CHAPTER III

REVIEW

OF LITERATURE

3. Review of Literature

3.1. Water resources in Nepal

Nepal has various fresh water wetlands and is broadly classified into two categories: natural and man-made. The natural wetlands include rivers, lakes, ponds, riverine floodplains, swamps, marshes and the man made are water storage areas and deep water agricultural lands. The total water bodies occupy 2.8 % of total area of the country. Natural water bodies consist of about 55% of total inland water resources (Shrestha, 1994). Due to the different altitudinal (Terai, Hills and Mountains) and climatological variation in water bodies, the various fish species are diversified (Shrestha, 2001, Shrestha, 2008). The country has approximately 6000 rivers and rivulets including permanent and seasonal rivers, streams and creeks (WECS, 2002). DoFD (2007) has estimated that water resources of Nepal occupy 817,100 hectares (Table 3.1).The major river systems namely the Mahakali, the Karnali, the Gandaki and the Koshi originate from the high altitude snow covered mountains and are 'permanent' in nature. On the other hand, rivers/rivulets originating in Siwalik/Terai are seasonal in nature. All the rivers of Nepal drain into the Ganges River System in India through the Terai region, giving rise to ox-bow lakes and floodplains.

Table 3.1. Estimated water surface area in Nepal (DoFD, 2007)

Resource details	Estimated Area (ha)	Percentage coverage (%)	Potential areas (ha)
1.Natural water	401500	49.14	
i. Rivers	395000	48.34	-
ii. Lakes	5000	0.61	-
iii. Reservoirs	1500	0.18	78000
2.Village ponds	6500	0.8	14000
3. Seasonal water			
i. Marginal swamps	11100	1.36	-
ii. Irrigated rice fields	398000	48.71	-
Total	**817100**	**100**	**92000**

The lentic water bodies are classified as glacial, tectonic and ox-bow types depending on their geological origin. The lakes that occur above 3500 m altitude are mostly of glacial

origin. There are 3,252 glacial lakes in Nepal (Mool *et al*., 2001). They are generally moraine dammed, but some like the Thulagi glacial lake of the Manaslu area are dammed by ice body (Hanisch and Hunger, 1998). The largest glacial lake is Rara (1036 ha) located at an altitude of 3000 m, followed by Shey Phoksundo (452 ha) and Tilicho Lake (4919 m, area 40 ha). Lakes and ponds of mid-hill and mountain regions (below 3000 m) of Nepal are considered tectonic in origin. Lakes Phewa, Beganas, Khaste, Rupa, Gunde, Dipang, Maidi and Kalpokhari of Pokhara valley are of tectonic category. Some notable hot springs (Tatopani) are situated in Sindhupalchok, some lakes of religious importance are Maipokhari of Ilam, Guphapokhari of Sankhuwasabha and Salpa pokhari at the junction of Bhojpur and Solukhumbu.

3.2. Physico-chemical conditions of water bodies

The physico-chemical parameters of water bodies influence directly or indirectly the aquatic organisms (the number, varieties, distribution, metabolic activities, growth, size, forms etc.) in various ways. Functioning of the aquatic ecosystem is regulated by the interaction among the physico-chemical and biological components of the system. Hence, it is essential to have the knowledge of physico-chemical parameters or identifying suitability and fertility of an aquatic ecosystem. Reid (1961) stated that the successful development and maintenance of a population of organisms depends upon harmonious ecological balance between environmental conditions and tolerance of the organism to variations in one or more of these conditions.

The physico-chemical parameters of a water body change due to seasonal changes, diurnal changes and pollutants. Mainly the air temperature, water temperature, dissolved oxygen, free carbon dioxide, transparency, pH, alkalinity, hardness, chloride and BOD determine the hydrological condition of water body.

Forel is considered as the founder of modern limnology. He worked on Swiss lakes and in 1892 published the book, “Le leman” (Forel, 1892). He also published first limnological text book, “The text book of limnology” in 1901 which provided an impetus for investigation in the limnological field (Forel, 1901). After him, Thienemann (1926) published the book “Limnologie”. Subsequently several authors published books on limnology (Welch, 1952; Ruttner, 1953; Hynes, 1960, 1971; Needham and Needham, 1962; Macan, 1963; Hutchinson, 1967; Wetzel, 2001).

Extensive works have been done by different workers on the physico-chemical and biological parameters of freshwater bodies. Forel (1892, 1901) and Thienemann (1926) provided an impetus for investigation in the limnological field. Moyle (1946) studied some chemical factors influencing the distribution of aquatic plants and reported nitrates along with phosphates as important factors in freshwater productivity. Ruttner (1953) published "Fundamentals of Limnology". Roy (1955) found that the higher pH is associated with the phytoplankton maxima. Barret (1957) revealed that water temperature affects other physico-chemical characteristics of water body. Das (1957) studied some physicochemical parameters of Kathura Tal, Lucknow and reported water temperature ranges (15 - 30) °C, pH (7.2 - 9.2), dissolved oxygen (4 - 6) mg/L and free carbon dioxide (17 - 39) mg/L. Chakaraborty *et al.* (1959) reported maximum dissolved carbon dioxide in monsoon months of the river Jamuna at Allahabad. Elmore and West (1961) stated that an increase in temperature of water results the decrease of dissolved oxygen and increase in sediment concentration which hampers photosynthesis and reduces dissolved oxygen level. Needham and Needham (1962) published "A Guide to Study Freshwater Biology".

A high value of dissolved oxygen was observed in winter season (Moitra and Bhattacharya, 1965). George (1966) reported direct correlation between chloride content and pH. Michael (1969) reported the seasonal trends in the physico-chemical factors of freshwater fish pond and their role in fish culture. He found direct correlation of carbon dioxide with the amount and nature of biological activity in water. Munawar (1970) observed that shallower water body change water temperature more quickly by comparative study of three ponds of Hyderabad. Cooke and Kennedy (1970) studied the eutrophication of North eastern Ohio lakes of Portage County, United States. Nasar and Munshi (1971) found direct correlation between bicarbonate alkalinity and pH. McColl (1972) studied water chemistry and trophic status of seven New Zealand lakes. Hannan and Young (1974) reported the influence of a deep storage reservoir on the physico-chemical limnology of a central texas river. Khalaf and Mc Donald (1975) studied the physico-chemical conditions in temporary ponds in the New Forest of southern England.

Zutshi and Khan (1977) reported highest Sechhi transparency in the month of January from Surinsar and Mansar Lake. A strong positive correlation was found between atmospheric and water temperature by Kant and Anand (1978). Swarup and Singh (1979) reported high chloride during summer season. Das (1981) studied seasonal fluctuation in physico-chemical

parameters of Nainital lakes. He reported maximum range of surface water temperature and pH in summer and minimum in winter. Alkalinity was maximum in winter and minimum in autumn. Singh *et al.* (1982) found high level of free carbon dioxide related to low level of oxygen and vice-versa in the river Brahmaputra at Guwahati.

Bhowmick and Singh (1985) mentioned that the low dissolved oxygen in the summer were mainly due to high temperature as well as microbial demand for oxygen in decomposition of organic matters. Wright *et al.* (1985) studied the effects of physico-chemical parameters and seasonal changes in macro invertebrates which significantly affects the community structure in some rivers in the Great Britain. Bhowmick (1988) reported temperature variations from (18-32)° C, pH from 6.8 to 9.1, total alkalinity from 68 to120 mg/L and dissolved oxygen (3 - 7.2) mg/L of ten lakes of West Bengal. Dobriyal and Singh (1989) observed negative correlation between free carbon dioxide and dissolved oxygen and positive correlation between free carbon dioxide and temperature. Lerman and Wevner (1989) found that the diffusion of carbon dioxide from the atmosphere and its storage in lakes cause increase in the alkalinity of water. Singh (1990) reported high bicarbonate alkalinity during winter and minimum transparency during monsoon period in the Brahmaputra river.

Jindal and Kumar (1993) observed inverse correlation of temperature with dissolved oxygen. Gupta and Shrivastava (1994) reported that pH increased when the dissolved oxygen and transparency decreased in Varuna River. Patralekh (1994) found higher dissolved oxygen and hardness in river than spring and pond, lower hardness during monsoon season in all the ecosystems and minimum amount of free carbon dioxide during summer in pond and maximum during winter in thermal spring. Haque and Khan (1994) reported maximum Secchi disc transparency in September and minimum in October, maximum total alkalinity during post winter months and low during monsoon months and dissolved oxygen concentration lower in winter than in spring. Sinha *et al.* (1994) studied the biodiversity and pollution status in relation to physico-chemical factors of Kawar lake of North Bihar and reported pH (6.3-7.23), dissolved oxygen (2.15-7.6) mg/L and free carbon dioxide (2.8-12.75) mg/L. Sharma *et al.* (1994) reported the range of pH (6.65-7.08), dissolved oxygen (2.15- 6.77) mg/L, free carbon dioxide (0.0 - 9.68) mg/L and total hardness (158.27-428.40) mg/L in Kawar lake Bihar. Singh (1995) recorded higher transparency during winter months.

Rawat *et al.* (1995) studied morphometry and physico-chemical profile of high altitude Lake Deoria Tal of Garhwal Himalaya from 1990 to 1991. Pandey and Lal (1995) studied seasonal variation in physico-chemical factors of Garhwal Himalaya hill stream Khandagad and found positive correlation between atmospheric temperature and water temperature. Turbidity, water velocity, water discharge were positively related with each other and were found maximum in monsoon. Maximum DO was in winter when temperature was low. Free carbon dioxide was found maximum during monsoon with high temperature and turbidity.

