Review of Literature

Disease is not a simple result of contact between host, pathogen and environment (Snieszko, 1974). Sublethal changes in water quality may stress fish sufficiently to predispose them to infectious disease (Wedemeyer and Wood, 1974). Various types of stresses have been implicated in epizootic of fish. 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.
- Signs of the disease.
- Etiology of the disease.
- Environmental factors.
- Histopathology
- Haematological studies.
- Prophylactic and therapeutic measures.

History of the disease

For more than thirty 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 in different areas. It is known as mycotic granulomatosis(MG) in Japan, red spot disease (RSD) in Australia and epizootic ulcerative syndrome (EUS) in South and South East Asia.

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

ulcerative syndrome is now recognized to be synonymous with mycotic granulomatosis described from Japan in 1971 and red spot disease discribed from Australia in 1972 (Chinabut and Roberts,1999).

In Japan an ulcerative condition of fish was first reported in the year 1971 in farmed ayu (*Plecoglossus altivelis*) in Oita prefecture. (Egusa and Masuda, 1971). The disease was named mycotic granulomatosis (Myazaki and Egusa, 1972). The disease affected various species of fish, ayu, goldfish, bluegill and some wild fish (Miyazaki and Egusa, 1972, 1973, a,b,c;). Hatai et al. (1977) isolated a fungus from infected cultured ayu and the fungus was named *Aphanomyces piscicida* (Hatai,1980). An epizootic characterized by shallow haemorragic ulcers named red spot disease(RSD) broke out affecting estuarine fish particularly grey mullet in Queensland, 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) ,NorthernTerritory (Pearce, 1990) and western Australia (Callinan, 1994a). Fraser *et al,* (1992) isolated an *Aphanomyces* fungus from diseased fish. After the out break of mycotic granulomatosis and red spot disease the fish disease characterised by dermal ulcer with large scale mortalities was reported in fresh water and estuarine fish 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). Epizootic haemorrhagic condition was reported in west, central and eastern Java in 1980 (Anon., 1981). Subsequently Wadagdo (1990) reported the out break of the disease with ulcers in snakeheads and cat fish in the Indonesian states of Sumatra, 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 sourthern peninsular Malaysia in 1979. Shariff and

Saidin (1994) described the status of the epizootic ulcerative syndrome after 1986. In Thailand the EUS outbreak occurred for the first time in 1981 and 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 ; Chulalongkorn University, 1983, 1985, 1987; 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). Soe (1990) reported that the subsequent attack of EUS were less extensive in Myanmar. 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 other provinces 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), Vietnam (Xuan, 1990) and Hong Kong (Wilson and Lo, 1992). EUS was first reported in fresh water and estuarine fish in western Sri Lanka 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 al.,* 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.

The epizootic ulcerative syndrome broke out for the first time in India in May 1988 in various states of north eastern India such as Tripura, Meghalaya and Assam (Das, 1988). Subsequently the disease spread to West Bengal

(Pal and Pradhan, 1990).Ultimately the disease spread to almost all the states of India except Gujarat, Punjab,Himachal Pradesh and Jammu and Kashmir by 1993 (Das and Das, 1993).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.1).

Epizootic ulcerative syndrome broke out for the first time in May 1988 in some North eastern states of India such as Tripura, Meghalaya and Assam (Das, 1988). In Assam severe attack of EUS was reported from Borak Valley districts of Cachar and Karimganj. The disease had also been reported from Nalbari, Jorhat, Kamrup, Naogao and Lakhimpur of Assam. The villages adjacent to Bangladesh border were most 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 1988 severe outbreaks of the ulcerative fish disease occurred in northern districts of West Bengal such as Coochbehar, Jalpaiguri, Dinajpur and Darjeeling 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. 2). By the year 1989, the disease had spread to almost all the districts of West Bengal except Purulia (Pradhan and Pal, 1990 ; Jain, 1990 ; Pradhan et al., 1991). In Bihar, Katihar and Kisanganj districts boardering the West Bengal were affected during April and May 1989 (Prasad and Sinha, 1990 ; Kumar *et al.* 1991). In Orissa the disease spread to the northern districts adjacent 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 (Fig. 3) and within 1993 almost all the states of India were affected except Jammu & Kashmir, Punjab, Himachal Predesh and Gujarat (Das and

Fig . 1 : Map showing the spread of EUS across the Asia-Pacific region.

