst like or nodule like granulomatous formations in huge numbers. Granuloma formations were also seen in the muscle layers of the skin. Most of the granuloma formations seemed to contain a highly basophilic material inside. Kidney haematopoietic tissue showed proliferation of macrophage cells especially indicating inflammatory reaction. Almost all the renal tubles and glomeruli were found necrotic. In the liver, most of the sinusoidal spaces and blood vessels were congested and wandering lymphocytes were plenty in the liver parenchyma. However, Das *et al.* (1990) did not find any significant changes in the liver except vacuolization in certain cases.

Mohan and Shankar (1995) observed non-septate fungal hyphae in dermis and epidermis with severe necrosis. In advanced ulcers massive invasion of the skeletal musculature by the fungal hyphae was consistent with myofibrillar necrosis. Pal and Pradhan (1995) reported the presence of different bacteria from the histopathological studies of experimentally infected *Clarias batrachus*

Viswanath *et al.* (1997) found that initially (Type I lesion) there were inflammatory changes in the section of the epidermis. Sections stained with Grocott's methanamine silver staining technique (GMS) revealed the presence of fungal hyphae in some of nodular structures. The fungal presence and associated inflammatory changes were restricted to the epidermis. In early stages the dermis and the skeletal musculater were free of pathological changes. In later stage (Type II lesion) they observed mycotic granulomas in the epidermis, dermis and the skeletal musculature associated with numerous non-septate fungal hypae. In the advanced stage of the lesion (Type III lesion), the epidermis and scales were completely lost with partial loss of dermis at the site of ulcer. In most cases the underlying musculature was exposed and was almost replaced by fungal granulomas and host inflammatory tissue. There was considerable myofibrillar necrosis.

Epizootic ulcerative syndrome has been described in more that 100 species of fresh water and brakish water fishes. It was expected that there will be variations not only in the clinical features but also in the histological picture of the ulcer and internal organs between different affected species. These variations in clinical and pathological feature depend on the degree of susceptibility and size of the fish concerned. Chinabut and Roberts (1999) have categorized EUS into five clinical types and described thoroughly the underlying histopathological changes.

Haematological studies

Das and Das (1993) showed higher counts of phagocytic cells in EUS affected fishes that reflected initiation of defence phagocytosis in blood

circulation. There was a decline in counts of RBC followed by a drop in haemoglobin content indicating anaemic condition. Prasad and Qureshi (1995) reported that there were remarkable changes in the numbers of RBC, WBC, differential count of WBC and haemoglobin content.

Declining trends of TEC and Hb contents in experimentally infected fishes, *Channa punctatus* (Pradhan and Pal, 1995) and (Saha, 1998) *Heteropneustes fossilis* with two Pseudomonads (R1 and R2) and one Aeromonad, *Aeromonas caviae* (R3) were reported. Pathiratne and Rajapaksha (1995) studied the total erythrocyte and leucocyte contents, haemattocrit and haemoglobin content, mean corpuscular volume, mean corpuscular haemoglobin concentration and differential leukocyte count in healthy and EUS positive fish, *Etroplus susatengis* in Sri Lanka. Results showed that EUS positive fish were anaemic through loss of blood and destruction of erythrocyte as shown by a significant reduction in TEC, haematocrit and Hb content. In addition, the total and differential counts of severely affected EUS positive fish indicated leucocytosis coupled with marginal increased in macrophages like cell population.

Control measures

Various types of prophylactic and theraputic measures were taken to avoid the disease attack or to minimize the disease attack.

Potassium permanganate

Jain (1990) reported that if the EUS affected fishes were given bath treatment with potassium permanganate @ 5 ppm the red spots on the body of the fishes turned blackish within a week and complete healing and regeneration of scales started within two weeks. Jhingran (1990) reported that the potassium permanganate @ 0.5-2ppm in water showed a good result in curing the ulcers.

Lime

Application of lime in the pond of EUS prone area @ 200-600kg. per hectare showed very good results either in checking the outbreak of EUS or in healing of the ulcers. (Jhingran *et al.* 1990). Jain (1990) reported that better results were found when liming @ 200-600kg per hectare was followed by bath treatment of the infected fish in 5ppm potassium permanganate solution or common salt at 3% to 4% solution.

Antibiotics

The most commonly used antibiotics to treat EUS affected fishes are erythromycin, nalidixic acid, oxytetracyclin, terramycin. To get effective result the above mentioned antibiotics were recommended @ 60-100mg per kg. of feed for 7 days (Jhingran, 1990).

CIFAX

A drug formulated by CIFA for application in EUS affected captive water @ 1 liter / hectare meter of water was reported to show encouraging results in controlling EUS (Das and Das, 1993).