Sharma (1996) studied ecology of the Koshi River in Nepal-India (North Bihar) from January 1992 to December 1993. He reported quite suitable physico-chemical characteristics of the Koshi river water with a high degree of ecological efficiency and enormous potential for biotic development. Jana (1998) has summarised the limnological data for about 60 lakes and reservoirs of India. Jain *et al*. (1999) reported positive correlation between pH and dissolved oxygen of water of a sacred lake Khecheopalri of Sikkim. They also reported positive correlation of water temperature with pH and dissolved oxygen but negative correlation with free cabon dioxide and alkalinity. Mishra *et al*. (1999) worked on limnology of a freshwater tributary during the year 1994 and reported maximum amount of dissolved oxygen, total alkalinity and chloride in winter season and free carbon dioxide in monsoon season. Sharma and Agarwal (1999) studied the water quality of the river Yamuna at Agra and the river was found to be highly polluted. Latifa and Acharya (2001) studied the physico-chemical parameters of a freshwater pond (Jagir pond) in Jagir, Manikganj, Bangladesh. They reported neutral to slightly alkaline pH (7-7.5), dissolved oxygen (2-5) mg/L and free carbon dioxide (6-9) mg/L. Palui *et al.* (2003) studied the eutrophication in Kaithkola Ox-bow lake, North Bihar. Sakhare and Joshi (2004) studied the physico-chemical properties of some reservoirs in Maharastra. Samal and Majumdar (2005) studied the hypolymnetic oxygen depletion scenario in the two national lakes, Rabindra sarobar and Subash Sarobar in India. Yousuf and Bhat (2006) reported the limnological features of the river Jhelum and its important tributaries in Kashmir Himalaya. Akin *et al.* (2008) reported the physicochemical, toxicological and ecological analysis of Gocekaya Dam lake. Singh *et al.* (2008) studied the impact of Lakhwar hydropower project on the physico-chemical parameters of water of the river Yamuna and revealed that the values of most of the river parameters were within the permissible limit of National River Standards and Bureau of Indian Standards (BIS).

Small, torrential, spring-fed river Relli of Darjeeling Himalaya in West Bengal, India was studied for assessing the physico-chemical status of water through space and time (Acharjee and Barat, 2011). Air temperature varied between 15.6^{o}C and 34.6^{o}C, while water temperature ranged from 13.3^{o}C to 27.8^{o}C. River water was slightly alkaline and specific conductance was low. DO level in water was high with a mean value of 9.7 mg/L whereas the free CO_2 level was found to be varying between 0.6 mg/L and 5.8 mg/L. The water of the river was weakly alkaline, with low total alkalinity, low total hardness and low chloride. Nutrient analysis showed low to moderate level of phosphate-P, ammonium-N, nitrite -N and nitrate-N. Mary Helen *et al.* (2011) focused upon the seasonal variation of physico-chemical parameters from coconut husk retting area of Parakkani river, Tamil Nadu and recorded pH (6.72-7.71), electrical conductivity (852-5787 µs/cm), PO_4(1.77-2.8) mg/L, F (0.2-0.4) mg/L,NO_3(3-12) mg/L, NO_2 (0.05-1.51) mg/L, SO_4(21-120) mg/L, Fe (1.53-3.76) mg/L and Mn (0.33-0.67) mg/L. The values were exceeded the permissible limit of WHO.

Aremu *et al.* (2011) revealed that Rivers Doma and Mada, Nigeria were unsafe for drinking and fish samples from these rivers were not desirable for consumption because of the presence of some toxic trace metals above the permissible level. Mondal *et al.* (2012) reported the physico-chemical status of surface water of Mirik Lake in Darjeeling from seven locations. Onada *et al.* (2015) analyzed four different physico-chemical parameters (Temperature, pH, Dissolved oxygen and Ammonia) of Nazarene Fish Farm, Ibadan, Nigeria and found that the values of dissolved oxygen and temperatures showed significant variations between the times of the day. Parisara (2015) studied the physicochemical parameters and its correlation of Konandur pond in Thirthalli, Karnataka, India and showed that mostly positive correlation so the water quality was not much polluted.

Though vast number of limnological investigations has been carried out in other countries, only a few works have been done in Nepal. Loffler (1969) reported the dominance of calcium among cations, low chloride and less than 1 µg/L phosphorous from the high altitude lakes of Mount Everest region. Lohman *et al.* (1988) studied pre and post monsoon limnological characteristics of lakes of Pokhara and Kathmandu valleys. They obtained low alkalinity and conductivity in the lakes of Pokhara valley. McEachern (1994) reported 8.4 pH, 0.03 mg/L phosphate and 8.5 mg/L dissolved oxygen in Narayani, a lowland (< 1000 m) river of Nepal. Aryal and Lacoul (1996) studied water quality and diversity of diatoms in Punyamati River. They reported high pH, total hardness, BOD at polluted site, where transparency and

dissolved oxygen were low. Ormerod *et al.* (1996) reported the pH range between 7.3 to 8, chloride 0.4 mg/L to 1.4 mg/L, nitrate 0.06 mg/L to 0.28 mg/L, and phosphate 0.02 mg/L to 0.04 mg/L from the highland (> 2000m) rivers of Nepal. The physico-chemical parameters of Koshi River were studied at the Kushaha area of Koshi River in the Koshi Tappu Wildlife Reserve (Thapa Chhetry and Pal, 2011). Ambient and surface water temperature ranged from 18^{o}C to 33.1^{o}C and 14^{o}C to 29.2^{o}C respectively. Both parameters showed a significant correlation with free carbon dioxide and biological oxygen demand and an inverse and significant correlation with pH, dissolved oxygen, total alkalinity, total hardness and chloride.

Thapa and Pal (2012) described the water quality of Baidya fish pond was normal except high fluctuation of chloride 1±0.241 to 29.84±0.260 mg/L and ammonia 1.55±0.088 to 18.7±0.061 mg/L during manuaring period. Thapa and Pal (2014) studied physico-chemical parameters of Singhia and Budhi rivers in Sunsari and Morang industrial corridor, Nepal.

3.3. Fish Disease

Fish disease occurs as a result of interaction between the environment, the host and the etiological agent. Stress makes the fish more susceptible to infection. When cultured more intensively, diseases appeared more frequently and become more prevalent. Stress is a condition in which an animal is unable to maintain a normal physiological state because of various factors adversely affecting its well-being. It is caused by placing a fish in a situation which is beyond its normal level of tolerance (Francis-Floyd, 2009). The current disease problems faced increased due to globalization of trade and markets; the intensification of fish-farming practices through the movement of brood stock, post larvae, fry and fingerlings; the introduction of new species for aquaculture development; the expansion of the ornamental fish trade; the enhancement of marine and coastal areas through the stocking of aquatic animals raised in hatcheries; the unanticipated interactions between cultured and wild populations of aquatic animals; poor or lack of effective biosecurity measures; slow awareness on emerging diseases; the misunderstanding and misuse of specific pathogen free (SPF) stocks; climate change and other human-mediated movements of aquaculture commodities (Bondad-Reantaso *et al.*, 2005).

Olivier (2002) described certain essential criteria in order for a disease to spread from either cultured fish to wild fish or vice-versa are presence of pathogen in both fish and water source; presence of susceptible host; viability, in terms of number and longevity of pathogen in the

environment and viable infection route. Infectious diseases of fishes are common occurrence. Causative agents of the infectious diseases are viruses, bacteria, fungi etc.

3.3.1. Viral disease

Piscine viral diseases sometimes caused serious problem in aquaculture (Ghittino *et al.,* 1984; Trust, 1986; Meguro *et al.,* 1991). Infectious carp dropsy or viral hemorrhagic septicemia needs special attention.

Viral hemorrhagic septicemia (Infectious carp dropsy)

Different European countries, as for example, German Democratic Republic (Schäperclaus, 1965, 1969), Rumania (St. Nicolau, 1951), USSR (Goncharov,1965), Yugoslavia (Tomasec, 1951; Tomasec and Fijan , 1965), Czechoslovakia (Volf and Havelka, 1965), France (Bellet, 1958, 1965), Italy (Ghittino,1965), Poland (Miaczyanski, 1965; Kocylowsky, 1965) have witnessed hemorrhagic ulcer disease of fishes. The disease have been named differently by different authors, e.g. infectious carp dropsy (Schäperclaus, 1965), hydropigenic neuroviross (St. Nicolau, 1951), rubella (Goncharov, 1965), pop eye sickness, viral hemorrhagic septicemia (Ghittino, 1965; Bellet, 1965), carp septicemia (Kocylowsky, 1965) etc. At the conclusion of the first European symposium on fish disease, Turin, Italy, October 20-24, 1962, it was decided that the disease should be named "Viral Hemorrhagic septicemia" (VHS). The symptoms of the disease are external lesions, ulceration, exophthalmia and dropsy.

For detection of viral pathogens among fishes several workers established fish cell lines (Chen and Kou, 1981; Chen *et al.,* 1983a, b; Yashushi *et al.,* 1991).

