Introduction

Fish have long been regarded as important protein source for human. Recently efforts have been made to increase aquaculture production by adopting intensive and semi-intensive methods of production to meet the demand of the ever increasing world population for more fish protein. The intensive rearing through high stocking densities, artificial feed and fertilizer use has created condition that favour the outbreak and spread of infectious disease. Outbreak of diseases hinder efforts to increase fish production. Fish are susceptible to various types of diseases. Fish disease is a global problem affecting fresh water and marine fish, wild, cultured, sport fish and even ornamental fish with large scale mortalities (Trust, 1986).

The vast and varied water resources of India are potentially one of the richest in the world. Apart from big rivers and their tributaries there are creeks, canals and long coastline. The dug up impounded water bodies eg. ponds and canals are most potential for pisciculture in India. The oxbowlakes locally known as beels and baors are also highly productive. The state, West Bengal has rich fishery resources and inland fish production is quite substantial. The state has the largest brackish water fishery resources in India. The rivers of West Bengal harbour twenty two species of economically important fish and the beel fishery comprises thirteen species of marketable fish (Jain, 1990). A large section of the rural population is dependent on aquaculture.

The outbreak of fish disease in India till 1988 had not been of alarming nature. The commonly occurring disease of fresh water fish in India were haemorrhagic septicaemia, dropsy, ulcerative disease, columnaris disease, microsporidiasis, dactlyogyrosis, gyrodactylosis, ligulosis, argulosis and saprolegniosis (Kumar and Dey, 1992; Das and Das, 1995). The most destructive of all fish disease ever witnessed by India is epizootic ulcerative syndrome (EUS). The epizootic ulcerative syndrome broke out for the first time in May 1988 in various states of north-eastern India such as Tripura Meghalaya and Assam (Das 1988). In October 1988 the disease spread to the northern districts of West Bengal such as Cooch Behar, Jalpaiguri, Dinajpur

and Darjeeling District (Pal and Pradhan, 1990). The incidence of the disease was reported from some South Bengal districts such as Murshidabad, Nadia, North and South 24 Parganas and Midnapore (Pradhan and Pal, 1990).

The disease affected almost all the districts of West Bengal except Purulia by 1989 and spread to other states like Orissa, Bihar, Uttar Pradesh by 1990 (Jhingran and Das, 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).

The severe outbreak of epizootic ulcerative syndrome during 1988-90 in West Bengal, created panic among the people and as a result the rate of fish consumption declined sharply. The fish farmers, fishermen and fish traders suffered heavy economic losses (Pradhan, 1992). The situation become so alarming that the West Bengal Government and Ministry of Agriculture, Government of India, had Organised the "National work-shop on ulcerative disease syndrome in fish" 6-7 March 1990 at Calcutta.

The name epizootic ulcerative syndrome (EUS) was adopted in 1988 at the consultation of Experts meeting on ulcerative Fish Disease in Bangkok (FAO, 1986). It was accepted that the condition was primarily an infectious disease and it was a complex condition involving certainly fungal and bacterial elements in its latter stages and probably one or more viruses. Various pathogenic organisms eg. Viruses (Frerichs *et al.*, 1986, Hedrick *et al.*, 1986; Ahne *et al.*,1988; Roberts *et al.*, 1989; Lilley and Frerichs, 1994; Kanchanakhan, 1996b; Lio-Po *et al.*, 2000), bacteria (Llobrera and Gacutan, 1987; Boonyratpalin, 1989, Callinan and keep, 1989; Pal and Pradhan, 1990; Subhasinghe, *et al.* 1990; Jhingran, 1990; Chakraborty and Dastidar, 1991, Lio-Po.*et al.* 1992, 1998; Yadav *et al.*, 1992; Saha and Pal, 2000) and fungus (Frazer *et al.*, 1992; Robert *et al.*, 1993; Chinabut *et al.*, 1995; Willoughby, 1999), have been isolated from affected fish. Various environmental factors such as lowering of water temperature, low alkalinity and pH fluctuations

have been recognised as potential predisposing factors (Bondad-Reantaso, 1992; Das and Das, 1993; Callinan *et al.,* 1995; Lumanlan-Mayo *et al.,* 1997).

