EPIZOOTIC ULCERATIVE SYNDROME IN FISHES

- Its present status in India

CENTRAL INLAND CAPTURE FISHERIES RESEARCH INSTITUTE

Indian Council of Agricultural Research

Barrackpore 743 101 West Bengal

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Foreword

One of the major factors hindering inland fish production now-a-days is various types of fish diseases. However, no other fish disease in India has been so menancing as Epizootic Ulcerative Syndrome. Transcending the confines of culture ponds, EUS has plagued the natural fish populations of the open water resources. The virulence and the trail of destruction to valuable fishes left behind by the epizootic have seriously affected the fisherman community, both economically and morally. Many vital clues regarding the aetiology of the disease are yet to be unravelled, inspite of intensive global research. An attempt is made here to document the present state of knowledge on the disease in India for benefit of research workers, aquaculturists and general public.

CIFRI, Barrackpore
11.02.1997

Dr. M. Sinha
Director

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INTRODUCTION

The dreaded fish disease Epuzootic ulcerative syndrome characterised by severe ulceration and causing heavy mortality in fishes has been a major concern since 1972 in different countries of the Asia-Pacific region. In India, Central Inland Capture Fisheries Research Institute (CIFRI) has been monitoring the disease since early 1988. The Institute had alerted the states in April, 1988 about the possibility of the disease outbreak, and the prediction came true in May 1988.

Every day may be fishing day but every day is not catching day This addage assumed a special significance, when Epizootic Ulcerative Syndrome struck the Indian fishes for the first time during May 1988. Fishermen cast their nets, but the sight of the repulsive ulcerated fish turned their obsession into a revulsion. In fact, conditions became so alarming that all fishery activities had come to a standstill and the disease had become a matter of grave concern for fishery scientists and administrators.

History of the disease

This dreaded fish disease has been a major concern in several countries of Asia-Pacific region (Fig. 1). In Queensland, Australia, an epizootic of marine and estuarine fishes characterised by shallow haemorrhagic ulcers occurred in 1972 with recurrence in subsequent years[1,2] . The disease was named 'red spot disease'. Papua New Guinea reported a similar type of disease characterized by dermal ulcer from the rivers of the south during 1975-76[3] and north during 1982-88[4]. Indonesia also reported similar type of disease in Bogor in 1980[5] which subsequently spread to West Central and Eastern Java. This disease was named infectious dropsy or 'haemorrhagic septicaemia'.

Malaysia reported the disease during 1981-83. The affected fishes had red or necrotic areas of ulceration all over their bodies and was called *Webak Kudes*. In early 1984, the disease was reported from fishing areas of Kampuchea along with a significant decrease in the natural fish stock. In 1984, a similar disease was reported from the southern and central parts of Laos. Burma experienced the outbreak of the disease during 1984-85 affecting both wild and cultured fish stock. In Thailand, the disease epizootic was first reported in 1980 in the natural water system and the disease recurred every year during 1980 to 1985 in different water bodies[4]. In Sri Lanka the disease was first reported in 1988 in the Kelani river, Dandugan Oya, and in streams nearby causing severe fish mortality. In Bangladesh, the first outbreak of the disease occurred during February/March 1988 in the rivers Meghna, Padma and Jamuna and adjoining water areas with enormous loss of the commercial fish stock. In India, the outbreak of the disease was first noticed in May 1988 among fishes of the rivers, canals, beels, paddy fields, and ponds of the North Eastern states. In 1989 Nepal was affected by the disease.

Areas affected by EUS

Fishes were afflicted with EUS in all types of water areas in India, namely rivers, floodplain wetlands (beels), lakes, irrigation canals, reservoirs and culture ponds (Table 1).

Time of occurrence

The disease is mostly observed during the post monsoon period which is different states, vary from May to February. In some states, for example Kerala which has two monsoon, *i.e.*, south west (June-August) and north east (October-November), the disease is prolonged and is observed throughout the year. Investigations carried out at disease prone sites in West Bengal showed that EUS outbreak occurs at the time of waning of rainfall and onset of gradual stagnation from September and fall in water temperature[6,7]. The details are given in Table 1.

Spread of the disease

Since May 1988, when the disease first appeared in the north-eastern States, it gradually spread to the eastern, central western, southern and northern states (Fig. 2). The disease in any particular area was severe during the first outbreak and gradually diminished in subsequent years, lasting up to three years. In the fourth year also in some areas it remained within pockets of minor incidence. In the north-eastern, eastern, central and some southern states the disease outbreak could be correlated to water-borne transmission. However, in many areas the transmission of the disease could be due to transplanting fish fry and fingerlings from disease-prone areas.