3.3.2. Bacterial fish diseases

Bacteria which are ubiquitous in nature can enhance the disease in poor nutrition, weakened immune system of fish and poor water quality. The most frequently occurring bacterial diseases are given below:

Outbreak of 'Sekiten byo' (red spot disease) in epizootic form during late spring to early summer of 1971 causing considerable mortalities among pond cultured eel in Shizuoka and Tokushima prefectures, Japan (Wakabayashi and Egusa, 1972). Petechial haemorrhage on the body surface was caused by the pathogen *Pseudomonas anguilliseptica*. Miyazaki and

Egusa (1977) had studied the histopathology of red spot disease of the Japanese eel and revealed that lesions appeared in dermis, subcutaneous adipose tissue, internal body musculature, vascular walls, bulbous arteriosus and heart. Various pathological changes in the internal visceral organs have also been recorded. Kuo and Kou (1978) isolated *P. angulliseptica* from red spot disease of *A. japonica* in Taiwan. Nakai *et al.* (1985) had recorded *P. anguilliseptica* in pond cultured ayu (*Plecoglossus altivelis*). Red spot disease was reported in Europian eel (*A. anguilla*) in Scotland (Nakai and Muroga, 1982). RSD was reported from Danish eel farm (Mallergaard and Dalsgaard, 1986) and several species of farmed salmonoid fish were infected by *P. anguilliseptica* in Finland (Wiklund and Bylund, 1990).

Motile Aeromonad disease of fishes caused by *Aeromonas hydrophila* was commonly known as motile aeromonas septicemia (AFS, 1975). The disease had been named as red pest for *A. anguilla* (Schäperclaus, 1934), red disease for *A. japonica* (Hoshina, 1962) and *Cyprinus carpio* (Egusa, 1978), red sore for *Micropterus salmonides* (Huizinga *et al.*, 1979) and Aeromonas disease in cyprinids (Takahashi, 1984b). Ulcer disease due to bacteria *Aeromonas hydrophila* has been reported in Indian major carps by Gopalakrishnan (1963), in *Catla catla* (Karunasagar *et al.*, 1986). Kumar *et al.* (1987) reported several outbreaks of such diseases.

Ulcer disease was described by Calkins (1989) and was common in various species of trout caused due to Gram negative motile rod, *Haemophilus piscium.* An extensive review on the disease in trout had done by Mawdesly - Thomas and Jolly (1968). Gold fish ulcer disease was first described in England in 1969 by Mawdesly-Thomas (1969). Ohtsuka *et al.* (1984) isolated *Aeromonas salmonicida* from ulcerative lesions of head part of diseased eels as the causative agent.

Vibriosis was described as worldwide infectious disease occurred mainly in farmed and wild marine and brackishwater fishes (Anderson and Conroy, 1970; Ghittino, 1972; Lewis, 1985). In Japan, Muroga and Egusa (1967) isolated the bacterium *Vibrio anguillarum* from ayu (*Plecoglossus altivelis*) in salt water Lake of Hamana. Afterwards the disease reported by other workers in Japanese eel, *A. japonica* (Jo and Muroga, 1972; Miyazaki, 1980), in yellow tail, *Seriola quinquirata* (Jo *et al.*, 1979). Bacterium isolated from first outbreak of cold water vibriosis (Hitra disease) from diseased fish in Norway in late seventies was *Vibrio salmonicida* (Egidius *et al.*, 1986). Vibriosis is characterized with haemorrahagic septicemia

and is frequently associated with superficial ulcers, haemorrhages at the base of fins and bloody discharges from the vent (Bullock *et al.*, 1971; Novotony, 1978).

Furunculosis is an infectious disease of many fish species caused by *Aeromonas salmonicida* (Ghittino 1972; Miyazaki and Kubota, 1975 and Ellis *et al.*, 1981).They described two types of furunculosis, i. acute type (without any external sign of disease), ii. sub acute type (skin or muscle lesion seen in many places).Various types of histopathological changes in the gills, gastro-intestinal tracts, kidney and spleen have been recorded by various authors (Ghittino, 1972; Miyazaki and Kubota, 1975; Boomker *et al.*, 1984).

Bacterial kidney disease is a chronic systematic infectious disease caused by *Renibacterium salmoninarum* (Sanders and Fryer, 1980). Histopathologically infected fish shows swollen, granular and purulent kidney. Sometimes ascitic fluid may be present in the peritoneum and within the opaque membrane covering the internal organs like kidney, spleen and liver (Wood and Yasutake, 1956; Smith, 1964). Bruno (1986) has experimentally induced the disease in rainbow trout and Atlantic salmon with viable *R. salmoninarum*.

Hawke (1979) described enteric septicemia of catfish externally characterized with haemorrhages, at the base of pectoral fins, on the lower edge of operculum. An enteric bacterium *Edwardsiella ictaluri* was identified (Hawke *et al.*, 1981; Chen *et al*., 1989). Miyazaki and Plumb (1985) reported that the disease can affect brain, liver, spleen and kidney of infected fishes. Baxa *et al.* (1985) reported that the diseased red sea bream possessed typical symptoms of Edwardsiellosis with haemorrhagic lesions on the caudal and dorsal fins and white spots on the liver and kidney. Baxa *et al.* (1990) found that *E. ictaluri* is a potential pathogen of salmonid fish also.

Myxobacteria and other external bacterial infectious disease in fish like “Columnar disease”, “Peduncle disease”, “Gill disease” and “Tail rot disease” are caused by bacteria (Mawdesley-Thomas and Jolly,1968). The most common agent is *Chondrococcus columnaris* but other organisms have also been considered for such diseases. Marks *et al.* (1980) isolated *Corynabacterium* sp. and *Flexibacter columnaris* from lesion of cat fish associated with “Columnaris disease”. Kumar *et al.* (1986) have reported “Columnaris disease” in *Labeo rohita* caused by *Flexibacter columnaris*.

Red Mouth disease is characterized with inflammation of buccal cavity, reddening of mouth, throat and opercula. Liver is often pale, the spleen and kidney are congested and become dark the causative agent is *Aeromonas hydrophila* (Mawdesley-Thomas and Jolly, 1968). *A. liquifaciens* and *Yersinia ruckeri* are also isolated from internal organs and blood (Grawinski, 1990).

Several other bacterial infections mainly the streptococcal disease in fishes has been reported by several authors (Kusuda *et al.*, 1976; 1978; Miyazaki, 1980). Tuberculosis in fishes caused by Gram positive rod, *Mycobacterium* sp. is well documented (Mawdesley-Thomas and Jolly, 1968). The fish Mycobacteriosis and fish Nocardiosis both belong to the same group of disease (Ghittino, 1972).

Kusuda *et al.* (1987) isolated fish pathogenic bacterium *Mycobacterium* sp. from an epizootic characterized haemorrhage and abdominal ascities with hypertrophy of the spleen and kidney with tubercles occurred in cultured yellow tail in Kochi prefecture, Japan. Bragg *et al.* (1990) also isolated *Mycobacterium fortuitum* from three species of fresh water fish in South Africa. Gelev *et al.* (1990) identified a bacterium *Halfnia alvei* responsible for the epizootic haemorrhagic septicemia in rainbow trout.

3.3.3. Epizootic ulcerative Syndrome (EUS)

3.3.3.1. History of the disease

Prior to 1971, there was no report of the outbreak of EUS in different parts of Asia and Asia-Pacific region. In 1971 from Japan an ulcerative condition of fish was first reported in farmed ayu (*Plecoglossus altivelis*) in Oita prefecture and it was named mycotic granulomatosis (MG) (Egusa and Masuda, 1971). In 1972 a similar ulcerative disease in fish was reported from Central Queensland, Australia with recurrence in subsequent years and the disease was known as red spot disease (RSD) (Rodgers and Burke, 1981).

During 1975-76 the ulcerative disease spread to Papua New Guinea (Haines, 1983). The disease spread to Indonesia in 1980 and it was named “haemorrhagic septicemia” (Roberts *et al.*, 1986). The ulcerative disease was reported from Malaysia in 1981-82 and by 1985 it spread to other countries like Thailand, Laos PDR, Cambodia and Myanmar (Ulcerative Fish Disease Committee, 1993; Tonguthai, 1985; Roberts *et al.*, 1986; Lilley *et al.*, 1992). A severe outbreak of EUS in December 1985 was reported from Laguna de Bay in the

Philippines affecting snakeheads, gobies, gouramies, catfish, crucian carp etc. (Llobrera and Gacutan, 1987).

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

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

India first experienced the outbreak of epizootic ulcerative syndrome in the month of May 1988 in some north eastern states of India such as Tripura, Meghalaya and Assam (Das, 1988). Naogao, Karimganj, Kamrup, Cachar and Silchar areas of Assam were initially affected. Severe outbreak of EUS was reported from Barak valley of Cachar and Karimganj districts of Assam (Jhingran and Das, 1990; Kar *et al.*, 1990; Kumar *et al.*, 1991). In India, the outbreak of the virulent EUS fish disease had been first encountered during July, 1988 among the fishes in the Freshwater bodies of North East (NE) India. In 1989, Nepal had also been affected by EUS (Das and Das, 1993; Kar *et al.*, 2007; Kar, 2013).

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

The disease gradually spread to Uttar Pradesh, Madhya Pradesh, Maharastra, Tamil Nadu, Andhra Pradesh, Kerela, Haryana, Rajasthan and Karnataka. By 1993, the disease spread to

almost all the states of India except Gujarat, Punjab, Jammu and Kashmir (Das and Das, 1993). Though the disease first appeared in wild water of rivers and canals but in the long run it affected all types of water bodies like reservoirs and culture ponds.