Epizootic ulcerative syndrome (EUS) was defined in 1994 at DFID Regional Seminar held in Bangkok as "a seasonal epizootic condition of fresh water and estuarine warm water fish of complex infections etiology characterised by the presence of invasive *Aphanomyces* infection and necrotizing ulcerative lesions typically leading to a granulomatous response" (Roberts *et al.,* 1994).

Heterogenecity of viral isolations eg. snakehead virus (Saitanu *et al.,* 1986), rhabdoviruses (Freirichs *et. al.* 1986, 1989; Roberts *et al.,* 1989, 1994, Lilley and Freirichs, 1994), a birna virus (Subramanian *et al.,* 1993), a retro virus (Freirichs *et al.,* 1991), and a reovirus (Freirichs, 1995) and the low recovery rate of viruses from diseased fish led to the conclusion by some workers that these were adventitious agents (Freirichs, 1995).

Bacterial pathogens have been claimed to play an important role in the outbreak of EUS (Llobrera and Gacutan, 1987; Boonyaratpalin, 1989, Costa and Wijeyaratne, 1989, Subasinghe *et al.,* 1990; Jhingran and Das, 1990; Pal and Pradhan, 1990; Chakraborty and Dastidar, 1991; Lio-Po. *et al.,* 1992, 1998).

Pal and Pradhan (1990) isolated two fluorescent Pseudomonads (R_1 and R_2) and one Aeromonad (R_3) and one coccus(C) from the ulcers of air breathing fish during the initial phase of the outbreak of the disease in North Bengal. R_1, R_2 and R_3 were found to be pathogenic in experimental healthy fish. At that time there was no report of the association of any fungus with EUS in India. Mohan and Shankar (1995) reported detection of non septate, highly invasive fungus in the histological section of EUS affected fish.Only a few Indian workers reported the presence of fungus in EUS affected fish (Karunasagar *et al.,* 1994; Quereshi *et al.,* 1995a; Pal, 1996; Viswanath *et al.,* 1997, 1998; Saha, 1998; Mohan *et al.,* 1999). No detailed work on water quality variables

has so far been carried out in the disease prone areas of North Bengal to find out any correlation with the recurring outbreaks of EUS in this area.

EUS is endemic in many countries and is still extending its geographical range. In 1996 it extended to Pakistan (EFID, 1998). AT present a declining trend has been observed mainly due to excessive application of various prophylactic and chemotherapeutic measures by cautious farmers. Outbreak of the disease is reported every year till today from various regions of North Bengal. Even during this winter season (2002-2003) we have collected EUS affected fish from different areas of North Bengal. Thus the primary source of the pathogen or the transmission factors are not yet controlled.

Under these circumstances it was considered worth while to study some physico-chemical parameters of the pond water of the disease prone areas and to study about the involvement of fungus and bacteria in epizootic ulcerative syndrome. The main objectives of this study are : (1) Studies on some physico-chemical parameters of pond water in epizootic ulcerative syndrome afftected areas of North Bengal. (2) Histopathological studies of naturally infected fish. (3) Studies on RBC count and haemoglobin content of naturally infected fish. (4) Isolation, characterisation and identification of bacteria from infected fish. (5) Isolation of fungus from infected fish. (6) Pathogenicity testing of bacteria isolated from EUS affected fish (7) Histopathological studies of experimentally infected fish by isolated pathogenic bateria, *Aeromonas hydrophila* (8) Histopathological studies of experimentally infected fish by isolated fungus *Aphanomyces* sp. (9) Studies on RBC count and haemoglobin content of experimentally infected fish by the isolated bacteria.