Fig 2 : Spread of EUS in India during 1988

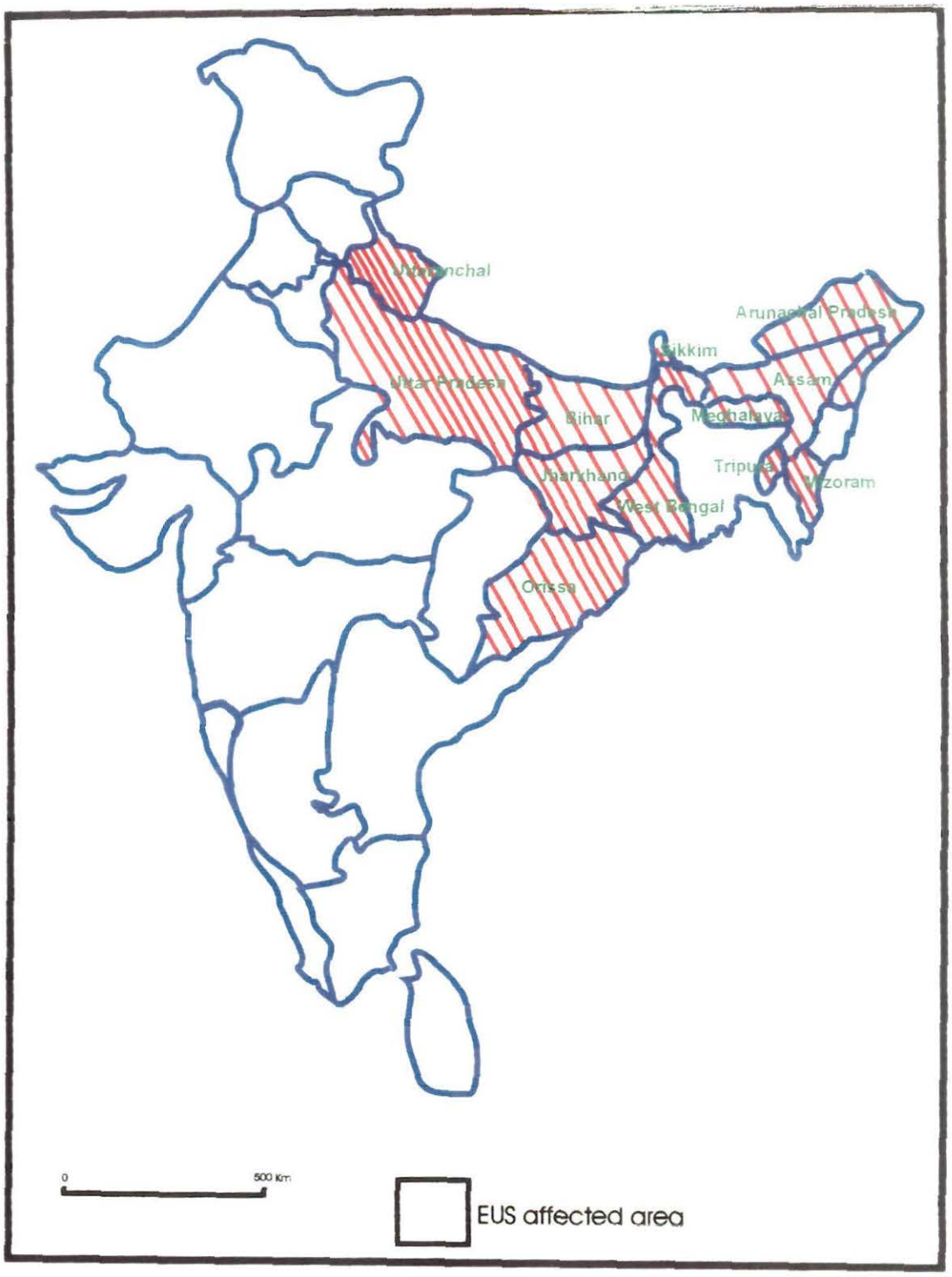

Fig3 : Spread of EUS in India during 1989

Das, 1993) (Fig. 4). Sardesai (1996) reported that the disease first broke out in Goa during August 1993. It was first noticed in Salcete Taluka in South Goa. Abdul Hamed (1996) reported that EUS was first occurred in the Cauvery river and its tributary Kabini river in T. Narasipura Taluk of Mysore district of Karnataka in August 1991 and then spread to other part of the state.