The initial outbreak of EUS in Nepal was reported in February 1989 from the eastern part of Nepal (Shrestha, 1994). EUS spread to several districts of Terai and Kathmandu Valley in 1998 and again it was reported from Terai in January, 2000 (DFID, 2001). By 1996, EUS had spread to the upper part of the Indus River in Pakistan (Kanchanakhan, 1996). In 1996, major outbreaks were reported in Pakistan (AAHRI, 1997).

In the USA, significant mortalities in Menhaden have been associated with a high prevalence of EUS lesions (Noga and Dykstra, 1986; Blazer *et al.*, 1999). The ulcerative mycosis (UM) has affected estuarine fish along the Atlantic coast of USA and is indistinguishable from EUS (Blazer *et al*., 2002). In Florida, infections with *A. invadans* in 21 species of estuarine and freshwater fish were reported (Sosa *et al.*, 2007a).

In the spring of 1998, the Florida Fish and Wildlife Research Institute received numerous reports of ulcerated fish disease primarily from the St. Lucie Estuary on the southeast coast of Florida. The diseased specimens had randomly distributed skin ulcers (Sosa *et al.*, 2007b). Saylor *et al*. (2010) reported mass mortality event of captive juvenile bulls eye snakehead (*Channa marulius*) collected from freshwater canals in Miami-Dade County, Florida. Clinical signs appeared within the first two days of captivity and included petechiae, ulceration, erratic swimming, and inappetence. Histological examination revealed hyphae invading from the skin lesions deep into the musculature and internal organs. Further investigation revealed the distribution and prevalence of *A. invadans* within the bullseye snakehead.

In Africa disease affected wild fish Bluegill sunfish (*Lepomis macrochirus*) and Cichlid (Cichlidae) in fresh water pond which was the first incidence of the disease confirmed in 2007. The disease was reported from Namibia in fish farms and in the wild from Zambia. The disease was reported from the entire Zambezi river with its upper tributaries in 2008 - 2009 , and the Kafue River and Okavango delta of Botswana in 2010. By 2011, the disease spread to Chongwe River, Zambia and the Western Cape Province of South Africa (Mudenda, 2012).

In Africa, the upper Zambezi floodplain at the confluence with the Chobe River spans the four countries of Botswana, Namibia, Zambia and Zimbabwe, making disease control a challenge (Huchzermeyer and van der Waal, 2012).

The disease was defined in DFID Regional Seminar in Bangkok in 1994 as "a seasonal epizootic condition of fresh water and estuarine warm water fish of complex infectious aetiology characterized by the presence of invasive *Aphanomyces* infection and necrotising ulcerative lesions typically leading to a granulomatous response (DFID, 1994).

The Fifth Symposium on Diseases in Asian Aquaculture held in Gold Coast, Australia where 36 EUS experts from Australia, India, Japan, Philippines, Sri Lanka, Thailand, and the United States of America re-examined the causal factors, case definition and nomenclature of EUS and proposed two new common names: epizootic granulomatous aphanomycosis (EGA) and ulcerative aphanomycosis (Baldock *et al.*, 2005).

EUS is also known as RSD, MG, UM and EGA. The Oomycete that causes EUS is known as *Aphanomyces invadans* or *A. piscida*. So far only one genotype of the EUS Oomycetes has been recognised, and is said to be the cause of the widespread outbreak. In addition, parasites and viruses (notably rhabdoviruses) had also been associated with particular outbreaks, while secondary Gram negative bacteria are usually associated with lesions due to EUS (OIE, 2000; FAO, 2009).The spread of the disease is shown in Figures (3.1-3.4).

Fig.3.1 EUS affected countries across the Asia-Pacific regions (Lilley *et al*., 1998)

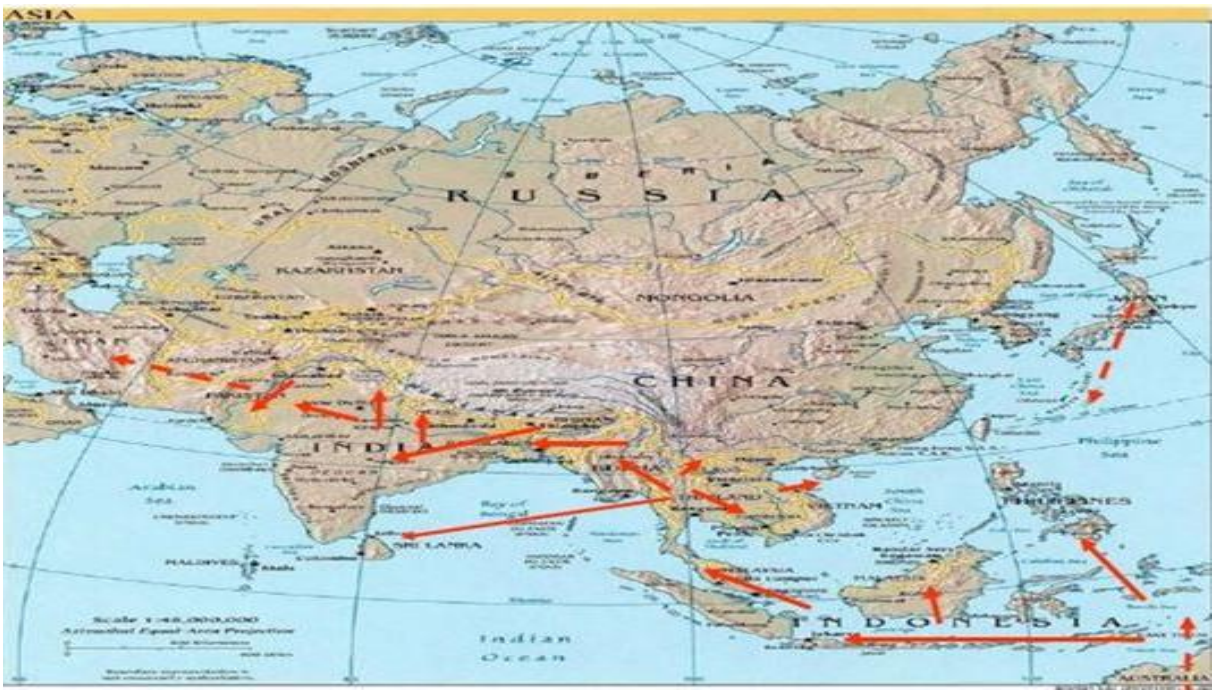

Fig.3.2 Original endemic areas of EUS (Mudenda, 2012)

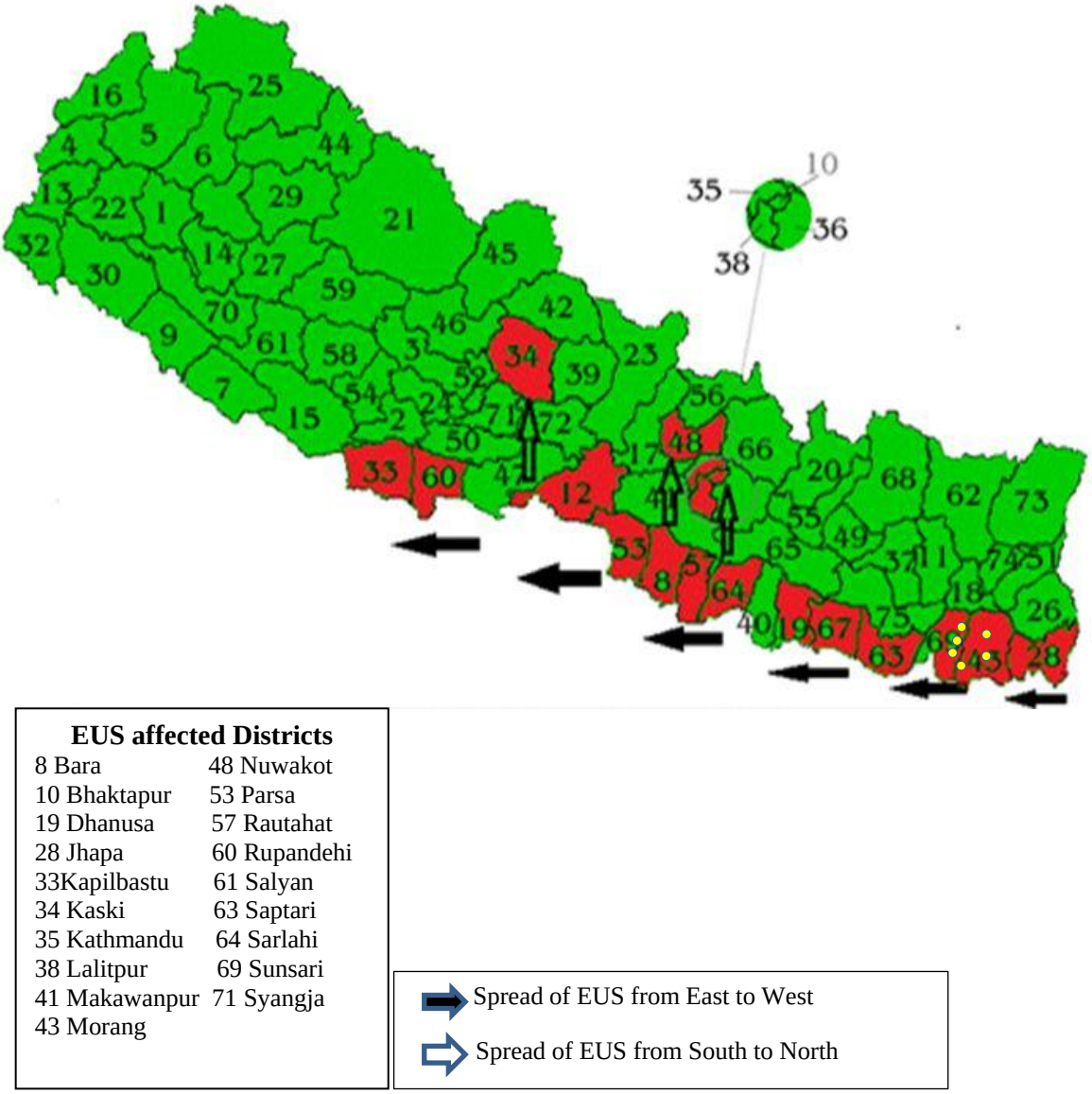

Fig.3.3 Spread of EUS in Nepal (Source: Nepal vista.com modified)

Fig.3.4 EUS affected areas in Southern Africa (Mudenda, 2012)

3.3.3.2. Species affected from EUS

EUS had been reported from 24 countries in both freshwater and estuarine environments throughout south, south-eastern and western Asia, the seacoast of North America, New South Wales (NSW) Australia, Northern Territory, Queensland and South Africa (FAO, 2009). 94 species of fishes have been confirmed to be naturally infected (OIE, 2013) and their numbers are still expanding (Table 3.2).