Major outbreaks

India witnessed the first major outbreak of EUS in May 1988 in the States of Tripura, Assam, Meghalaya and West Bengal. It gradually spread and affected major outbreaks till 1992 in the States of Orissa, Bihar, Uttar Pradesh, Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka, Haryana and Rajasthan[7] (Table 1).

Fish species affected

Thirty species of freshwater and brackishwater fishes have been recorded to be afflicted by EUS out of which four are exotic and the rest, indigenous (Table 2). The range of incidence of the disease recorded from the various species of fishes and from different types of water bodies (Table 3) reveals that certain genera of fishes, such as *Channa, Puntius, Mastocembelus, Mystus, Glossogobius, Anabas, Clarias* and *Heteropneustes* are highly susceptible to EUS.

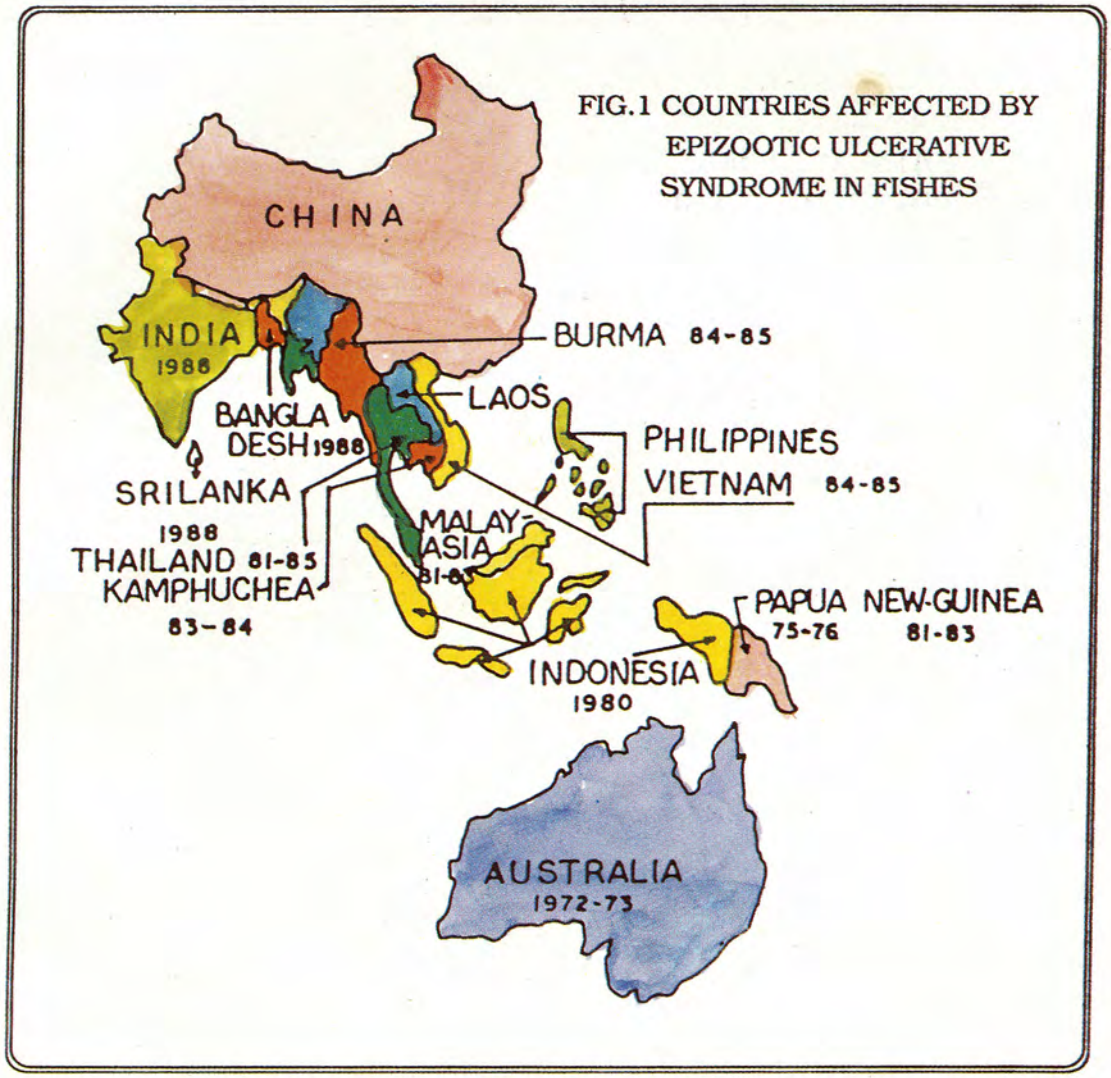