At present a declining trend of the EUS occurance is observed in different states of India. In West Bengal also the occurance of EUS in the districts of South Bengal is not a major factor but 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).

Fish species affected

The epizootic ulcerative syndrome have affected more than 100 fish species of both fresh water and brackish water fish of wild and cultured variety (Lilley *et al.*,1992). Das and Das (1993) found that the occurance 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. A study in the state of Karnataka conducted by Mohan and Shankar (1994) showed that EUS first attacked the bottom dwelling snakeheads (*Channa* sp.) and then attacked the catfishes (*Mystus* sp. and *Wallago* sp.) ,minor carps (*Puntius* sp.) ,featherbacks (*Notopterus* sp.) etc. The indian major carps were mostly unaffected in the state of Karnataka. The other major fish species affected in India are *Ctenopharyngodon idella, Hypophthalmichthys molitrix, Nandus nandus, Rasbora* sp., *Ompak* sp., *Mugil cephalus, M. parsia, Sactophagus* sp., *Epinephelus* sp. , *Catla catla, Labeo rohita, L. calbasu, C. mrigala, Mystus* sp. (*Jhingran* and Das, 1990 ; Pal and Pradhan, 1990 ; Kumar *et. al.,* 1991 ; Abdul Hameed, 1996 ; Mukherjee, 1996 ; Das, 1997). Saha (1998) reported that about 46 fish species were affected (Table -1).

Fig 4 : Spread of EUS in India during 1993

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

In Philippines the affected fish species were snakehead (*O. striatus*), catfish (*Clarias batrachus*), gourami (*T. pectoralis*) ,goby (*Glossogobius girus*) , crucian carp (*Carassius carassius*) ,Manila sea catfish (*Arius manilensis*) and silvery theraponid (*Therapon plumbius*) (*Llobrera*, 1987). During the outbreak of 1990, the estuarine fish species such as mullet (*Mugil* sp.), flatfishes (*Platycephalus* sp. and *Psethodes* sp.) , goat fish (*Upeneus bensasi*) , croaker (Johnius sp.) and spadefish (*Scaptophagus* sp.) were affected (Bondad-Reantaso, 1991). In Pakistan different snakeheads and *C. mrigala* were reported to be affected by EUS (Callinan *et al.*, 1997; DFID, 1998). In Australia, yellow fin bream (*Acanthopagrus australis*) and striped mullet (*M. cephalus*) were among the affected species (Callinan *et al.*, 1995a). In Sri Lanka, *O. striatus, O. punctatus, Heteropneustes fossilis* were the commonly affected fish (Subasinghe *et al.*, 1990). In Bangladesh the Thai silver barb, *Puntius gonionotus* (Bleeker) was among the most susceptible species (Ahmed and Rab, 1995). The Nile tilapia, *Oreochromis niloticus* (L), milkfish and common carps were resistant to epizootic ulcerative syndrome (Bondad-Reantaso *et al*,. 1992).

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 only very few accurate estimate of losses.

Table 1 : Fish species affected by EUS in India

Wild	Cultured
FRESH WATER	
Anabas testudines	*Catla catla*
Acrossocheilus hexagonolepsis	*Cyprinus carpio*
Ambasis ranga	*Cirrhinus mrigala*
Amblypharyngodon mola	*Ctenopharyngodon idella*
Amphipnous cuchia	*Hypophthalmichthys molitrix*
Ailia coila	*Labeo rohita*
Clarias batrachus	*L.bata*
Channa punctatus	*L. calbasu*
C. gachua	*Puntius javanicus*
C. striatus	*P. sarana*
Callichrous pabda	
Chanda chanda	
Colisa fasciata	
Gadusia chapra	
Glossogobius giuris	
Heteropneustes fossilis	
Mystus sp.	
Mastocembelus sp.	
Macrognathus aculeatus	
Mastocembelus armatus	
Monopterus cuchia	
Nandus 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.