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

Table 3.2: EUS affected fish species in the world.

S.No.	Species affected	S.No.	Species affected
1	*Mystus tengra*	48	*Notopterus* sp.
2	*Puntius ticto*	49	*Catla catla*
3	*P. sarana*	50	*Labeo calbasu*
4	*P. chola*	51	*Trichogaster* sp.
5	*Nandus nandus*	52	*Puntius javanicus*
6	*Labeo rohita*	53	*Chanda chanda*
7	*Cirrhinus mrigala*	54	*Glossogobius giuris*
8	*Anabas testudineus*	55	*Gadusia chapra*
9	*Channa punctatus*	56	*Macrognathus aculeatus*
10	*Wallago attu*	57	*Rhinomugil corsula*
11	*Clarias batrachus*	58	*Acrossocheilus hexagonolepis*
12	*Macrognathus aral*	59	*Ailia coila*
13	*Salmostoma bacaila*	60	*M. cephalus*
14	*Ophiocephalus striatus*	61	*M. subviridis*
15	*Labeo bata*	62	*Sillago* sp.
16	*Heteropneustes fossilis*	63	*Scatophagus* sp.

Table 3.2: EUS affected fish species in the world (cont'd)

S.No.	Species affected	S.No.	Species affected
17	*Mastacembelus armatus*	64	*Epinephelus* sp.
18	*Channa gachua*	65	*Etroplus* sp.
19	*Channa micropeltis*	66	*Platycephalus* sp.
20	*Oxyeleotris marmoratus*	67	*Monopterus cuchia*
21	*Trichogaster trichopterus*	68	*Bidyanus bidyanus*
22	*Trichopsis vittatus*	69	*Carasius auratus*
23	*Betta splendens*	70	*Channa*(*Ophiocephalus*) *pleurophthalmus*
24	*Dermogenus pustillus*	71	*Esomus* sp.
25	*Mugil parsia*	72	*Acanthopagrus australis*
26	*Tridentigerobscurus obscures*	73	*Fluta alba*
27	*Scardinius erythropthalmus*	74	*Johnius* sp.
28	*Rohtee* sp.	75	*Lepomis macrochirus*
29	*Puntius gonionotus*	76	*Sillago ciliate*
30	*Toxotes chartareus*	77	*Macquaria ambigua*
31	*Lates calcarifer*	78	*Mastacembelus pancalus*
32	*Nematolosa erebi*	79	*Xenentodon cancila*
33	*Ambassis Agassiz*	80	*Valamugil* sp.
34	*Arius* sp.	81	*Oncorhynchus mykiss*
35	*Strongylura kreffti*	82	*Notopterus notopterus*
36	*Lutjanus argentimaculatus*	83	*Ophronemus gorami*
37	*Glossamia aprion*	84	*Terapon* sp.
38	*Liza diadema*	85	*Oxyeleotris* sp.
39	*Toxotes lorentzi*	86	*Platycephalus fuscus*
40	*Scatophagus argus*	87	*Upenius bensai*
41	*Scleropages jardini*	88	*Plecoglossus altivelis*
42	*Melanotaenia splendid*	89	*Psettodes* sp.
43	*Oxyeleotris lineolatus*	90	*Rhodeus osscilatus*
44	*Leiopotherapon unicolor*	91	*Kurtus gulliveri*
45	*Rasbora* sp.	92	*Amniataba percoides*
46	*Amblypharyngodon mola*	93	*Ompok* sp.
47	*Aorichthys aor*	94	*Hypophthalmichthys molitrix*

Pal and Pradhan (1990) collected 129 *A. testudineus,* 16 *H. fossilis* and 11 *Clarias batrachus* showing haemorrhagic ulcerative lesions in their body surface from North Bengal region during the initial outbreak of the disease in the state of West Bengal, India. Pradhan *et al.* (1991) reported ulcerative disease in Indian major carps.

Das and Das (1993) reported that in India, 30 species of fishes have been recorded to be affected by EUS out of which 4 species were exotic and the rest indigenous. They found that the incidence percentage was highest in the genera *Channa* (5-100%) and *Puntius* (20-100%). Other highly susceptible genera were *Mystus, Mastacembelus, Glossogobius, Anabas, Clarias* and *Heteropneustes.* The percentage of incidence was low in case of carps. It was reported that species like *Channa striatus, C. Punctatus, Clarias batrachus* and *Anabas testudineus* have been severely affected by EUS in Assam since 1995 and the outbreak has been occurring during the period from November to March. This indicates that there is a differential pattern of species susceptibility as EUS has been spreading through different years (Kar, 1999; 2000; Kar and Dey, 1990 a, b; Kar *et al*., 1990, 1993, 1994, 1995a, b, 1996, 1997). An epidemiological analysis of EUS in the state of Karnataka India conducted by Mohan and Shankar (1994) revealed that the disease first affected the bottom dwelling snakeheads (*Channa* spp.). Next to be affected were the catfishes (*Mystus* spp. and *Wallago* sp.), minor carps (*Puntius* spp.) and featherbacks (*Notopterus* sp.). Among cultivated freshwater spp. *Catla catla, Labeo rohita, Labeo calbasu, Cirrhinus mrigala, Puntius javanicus, Chanda chanda, Glossogobius giuris, Gadusia chapra, Macrognathus aculeatus, Rhinomugil corsula, Trichogaster* sp., *Acrossocheilus hexagonolepis, Salmostoma bacaila, Monopterus cuchia, Ailia coila* and among brackish water spp. *Mugil parsia, M. cephalus, M. subviridis, Sillago* sp., *Scatophagus* sp., *Epinephelus* sp., *Etroplus* sp. and *Platycephalus* sp. were infected. In the estuarine region, bottom dwelling mullets were found to be highly susceptible to the disease followed by the other species. The Indian major carps were mostly unaffected in the state of Karnataka. Other major fish species to be affected in India were *Ctenopharyngodon idella, Hypophthalmichthys molitrix, Nandus nandus, Notopterus* sp., *Rasbora* sp., *Wallago* sp., *Ompok* sp. etc. (Abdul Hameed, 1996; Mukherjee, 1996).

In the Philippines, the affected species in the Laguna Lake were snakehead (*Ophiocephalus striatus*), catfish (*Clarias batrachus*), gouramy (*Trichogaster pectoralis*), goby (*Glossogobius giuris*), crucian carp (*Carassius carassius*), Manila sea catfish (*Arius manilensis*) and silvery theraponid (*Therapon plumbius*), mullet (*Mugil* sp.), flatfishes (*Platycephalus* sp. and

Psethodes sp.), goatfish (*Upeneus bensasi*), croaker (*Johnius* sp.) and spadefish (*Scaptophagus* sp.) were affected (Llobrera, 1987; Reantaso, 1991).

In Srilanka, Subhasinghe *et al.* (1990) found 19 affected fish species which included *Ophiocephalus striatus, Channa (Ophiocephalus) punctatus, Heteropneustes fossilis* and *Mastacembelus armatus* etc.

Ahmed and Rab (1995) reported that Thai Silver barb, *Puntius gonionotus* was the most susceptible fish species in Bangladesh.

In Australia, yellow fin bream (*Acanthopagrus australis*) and striped mullet (*Mugil cephalus*) were among the affected species (Callinan *et al.*, 1995a). Humphrey and Pearce (2004) reported that archer fish (*Toxotes chartareus*), barramundi (*Lates calcarifer*), bony bream (*Nematolosa erebi*), chanda perch (*Ambassis agassiz*), fork-tailed catfish (*Arius* sp.), long tom (*Strongylura kreffti*), mangrove jack (*Lutjanus argentimaculatus*), mouth almighty (*Glossamia aprion*), mullet (*Liza diadema*), primitive archer fish (*Toxotes lorentzi*), red scat (*Scatophagus argus*), saratoga (*Scleropages jardini*), rainbow fish (*Melanotaenia splendida*), sleepy cod (*Oxyeleotris lineolatus*), spangled perch (*Leiopotherapon unicolor*), striped grunter (*Amniataba percoides*) and nursery fish (*Kurtus gulliveri*) were affected in the Northern Territory of Australia.

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

Some fish, such as common carp (*Cyprinus capio*), Nile tilapia (*Oreochromis niloticus*) and milk fish (*Chanos chanos*) have been considered to be naturally resistant to infection with *A. invadans* (Lilley *et al.*, 1998). Oidtmann *et al.* (2008) reported that European eel (*Anguilla anguilla*) was also resistant.

Dahal *et al.* (2008) reported 13 fish species (2 cultured and 11 wild) were EUS positive fishes on the basis of histopathological study in Kapilvastu district of Nepal. Two cultured species were *Labeo rohita* and *Cirrhinus mrigala;* other 11 wild species were *Mystus tengra, Puntius*

ticto, P. sarana, P. chola, Anabas testudineus, Channa punctatus, Wallago attu, Clarias batrachus, Nandus nandus, Macrognathus aral and *Salmostoma bacaila.*

Sosa *et al*. (2007a) reported that 21 species of estuarine and freshwater fish in Florida were infected with *A. invadans*. Bluegill sunfish, Cichlid and fresh water sharp tooth catfish were affected in South Africa (Mudenda, 2012).

3.3.3.3. Signs of the disease

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

Pal and Pradhan (1990) during the initial outbreak of the disease in the plains of North Bengal observed keenly a considerable number of EUS affected air breathing fishes. They reported that the disease first appeared as a red spot on the skin of the fish without scales. Later ulcers developed in affected areas damaging badly underlying muscle layer. But in scaly fishes, initial damage of mucous layer covering the scales were followed by appearance of red spots and finally sloughing of scale took place with development of ulcers.

Kumar *et al.* (1991) reported that distribution of severe ulcerative skin lesions varied from species to species. In murrels the ulcerations were mostly pronounced and developed in the head and caudal areas. In advanced stages the tail lesions could erode the affected areas to such an extent that there was total loss of peduncle portion. Sometimes the erosion progressed deep into the body exposing the abdominal cavity. In *Puntius* sp. dark red haemorrhagic, superficial ulcers area found on either side of the body. But in Indian major carps, long stripped haemorrhagic lesions were found in the region of caudal peduncle.

Pradhan *et al.* (1991) reported infection of different stages of development, i.e. from single or multiple haemorrhagic spots to deep and necrotic ulcers in Indian major carps like *Catla catla, Cirrhinus mrigala* and *Labeo rohita.*

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

Type 1 lesions were like tiny red spots on the body surface with no observable haemorrhages and ulcerations.
Type 2 lesions were large (2-4 cm) and appeared as dark raised discoloured areas on the body surface. Scales and skin were not affected.
Type 3 lesions appeared as circular to oval open dermal ulcers extending into skeletal musculature. At these advanced stages, haemorrhagic and necrotic open ulcers devoid of epidermis and scales were also found.

Chinabut and Roberts (1999) classified the EUS disease into five types of infectious process:

Type I. It is characterized by a highly distinctive dark brown lesion on one or both flanks and the affected fishes float on the surface of water and grasping air before their expiring.
Type II. This type was mostly found in top order predator air breathing fish, such as the snakeheads. The infections were characterized with chronic and extensive lesions.
Type III. In this case the ulcers were chronic and extensive and at early stage of infection a small red rosette or an ulcerated scale bed were found. It depends on the age and size of fish. This type is also found in snakeheads.
Type IV. This type of lesions is found in the grey mullet (*M. cephalus*). It is characterized by small necrotic erosions of one or more scale beds which gradually extended to produce a shallow ulcer with a red centrum, white rim surrounded by black edge.
Type V. This type is common in some large Indian carp. In this type a single, small, areas of haemorrhagic ulceration on fin or back was found.

3.3.3.4. Etiological Investigation

FAO consultation of experts meeting was organized in Bangkok where the name epizootic Ulcerative syndrome was adapted and it was also accepted that the condition was primarily

an infectious disease of mixed etiology (FAO, 1986). The investigation carried out by various workers on the etiology of disease will be discussed on the following headings: i. Virus, ii. Bacteria, iii. Fungus and iv. Animal parasites.

i.Virus

Virus like particles was detected in different tissues of affected fish during 1982-83 outbreaks in Thailand (Rattanaphani *et al.,* 1983; Wattanavijaran *et al*., 1983 a, b and 1984). Rhabdovirus was isolated from diseased fish in some south-east and south Asian countries by Freirichs *et al.* (1986, 1989). They suggested that it could be the initiating factor in the outbreak of EUS. But Freirichs *et al.* (1986, 1989) could isolate the virus from not more than 5% fish examined and the virus could not induce the disease in healthy fish experimentally. Saitanu *et al.* (1986) isolated a new virus, snakehead fish virus (SHV) from infected *O. striatus, C. micropeltes, Oxyeleotris marmoratics, T. trichopterus, T. vittatus* etc. SHV also produced cytopathic effects, rounded cells and complete destruction of cell sheet on BB, BB_2 and FHM cells. Hedrick *et al.* (1986) from cultured sand goby (*Oxyeleotris marmoratus*) and Subramaniam *et al.* (1993) in Singapore from infected fish isolated birna virus. Ahne *et al.* (1988) also isolated a rhabdovirus from snakehead (*O. striatus*) in Thailand. Rhabdoviruses were also isolated from the diseased fish collected from Thailand, Myanmar, Australia (Roberts *et al*., 1989; Lilley and Freirichs, 1994) and the viruses were named as ulcerative disease rhabdovirus (UDRV).

Sitdhi (1989) carried out virological studies on EUS affected fish species in Assam, West Bengal and Tripura. He found no cytopathic effect on snakehead cell line up to 14 days after exposure to tissue extracts of EUS affected fishes. Electron microscopic studies by Kar *et al.* (1990) showed the presence of viruses in muscles and gills of ulcerative disease affected fish in Assam. Kumar *et al.* (1991) showed cytopathic effect within seven days in culture from affected *Channa* sp., *Puntius* sp. and *Mastacembelus* sp.. Microscopic studies showed spherical virus particles. Freirichs *et al.* (1991) reported that the isolation of retro virus capable of inducing cytopathological effects (CPE) in a wide variety of tissue culture. As a confirmation for the presence of viruses in the EUS affected tissues, BF2 fish cell lines was used by infecting the growing cells with filtrate from homogenized tissues of ulcerated *Clarias batrachus*. A progressive CPE was noticed even at dilution of 10 of a 10% tissue homogenate (Kar, *et al.,* 1993).

ii. Bacteria

Bacterial pathogens have been claimed to play a major role in the disease outbreak by scientists working both in India and abroad. Studies on affected fishes in different countries recorded a wide range of pathogenic bacteria in the ulcerated area and in the internal organs such as kidney, liver, intestine and gills of affected fishes.

Llobrera and Gacutan (1987) reported the consistent association of *Aeromonas hydrophila* with necrotic ulcers and lesions in snakehead (*Ophiocephalus striatus*), Thai catfish (*Clarias batrachus*), crucian carp (*Carassius carassius*) and goby (*Glossogobius giuris*) in Laguna de Bay, Philippines from December, 1985 through February 1986. The bacteria were isolated from body lesions and ulcers of all fishes examined and rarely from the kidney and liver of carp and catfish.

Boonyaratpalin (1989) reported that the EUS involving both wild and cultured fish in Burma, Indonesia, Lao peoples' Democratic Republic, Malaysia, Singapore and Thailand was associated with bacterial pathogens, primarily *Aeromonas hydrophila* and occasionally *Pseudomonas* sp.. *Aeromonas hydrophila* was also reported to be associated with EUS affected fishes in Sri Lanka (Costa and Wijeyaratne, 1989). Subasinghe *et al.* (1990) examined 19 species of fish from Sri Lanka including *Ophiocephalus striatus*, *Ophiocephalus punctatus*, *Heteropneustes fossilis*, and *Mastacembelus armatus* and during these examinations, the consistent association of *Aeromonas hydrophila* with the haemorrhagic lesions and open necrotic ulcers on the body surface was revealed.

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

Four types of bacteria, two fluorescent Pseudomonads (R_1 and R_2), one Aeromonod (R_3) and one *Micrococcus* sp. (C) were isolated from skin lesions of air breathing fishes by Pal and Pradhan (1990) where R1, resembled *Pseudomonas fluorescens*, R2 resembles *Pseudomonas aeruginosa* and R3 showed strong resemblance with *Aeromonas caviae* (Pradhan, 1992). When a mixed culture of bacteria was inoculated in *Anabas testudineus* and *Channa punctatus*, severe ulcers were produced but pure cultures of the fluorescent Pseudomonads and Aeromonads induced only superficial ulcers while pure culture of *Micrococcus* sp. did

not produce any ulcers (Pal and Pradhan, 1990; Pradhan and Pal, 1990). Pradhan *et al.* (1991) isolated two Pseudomonads (R_4 and R_5) which resembled *Pseudomonas fluorescens*, one Aeromonad (R_6) and another coccus (C_1) from the Indian freshwater major carp, *Cirrhinus mrigala. Aeromonas hydrophila* was only isolated from EUS affected fishes of more than 70 species by Chattopadhya *et al.* (1990). Several researchers in India and abroad reported associations of bacterial pathogens with EUS (McGarey *et al.*, 1991; Ali and Timuli, 1991; Mukherjee *et al.*, 1991; Lio-Po *et al.*, 1992). Chakraborty and Dastidar (1991) repeatedly isolated chemoautotrophic nocardiofarm (CAN) bacteria from different types of skin lesions of EUS affected fishes.