Llobrera (1987) reported that the first outbreak of EUS in Philippines affected 15,000 lake shore families in Laguna Lake with a 30% decrease in average daily income of fishermen. 75,000 people depended on 5,000 lake, Mangabol swamp in Pangasinan province, Philippines for food and income from capture fishery and pond aquaculture suffered over 50% and 40% losses during 1989 and 1990 outbreaks of EUS respectively (Bondad-Reantaso *et. al.*, 1994). They also reported about great panic created among the consumers and farmers in Philippines.

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

In 1988, in some communities, a wide spread but unfounded fear of disease transmission to 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 effected 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 118 million and 88.2 million Taka in Bangladesh in the year 1988 and 1989 respectively (Barua,1990).

In Sri Lanka the fishermen 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 Sri Lankan Rupees (Balasuriya, 1994).

About 15-20% of total fish production was lost in Nepal during initial outbreaks. During 1989-90 the total economic loss in eastern Nepal was about of Rs. 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 though all the incidents of EUS outbreak were not reported and estimated accurately from different part of the country the assumption of 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).

Study in Assam showed that the total loss of fish due to EUS had been estimated at 10,625 metric tones affecting 81,400 numbers of fish farmers (Das, 1996). After the initial outbreak, investigations carried out in five districts of West Bengal showed that 73% aquaculture units were adversely affected by EUS. This lowered the fish consumption rate by 28.7% ; 23.3% and 20.5% in urban, suburban 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 fish were lost. 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. 5,000, while 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.In Bihar the loss of fish costing about Rs. 48.0 lakhs was reported during initial outbreaks of 1989-90 (Prasad and Sinha, 1990). Orissa suffered loss of Rs. 30.0 lakhs during 1989-91 (Das, 1994).A study conducted in Kerala showed that the EUS completely paralised the fish market and the fishermen (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 million (Das, 1994).Sardesai (1996) reported that in Goa farmers sustained losses 20 metric tones fish amounting to Rs. 8.0 lakh in 1993. The fish losses were

30 metric tones amounting 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 fish valued at approximately Rs 5.00 lakhs in about 20 tanks in three taluks of Dharwad district of Karnataka during February 1994 was destroyed by EUS. The occurance of the disease in India had caused a decrease in the demand even for healthy fish also. The situation was so serious that both the fisheries department and the health department of severely affected states took up special publicity programme through different media to apply lime in precise dose in the ponds which would act as water purifiers and in this connection lime was distributed free of cost to the fish farmers (Prusty and Nayak , 1990). It can be said that the EUS had made a closer links between the fisheries department and the fish farmers. Another positive impact of EUS has been the increased funding allocated to fish disease research and diagnostic facilities in Asia by Governments and International Organisations.

Signs of the disease

After the initial outbreak of EUS in India Jhingran and Das (1990) reported that the signs and other characteristics of EUS were conspicuously different from the other low level ulcerative conditions reported earlier. Fish in the rivers as well as in confined waters exhibited abnormal swimming behaviour with head projected out of water. In the river the fish floated near the bank with abnormal swimming behaviour. In the initial stage of the disease the infection usually occured in the form of multiple inflamatory 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 *A. testudineus*, 16 *H. fossilis* and 11 *C. batrachus* from different affected areas of North Bengal.

They observed that in case of fish without scales the signs 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 spots 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 from 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 *C. catla, L. rohita* and *C. mrigala*. In some fish infections were at the primary stage with single or multiple haemorrhagic red sopts on the body. Some fish showed superficial ulcers and a few showed deep haemorrhagic ulcers. In advanced stage ulcers were deep and necrotic. They observed some fish showed abnormal swimming behaviour and occasional jumping out of the water in a pond.

Pradhan (1992) noticed that in severe infection the lesion eroded the total peduncle portion of a *C. 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.

After investigating over 300 EUS affected fish from affected fresh and brackish water bodies of Karnataka, 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. These

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 cm.) 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 into 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 fish had a highly distinctive dark red to brown lesion on one or both flanks and may float on the surface grasping for 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 fish, such as the snakeheads.

Type III This type of disease depended on the age of 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 an ulcerated scale bed were found. This type was also found in the snakeheads.

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

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

Etiology of the disease

Epizootic ulcerative syndrome has been accepted as an infectious disease and it is a complex condition involving certainly fungal and bacterial elements in its latter stages and probably one or more viruses (Chinabut, 1995). Etiological studies have been the subject of major research in the affected countries. The FAO consultation of experts recommended further research in different fields viz. Virology, bacteriology and mycology associated with different outbreaks. The investigations carried out by various workers will be discussed in the following headings : (i) virus (ii) bacteria (iii) fungus (iv) animal parasites.

(i) 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 ; Wattanavijarn *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). Frerichs *et al.*, (1986 , 1989) isolated rhabdoviruses from diseased fish in southeast and south Asia and suggested this could be the initiating factor in the outbreak of EUS. Though this virus was not isolated from more than 5% of the diseased fish 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*, *Oxyeleotris marmoratus*, *T. trichopterus*, *T. vittatus*, *B. splendens* and *Dermogenus pustillus*. This virus was not affected by ether or chloroform and was resistant at 60°C for 30 min. SHV produced cytopathic effects, rounded cells and complete destruction of cell sheet on BB, BF2 and 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 one 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. Sidhi (1989) conducted 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. armatas,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 microscopy in the muscle and gills of ulcerative disease affected fish in Assam. Kumar et al. (1991) reported that inocula 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) Kanchanakhan (1996b) showed that the rhabdovirus strain, T9412, isolated in Thailand in 1994, resulted in substantial lesions in striped snakeheads depending upon the temperature, fish species and fish age. All snake head fry died when injected with T9412 at 20°C but no mortality was recorded at 29°C or in other species of fish at either temperature. A virus from snakehead *O. striatus* from Laguna de Bay, Philippines was isolated in January 1991 by using snakehead spleen cells (Lio-po *et al.*, 2000).

(ii) Bacteria : Various scientists claimed that bacteria played important roles in the outbreak of EUS. Different types of pathogenic bacteria were isolated by different workers from the ulcers as well as from various internal organs of the EUS infected fish. Though the bacteria isolated from different fish varies from species to species but *Aeromonas* sp.were most predominant pathogenic

bacteria. *A. hydrophila* was 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 Peoples Democratic Republic, Malaysia, Singapore and Thiland. Costa and Wijeyaratne (1989) reported association of *A. hydrophila* with EUS affected fish in Sri Lanka. Subasinghe *et al.*, (1990) examined 19 species of fish infected with EUS from Sri Lanka such as *O. striatus, O.punctatus, H. fossilis* and *M. armatus* and found *A.hydrophila* in all affected fish *P. fluorescens* and some unidentified bacteria were also found occasionally. Jhingran and Das (1990) isolated *Micrococcus* sp. from the lesions of affected fish. Kar et al. (1990) found *Pseudomonas aeruginosa* in the surface muscle lesions and gill tissues. Pal and Pradhan(1990) isolated four types of bacteria, two fluorescent pseudomonads (R1 and R2), one aeromonad (R3) and one *Micrococcus* sp. (C) from the ulcer of air breathing fish. R1 resembled with *Pseudomonas fluorescens*, R2 resembled with *P. aeruginosa* and R3 was found to be *Aeromonas caviae* (Pradhan ,1992). 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. Similar results were reported in healthy *C. punctatus* also (Pradhan and Pal, 1990). Pradhan *et. al.*, (1991) also isolated two Pseudomonads (R4,R5) which resembled with *P. fluorescens*, one Aeromonad (R6) and another coccus (C1) from the Indian major carp *C. mrigala*. 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 , India. Mukherjee *et al.*, (1991) isolated five distinct strains of *A. hydrophila* from 182 EUS affected fresh water and brackish water fish. 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. Qureshi *et al.,* (1995b) isolated nine types of bacteria, after conducting examination of EUS affected fish, of which three are Pseudomonads (*P. fluorescens; P. aeruginosa* and *Pseudomonas* sp.), two Aeromonads (*A. hydrophila* and *Aeromonas* sp.), one Cytophaga 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. The 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. Lio-Po *et al.,* (1998) isolated four types of bacteria such as *Aquaspirillum* sp, *Pseudomonas* sp. *Streptococcus* sp. and *A. hydrophila* from the ulcers of EUS affected fish of Philippines and Thailand. 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. striatus* and *Streptococcus* sp. induced slight ulcer which healed rapidly. 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*. Among these bacteria only six strains of aeromonads and pseudomonads were pathogenic and the rests were non-pathogenic. Saha and Pal (2002) showed that four Aeromonads out of sixteen strains induced ulcers in healthy *A. testudineus* when injected intramuscularly.