Torres *et al.* (1993) performed virulence screening of 54 species of *Aeromonas* and found the *Aeromonas hydrophila* was the most pathogenic. Qureshi *et al.* (1995) also performed the virulence test of eight bacterial isolates from EUS affected fishes and found *Aeromonad* and *Pseudomonad* were highly pathogenic while micrococcus and cytophogans were less pathogenic. Lio-Po *et al.* (1998) isolated four species of bacteria from EUS affected fishes from Philippines and Thailand and *A. hydrophila* was proved to be most pathogenic. Regular isolation of bacterial flora from the surface lesion of EUS affected fishes as well as from their muscles, gut, liver, gills, heart, kidney and gonads revealed the occurrence of haemolytic strains of *E. coli, A. hydrophila, Pseudomonas aeruginosa, Staphylococcus epidermitis* and *Klebsiella* sp.. Saha and Pal (2000) isolated 16 strains of bacteria belonging to the genus *Pseudomonas, Aeromonas, Micrococcus, Bacillus, Vibrio* and *Moraxella* from the ulcers of infected fishes, *C. punctatus, Puntius* sp. and *Mystus* sp.. Only six strains belonging to the genus *Aeromonas* and *Pseudomonas* were pathogenic while the rest were non pathogenic among isolated strains. Saha and Pal (2002) tested the virulence of two florescent Pseudomonads and one Aeromonad isolated from the extraperitoneal lesions of diseased fishes injecting into the healthy *H. fossilis*. The EUS disease symptoms were visible after 48 hours of injection and the injected bacteria could be reisolated from the external lesions, kidney and liver of affected fish. Das *et al*. (2007) isolated eight bacteria (seven *A. hydrophila* and one *A. caviae*) from the lesions of EUS affected fish *Cirrhinus mrigala* from an affected pond in Jalpaigudi district of West Bengal. All these Aeromonas bacteria were found to be pathogenic after intramuscular injection of these isolates to the healthy *Channa punctatus*. Das *et al.* (2009) reported isolation of 15 Aeromonas from the ulcers of EUS affected fishes, Katla (*Catla catla*), Mrigal (*Cirrhinus mrigala*) and Puntius (*Puntius* sp.). Khalil *et al.* (2010) isolated the most pathogenic bacteria *Pseudomonas septicemia* and *P.*

aerugenosa from diseased *Oreochromis niloticus* characterized by darkening of body, loss of scales, tail rot and congestion of all internal organs.

iii. Fungi

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

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

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

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

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

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

In India also workers most frequently isolated *Saprolegnia* sp. and *Aspergillus* sp. from EUS affected fishes (Das *et al.,* 1990; Kumar *et al.,* 1991). Mohanta and Patra (1992) detected *Saprolegnia parasitica* in the infected specimen of *Anabas testudineus* in India. Karunasagar *et al.* (1994) traced out the existence of fungi deep into the musculature below the EUS affected ulcers in both freshwater and estuarine fish, but no existence of fungi was detected in early stages of lesions prior to development of ulcers.

Histopathological studies of EUS affected freshwater and estuarine fishes showed the presence of numerous non septate, highly invasive fungal hyphae (Mohan and Shankar, 1995). Qureshi *et al.* (1995a) isolated seven species of fungi belonging to the genera *Saprolegnia, Aphanomyces* and *Achlya* from lesions of EUS affected fishes from Bhopal. Pal (1996, 1997) also isolated three species of fungi but he stated that no fungi were found in the primary stage of ulcer formation. Viswanath *et al.* (1998) assumed that EUS specific fungus can only enter into the fish after the primary damage to the skin. Mohan *et al.* (1999) suggested that an invasive fungus *A. invadans* is the primary pathogen of EUS. Roy and Pal (2003) reported isolation of an aseptate fungus, *Aphanomyces* sp. from infected *C. mrigala* and experimentally fungal zoospores induced ulcer in healthy *C. punctatus*. Routh (2006) reported isolation of *Apanomyces* sp. from infected *C. striata, C. punctatus, L. rohita* and *L. bata.* Pathogenicity studies with the zoospores of fungus, *Aphanomyces* sp. (Fcs1) isolated from ulcer of *C. striata* induced ulcer at the site of injection.

Detailed histopathological studies (Kar and Upadhyaya, 1998) revealed focal area of increased fibrosis and chronic inflammatory cell infiltration in muscle; focal areas of fatty degeneration of the hepatocytes surrounding the portal triads and occasional infiltration by mononuclear cells in the liver. Preliminary histological studies revealed higher DLC and ESR but low Hb content in the blood of EUS affected fishes as compared to the corresponding healthy fishes of the same species (Kar *et al*., 1994). Likewise from some ther histochemical studies (Kar and Das, 1999) revealed interruption of glycogen synthesis in the liver and blockade of respiratory pathways in the gills. Preliminary enzymological studies (Kar, 1999) in the EUS- affected fishes revealed high values of alkaline phosphatase, SGOT, SGPT and LDH.

A. invadans was isolated from bluegills (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*) and American shad (*Alosa sapidissim*) but granulomas were not observed despite fungal hyphae in histological sections of dermis and skeletal muscle (Sosa

et al., 2007a). Similar observations were made in channel catfish (*Ictalurus punctatus*), black bullhead (*Ameiurus melas*) (Hawke *et al.*, 2003) and European catfish (*Silurus glanis*) (Oidtmann *et al.*, 2008). Afzali *et al.* (2013) isolated and identified fresh water fungi species including *Aphanomyces* sp. from the Malaysian natural water bodies and fish farms.

iv. Animal parasites

Animal parasites associated with EUS were *Palesintis* sp., *Trianchoratus* sp., *Gyrodactylus* sp., *Dactylogyrus* sp., *Epistylis* sp., *Trichodina* spp., *Ichthyophthirius* sp., *Trpartiella* sp. and several myxozoans like *Henneguya* sp. and *Thelohania* sp. (Jhingran, 1990). However, he opined that parasites are not the primary cause of the ulceration. Likewise, several workers (Mandal *et al.*, 1990; Kumar *et al.*, 1991; Ram, 1992, Subashinghe, 1993 and Callinan *et al*., 1997) mentioned that protozoans, myxozoans and crustaceans (*Lernaea* sp.) were associated with the EUS.

3.3.3.5. Environmental factors associated with EUS outbreak

The environment of cultured fish is composed of water and its holding system like tanks, ponds, cages, pens etc. Stability of the environment, especially in the physico-chemical parameter of the water brought about by the fish culture activity itself or by natural causes, will determine the health of the fish. Fluctuations in temperature, pH, salinity or dissolved oxygen beyond the optimum range for the host may lead to stress and disease. The key to successful fish culture is to understand and manage the environment of the host organism. Understanding the role of the environment in affecting the nature and fish pathology is often explained in the light of sudden changes in the subtle reaction between fish, their environment and the potential pathogenic organism. The environmental role is considered as prime importance by different authors (Snieszko, 1974; Wedemeyer, 1970; Wedemeyer *et al.*, 1977; Csaba *et al.*, 1981; Ahne *et al.*, 1982; Schaperclaus, 1986, 1991). Like other fish diseases, variations in the environmental parameters are suspected to act as predisposing factors in the outbreak of the EUS. Roberts *et al.* (1986) reported that outbreak of EUS occurred in a cyclic manner when temperature falls especially after a heavy rainfall, low alkalinity and pH fluctuations.

Snieszko (1974) stated that an overt infectious disease occurs when a susceptible host is exposed to virulent pathogen under stress. Organic pollution of water and soil may not be considered as the principal cause of EUS outbreak. There has always been severe fall in total

alkalinity (TA) values in these water bodies prior to EUS outbreak and causing 'stress' condition for fishes in the water and making them susceptible to attack by the pathogens leading to the outbreak of EUS (Kar and Dey, 1990a). Estimation of mercury, lead, arsenic and cadmium (by atomic adsorption spectrophotometry), did not reveal any appreciable differences in the quantity of these elements in the water and soil samples of the EUS affected and unaffected water bodies of Assam which indicated absence of trace element contamination in the outbreak of EUS (Kar and Dey, 1990b).

An extensive survey of the environmental factors in the affected states in India showed that incidence of disease outbreak was as high as 65% in waters having low alkalinity (13-30 ppm) and hardness (6-45 ppm) in comparison to 20% to 30% in water bodies having higher alkalinity (76-200 ppm) and hardness (62-190 ppm) (Jhingran,1990). Zachariah (1992) studied the effect of some physicochemical factors on EUS in the Vembanad lake Kerala, India. He noted that the significant changes in the water quality parameters of the EUS zone may cause the stress leading to the outbreak of EUS. After the devastating cyclone in 1988, EUS was detected in many confined waters polluted by carcass of domestic animals and rotten leaves of plants in Sunderban (Saha *et al.*, 1992).

Mohan and Shankar (1994) reported that EUS was first seen in Karnataka in the Cauvery river system during August –September 1991, immediately after flood. The rapid spread of EUS in Bangaladesh resulted due to flood (Barua, 1994). Das (1996) mentioned that the EUS initially occurred during the summer season when the flood waters entered the pond, canal and ditches during the period of May to October, 1988 in Assam. According to Abdul Hameed (1996) the predominant reason of EUS outbreak during four continuous years from 1991-1994 in Karnataka was after major floods during monsoon from July to September. Sardesai (1996) reported that the disease initiation occurring from last week of July – September, 1993 in Goa salinity in estuarine water bodies at the outset of the monsoon was reaching to zero and the disease spread in several parts of Goa affecting water bodies during monsoon season of the year 1994-1995. Data collected by Das (1997) revealed that the disease prone areas in the severely affected states of India had low alkalinity and hardness but with lesser intensity. In West Bengal, the disease outbreak occurred after the monsoons, at the stage of declining rainfall and onset of gradual stagnation from September and fall in winter temperatures (Das and Das, 1993).