(iii) Fungus : Involvement of fungi in the etiology of epizootic ulcerative syndrome was suspected when severe chronic granulomatous mycosis was found in histological sections of affected fish in Thailand (Limsuwan and Chinabut ,1983) . A wide range of Saprolegniaceae, including *Achlya* sp.

Saprolegnia sp. were identified from the affected fish (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 fish of Thiland.When a mycelium from these strains was placed below the dermis of healthy fish, it caused an inflammatory response and proceed to migrate down into the tissues of the fish, inducing severe myonecrosis with chronic epithelial reaction.

Spore suspension of the specific pathogen, *Aphanomyces* induced histological changes in the muscle of injected fish kept at three different temperatures,19,26 and 31°C. The mortalities and myonecrosis were considerably higher in the fish kept at 19°C than in the fish kept at 26 and 31°C (Chinabut *et al.,*1995) . 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 fish in Japan . As their publications 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 altivelis*s Shiga Prefecture, Japan and it was named *Aphanomyces piscicida* (Hatai, 1980). Association of *Aphanomyces* sp. was reported from R.S.D. outbreaks in Australia in 1989 (Fraser *et al.,*1992). Paclibare *et al.* (1994) reported fungal isolation from EUS affected fish in Philippines. Callinan *et al.* (1995 a,b) reported isolation of *Aphanomyces* sp. from EUS in Philippines and R.S.D.affected fish in Australia.

Aphanomyces spp. were also obtained from the Philippines , Indonesia, Bangladesh (Lilley and Roberts, 1997).

The *Aphanomyces* sp. from various countries have been compared directly 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 *A. piscicids* (Hatai, 1980) A. *invadaris* (Willoughby *et al.* 1994) and EUS related *Aphanomyces*, ERA (Lumanlan-Mayo *et al.,* 1997). *Aphanomyces invadaris* was renamed to *A. invadans* (David and Kirk , 1997). Lilley *et al.,* (1998) has confirmed that a single species of *Aphanomyces* is a necessary cause of EUS. It occurs in all outbreaks and in some outbreaks it may be the only biological factor required for disease to occur. Willoughby (1999) suggested that *A. 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) have 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 Chesapeake Bay and the Pamlico Estuary, North Carolina, that all ulcers harboured a deeply invasive, highly pathogenic fungus now known to be *A. piscicidans.* Kurata *et al.,* (2002) recently purified galactose-binding protein (GBP) from *A. invadans* and the molecular weight of this GBP is 40 KD. 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.

In India the fungal species most frequently isolated was *Saprolegnia* sp. (Das *et. al.,* 1990). Kumar *et. al.,* (1991) reported presence of *Aspergillus* sp. from EUS affected fish. *Saprolegnia parasitica* was detected by Mohanta and Patra (1992) from the infected specimens of *A. 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 fish. 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 fish 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 brackish water fish of Karnataka and observed that numerous non-septate , 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 *M. cavasius* collected from Bhopal , India 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 fish.

Pal (1996,1997) mentions isolation of three species of fungi but no fungus was detected at the initial stage of 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 *A. invadans* associated with EUS is a primary pathogen. No experimental work on the induction of ulcer by the isolated *Aphanomyces* fungus has been done in India.

(iv) Animal parasite : Reungprach *et al.,*(1983) reported association of several metazoans (*Dactylogyrus* sp, *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 Thailand. A large number of protozoans (*Epistylis* sp.) were reported from several fish with tiny red spots on the skin before the second outbreak in Thailand (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 fish in India. He also reported that as these parasites were found at a very low intensity they could not be the primary cause of ulceration. Mandal *et al.* (1990) reported the presence of *Costia necatrix* in the ulcer of EUS affected fish in West Bengal, India. Myxozoan parasites in the skin and kidney of *C. 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 *C. 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 cohabitat 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). Sublethal changes in water quality may stress fish sufficiently to predispose them to infectious diseases (Wedemeyer,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 a pronounced similarity in the geo-climatic conditions of the affected countries, which generally have rainfall followed by a dry season. Jhingran (1990) and Ahmed and Hoque (1998) reported a noticeable decrease in water temperature. 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 tempareture was below the annual mean daily temperature. Lumanlan-Mayo *et al.* (1997) mentioned low water temperatures <30°C has important role in EUS outbreak.

Immunosuppression at low temperatures was suggested as a likely mechanism for the seasonality of EUS (Chinabut *et al.,* 1995). Several other wokers 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; 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), Palisoc and Aralar (1995) and Sanaullah (2001) reported reduced alkalinity and chloride at the time of EUS outbreak. 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 Kanoda districts of Karnataka, India. 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 settings.

Sardesai (1996) mentioned that the disease started occurring last week of July to 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.

Pathiratne and Jayasingha (2001) observed that declining dissolved oxygen concentration in water coincided with initiation of EUS outbreak in Bellancoila- Attidiya wetlands in Sri Lanka.

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 fish 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 fish. 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

. 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. Pradhan (1992) found various degree of degeneration in the liver of diseased fish. Sections of spleen showed vacuolation and necrosis in some region. In the kidney, tubular degeneration, tubular breakage and vacuolation of tubular cells were the most frequent changes. The epidermis of the skin of infected *C. batrachus* were completely destroyed in the ulcer area. The dermis lost its original compactness due to necrosis and the hypodermis appeared in the form of a network of thin fibrils.

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 *C. batrachus*. Histopathological examinations showed the vacuolation and necrosis in the liver and tubular vacuolation and necrosis in the kidney. Vacuolation and necrosis were also detected in the spleen in fish treated with bacteria in mixed condition only.

Histopathology of experimentally infected *C. striata* with EUS associated *Aphanomyces* sp. at three different temperatures, 19°C, 26°C and 31°C were studied by Chinabut et al. (1995). In the early stages of the disease, degenerative changes were observed in all samples, but inflammatory infiltrate was much more marked in fish kept at 26°C and 31°C.by 8 days post injection, the mycotic granulomata in the 26°C sample were extensive and had progressed into the para-vertebral muscles and on the contra-lateral side of the spine to the injection site.In the fish kept at 31°C, the hyphae starting to be walled off in a whirling pattern of macrophages. Fibrosis and many new blood vessels were observed at the site of injection in the specimens kept at 26°C and 31°C. The specimens kept at 19°C showed a more limited host response. Severe myonecrosis of large areas of the myotome developed in these specimens with haemorrhages and small inflammatory foci around the fungal hyphae . The hyphae continued to progress through the myotome and had invaded the peritoneal cavity by 14 days post injection . A limited macrophage response was observed at this stage. From 14 to 28 days post injection, the healing process became well established in the specimens kept at 26°C and 31°C. Hyphae were walled off by a whirling pattern of macrophages. Melanin-containing cells were observed in increasing numbers in the fibrous stroma as healing progressed. In the late stages of infection, regenerating muscle fibres were observed replacing the fibrous tissue. Surviving fish kept at 26°C and 31°C appeared to have recovered completely by 28 days post injection. There were no signs of any healing response at 21days post injection in the specimens kept at 19°C and subsequently all the fish died.