Virgona (1992) studied the environmental factors influencing the prevalence of a cutaneous ulcerative disease (red spot) in sea mullet (*Mugil cephalus*) in the Clarence River, New South Wales, Australia. He found significant correlation between weekly rainfall in the lower catchment and the prevalence of early stage lesion. Progress to the later stages of the disease occurred after heavy rainfall and high flow of river. Bondad-Reantaso *et al.* (1992) following regular monitoring of water qualities in the Philippines revealed that there were variations in temperature, chloride, rainfall and hardness of water at the time of EUS outbreak. Palisoc and Aralar (1994) observed that the depth, Secchi disc transparency, temperature, chlorides and alkalinity were significantly correlated with EUS outbreaks from January 1988 to December 1989 in Laguna Lake, Phillipines. Callinan *et al.* (1995a) observed that in both Phillipines and Australia, EUS outbreaks in estuarine fish occurred only from estuaries having significant areas of acid sulphate soil in the catchments. They found that in the Richmond River, New South Wales, EUS prevalence was highest in those parts of the river fed by tributaries draining acid sulphate soil areas. Localized EUS outbreaks were observed after a rain fall and associated with pH values between 5 and 6.3. Furthermore, runoff water from acid sulphate soil areas may cause epidermal damage in exposed fishes, thus making the fishes susceptible to infection (Sammut *et al.*, 1995).

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

Water temperature is an important factor in the development of EUS. Generally, EUS occurs when the water temperatures are comparatively low either because of a sudden drop in temperature associated with massive rainfalls or in the cold season of the year (Chinabut *et al.*, 1995; Lilley *et al.*, 2002). So far, the majority of fish species affected by EUS are tropical species and in those climates, temperatures considered 'low' can be as high as 25ºC (Pathiratne and Jayasinghe, 2001). Sanaullah *et al*. (2001) recorded that EUS occurred between October and March every year in Faridpur, Bangladesh. They showed highest prevalence in snakeheads (30 %). Results of ANOVA was significant to low chloride ($p < 0.045$), alkalinity ($p < 0.043$)

and temperature ($p < 0.00065$) during EUS outbreak. The role of total ammonia at low chloride concentration was also related with outbreaks. However, EUS has been observed in a wide temperature range: the disease occurred in freshwater ponds in Louisiana in the winter months at water temperatures from 10 to 15ºC (Hawke *et al*., 2003) and in fish pond in the Philippines at temperatures as high as 33ºC (Bondad-Reantaso *et al*., 1992).

Roy and Pal (2003) carried out the experiment for three years (July 1996-June, 1998) to determine the relationship between the physicochemical properties of EUS affected pond water and outbreak of the disease in Malbazar, Salbari and Singhimari of the Jalpaiguri disrrict of West Bengal. The study showed that the outbreak of EUS occurred in these ponds when dissolved oxygen content, hardness and total alkalinity of pond water remained low. Epidemiological studies in Bangladesh and Nepal identified a range of factors that increased the risk of EUS infection. Most significant of these was connection of ponds to natural water bodies and presence of wild fish in the pond. This was supported by prevalence data, which showed that EUS is endemic in natural water bodies in most of the areas examined (DFID, 2001).

The annual cycle of flooding of Zambezi flood plain brings about changes in water quality that are thought to favour the infectivity of *A. invadans*, with diseased fish appearing soon after the plains become flooded. In 2006 the disease has spread rapidly upstream along the upper Zambezi and its tributaries. In 2010 the disease was reported from the Okavango Delta in Botswana and in 2011 from the Western Cape Province of South Africa (Huchzermeyer and van der Waal, 2012).

In Uttar Pradesh, India (2010-2011) EUS, outbreak has occurred in some fish farms in March and it continued till end of May (peak summer season) when the mean temperature was 31.6± 0.65ºC. In those farms pH and DO were 9.13 ± 0.05 and 9.66 ± 0.4 mg/L respectively, which were above the trigger values. Therefore, it seems unlikely that any specific environmental factor is always associated with all EUS outbreaks. More epidemiological studies are required to get an insight into the role of various environmental risk factors responsible for EUS (Pradhan *et al*., 2014)

3.3.3.6. Influence of pesticides, heavy metals and other agro chemicals

High incidence of the disease in the rice field areas in India as in case of other countries especially in areas of indiscriminate pesticide application suggest 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 in affected water as well as in the muscle of affected fish. Studies conducted by Kurup (1992) in the EUS struck regions of north eastern Kuttanad in Kerala, India reveal that indiscriminate pesticide application in the rice fields have aggravated water pollution problem. The concentration of pesticide in water was found to be above toxic levels and he suggested that this may create a stress condition for aquatic life and this may be a predisposing factor for EUS outbreak. Analysis of pesticide residue in water, fish and plankton of some specific EUS affected water areas in India were carried out by Chowdhury *et al.* (1994). Analysis of residues of organochlorine pesticides such as BHC, DDT and their metabolites and endosulphan, methyl parathion etc. in water bodies near rice field areas indicate that although occasionally higher concentrations of organochlorine and organophosphorus pesticides have been found in water and fish samples, no correlation could be made with the presence of pesticide residue and disease outbreak. Jhingran and Das (1990) analysed the concentration of various metals like Fe, Zn, Cu, Cr, Cd, Pb and Hg in the water of affected areas. The available information from the study didn't suggest any perceptible role of the heavy metal contamination in creating stress to the fish leading to outbreak of disease.

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

3.3.3.7. Socio-economic impacts of EUS

There were great social and economic impacts of the diseases. In Asia and Africa, the spread of the disease has substantially affected livelihoods of fish farmers and fishermen and in some cases threatened the sustainable food supply for local populations depending on fish as a relatively affordable source of animal protein (Lilley *et al.*, 2002; FAO, 2009). In India, the investigations carried out in five districts of West Bengal immediate after the first outbreak revealed that about 73% aquaculture units were adversely affected by EUS and some farmers were forced to search alternate jobs and 88.9% fish traders were also victimized due to losses to some extent during the affected period (Bhowmick *et al.*, 1991). In Assam, study of impact

from EUS revealed that the total loss of fish from this area had been estimated at 10,625 metric tons affecting 81,400 numbers of fish farmers as well as member of the fishing community (Das, 1996). In Kerala state, spread of EUS completely paralyzed the inland fish markets and threw the fishermen out of their occupation. The women fish vendors were particularly subjected to severe hardship. They had to seek alternate employment without much success (Sanjeevaghosh, 1992). The occurrence of the disease in India had caused a decrease in the demand even for healthy fishes. There was a false but wide spread apprehension of disease being transmitted to human being (Jhingran, 1990). Fishery and health both departments launched the awareness programme through different media and advised to farmers of affected area to use prescribed doses of lime in ponds which was distributed free of cost (Prusty and Nayak,1990).

In Philippines, the first outbreak of EUS affected 15000 lakeshore families in Laguna lake with the 30% decrease in average daily income of fishermen (Llobrera, 1987). In Bangladesh fish traders suffered heavy losses during 1988 and 1999 due to price reduction of fish up to 75%. The total economic losses were about 118 and 88.2 million Taka in Bangladesh in the year 1988 and 1989 respectively (Barua, 1990).

In Nepal, losses due to EUS was not recorded (Shrestha, 1990). It was due to inadequate research in that field and hesitation of farmers to provide information about its occurrence from the fear of price down. On the part of the fish farmers, there was a growing fear concerning the eventual decline of the natural fish population with continual annual recurrences of EUS. Subsequently it was reported that EUS had caused a loss about 15-20% of total fish production with estimated worth of Rs.300 million (ADB/NACA, 1991). In Philippines, the panic created mainly by the press gradually subsided with the improvement of the situation through government's education program and farmers learned to accept the occurrence of EUS at particular times of the year (Bondad-Reantaso *et al.*, 1994). In countries affected by EUS (Botswana, Namibia and Zambia), EUS negatively impacted on the livelihood and food fish source of the communities dependent on subsistence farming (Bondad-Reantaso *et al.*, 2012).

3.3.3.8. Management of EUS

Management of EUS includes both prophylactic and therapeutic measures.

i. Potassium Permanganate

Jain (1990) reported that after bath treatment of EUS affected fishes with potassium permanganate@ 5ppm 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) reported that the potassium permanganate @ 0.5-2 ppm in water showed a good result in curing the ulcers.

ii. 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 healing of the ulcers (Jhingran *et al.*, 1990). Jain (1990) suggested that better result has been obtained 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 3-4% solution. Jhingran *et al.* (1990) suggested that liming in water is more effective because it raises the pH value of the water to neutral or slightly alkaline value precipitates suspended or soluble organic materials promote biological productivity by enhancing the breakdown of organic substances by bacteria and killed most of the undesirable microorganisms due to its caustic reaction.

iii. Antibiotics

The most commonly used antibiotics to treat EUS affected fish are erythromycin, nalidixic acid, oxytetracycline and terramycin. The prescribed dose for effective result of the above mentioned antibiotics were recommended @ of 60-100 mg /Kg of feed for seven days (Jhingran, 1990).

iv. CIFAX

Application of CIFAX, a drug formulated by CIFA (Central Institute of Freshwater Aquaculture) in EUS affected captive water @ 1L/ hectare meter of water showed encouraging results (Das and Das, 1993).