Viswanath *et al.,* (1997) found that initially (Type I lesion) there were inflammatory changes in the sections of the epidermis. Sections stained with Grocott's methenamine 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 musculature were free of pathological changes. In later stage (Type II lesion) they observed mycotic granulomas in the epidermis, dermis and skeletal musculature associated with numerous non-septate fungal hyphae. 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. Chinabut (1990), Wada *et al.* (1994), Ahmed and Hoque (1998), Viswanath et al.(1998) found that in some advanced lesions, fungal hyphae can be seen invading the abdominal viscera which would almost certainly be the ultimate cause of death. A large number of mycotic granulomas had been demonstrated in the kidney, liver and digestive tract of several fish like *C. mrigala, C. lalia , Channa* sp., *Puntius* sp., *Esomus* sp., *Mugil* sp., *Valamugil* sp., *Therapon* sp., *Glossogobius* sp. and *Sillago* sp.

Viswanath *et al.,* (1998) further demonstrated fungus penetrating the oesophagus and spinal cord of mullet and intermuscular bones of *Puntius* sp. Histopathological examinations of the ulcer region of the EUS affected fish conducted by Saha and Pal (2000) showed complete loss of the epidermis. Necrotic granulomatous response was noted in the dermal layer. Lilley et al. (1997) examined the muscle of fish histopathologically after 7 days of inoculation where fungal spores were inoculated and found that there was evidence of some mild traumatic damage such as sarcoplasmic degeneration, haemorrhage and cellular inflammatory infiltration in all cases.

Epizootic ulcerative syndrome has been described in more than 100 species of fresh water and brackish water fish. It is 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 features 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. According to them the Type-I clinical and histopathological pictures consist of severe acute invasive myositis.Flocculent myofibrillar necrosis below the skin lesion without host inflammatory response was the important diagnostic feature. Fungal hyphae , very delicate and of varying diameter , was seen immigrating along fascial planes and between myofibrils with areas of myonecrosis around the fungal hyphae. Type II infections were characterized by rapid early invasion of the dermis, musculature with an acute host inflammatory response.Granuloma formation is an important identifying characteristic feature of this Type II lesion. Type III clinical and histopathological picture is typical in case of infected snakeheads. The pathological picture depends on the age of infection and the size of fish.Granuloma formation is consisting of epitheloid macrophages and muscles are extensively destroyed. The grey mullet (*M. cephalus*) show the Type IV lesion. Type V lesion and histological pictures are found in some fish which have appeared refractory to it e.g. tilapia, Chinees carps and Europian carps. They show small, often single haemorragic ulceration on fin or back and histologically it comprises localized inflammatory lesion.

Haematological studies

Das and Das (1993) conducted a study on haematological parameters of EUS affected fish which showed higher counts of phagocytic cells 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. Pradhan and Pal (1995) observed erythrocyte count and haemoglobin content of *C. punctatus* for seven days after

intraperitoneal injection of four bacteria, two fluorescent Pseudomonads (R_1 and R_2), one Aeromonad, *Aeromonas caviae* (R_3) and one coccus, *M. varians* (C), isolated from naturally infected air breathing fish. They observed that the R_1, R_2 and R_3 injected fish showed significant decrease of TEC and Hb content while the TEC and Hb content of fish treated with coccus (C) and control fish showed no significant change even after 168 hours of injection.

Saha (1998) observed similar type of declining trends of TEC and Hb contents in experimentally infected fish, *H. fossilis* with two Pseudomonads (R_1 and R_2) and one Aeromonad , *A. caviae* (R_3).

Ptheratue 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 increase in macrophages like cell population.

Prophylactic and therapeutic measures

When environmental qualities conducive to fish health deteriorate, fish experience tremendous stress making them vulnerable to various pathogens (Jhingran, 1990). To avoid the disease attack or to minimize the disease attack the following prophylactic and therapeutic measures were taken.

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

Lime : Application of lime in the pond of EUS prone area @ 200 -600 Kg 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) suggested that better result has been recorded when liming @ 200 -600 Kg per hectare 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) also suggested that liming in water are more effective because it raises the pH value of the water to neutral or slightly alkaline value, precipitates suspended or soluble organic materials , promotes biological productivity by enhancing the breakdown of organic substances by bacteria and kills most of the undesirable microorganisms due to its caustic reaction.

Antibiotics : The most commonly used antibiotics to treat EUS affected fish are erythromycin, nalidixic acid, oxytetracyclin, terramycin. To get effective result of 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 L/ hectare metre of water is reported to show encouraging result in controlling EUS (Das and Das, 1993).