FIG. 1 COUNTRIES AFFECTED BY EPIZOOTIC ULCERATIVE SYNDROME IN FISHES

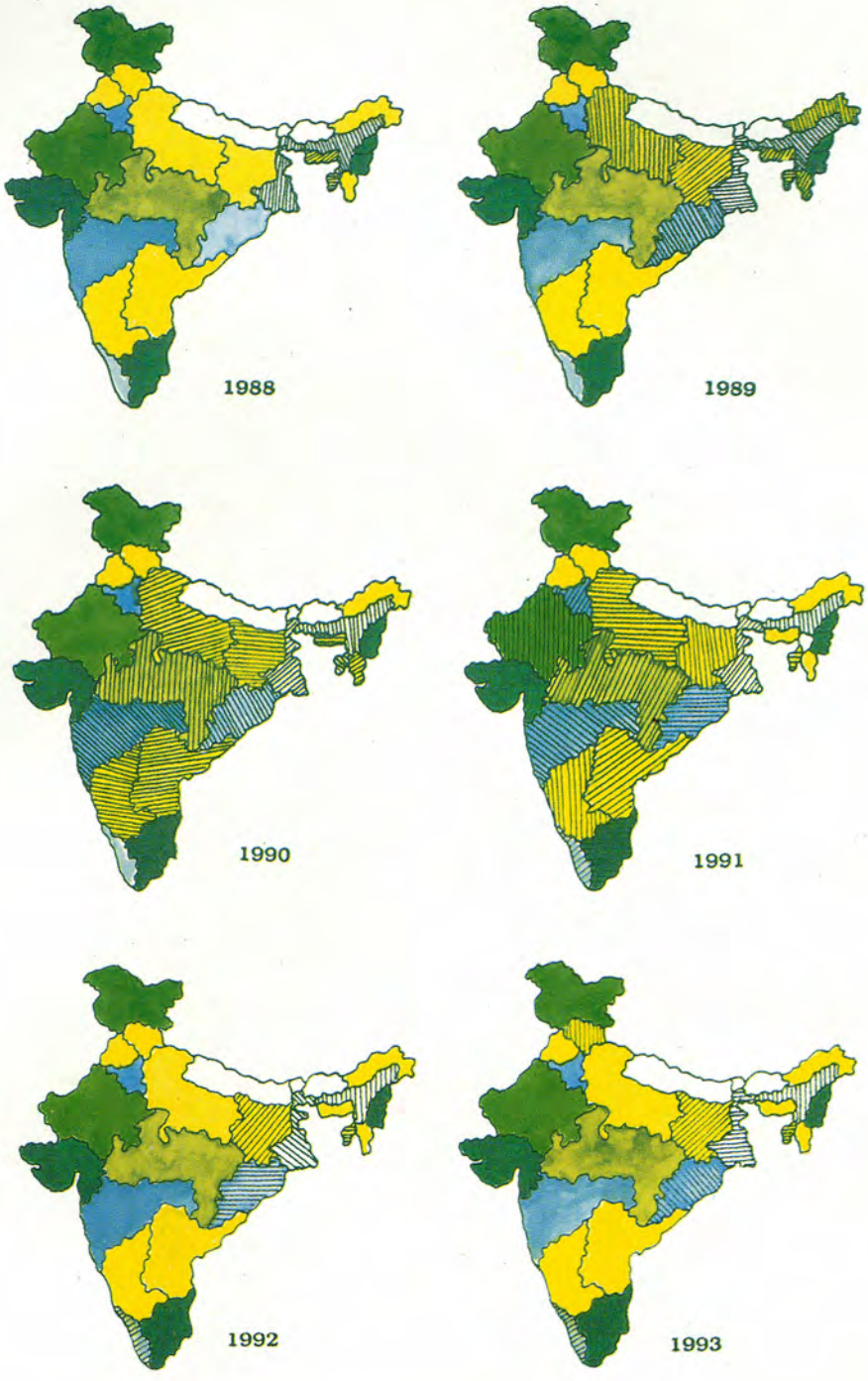


Fig. 2 Spread of EUS in the States of India from 1988-1993

Table 1. Details of EUS outbreak in different states

State	Period of outbreak	Duration	District affected	Water areas
Tripura	1988 - 1991	May to Sept.	North, South & West Tripura	Rivers, lakes, reservoirs, paddy fields, ponds
Assam	1988-1991	May to Dec.	All districts	-do-
Meghalaya	1988 - 1990	May to Dec.	East Khasi & Garo Hills	Rivers, streams, paddy fields
Mizoram	1989,1990	June to Sept.	Large ponds	Large ponds
Arunachal Pradesh	1989	Sept. to Dec.	Itanagar	Rivers, ponds
Manipur	1989	December		Ponds
West Bengal	1988 - 1991	Sept. to Dec.	All districts	Beels, reservoirs, paddy fields, ponds
Orissa	1989 to 1992	Oct. to Jan.	Cuttack, Puri Balasore, Mayur-bhanj, Bhadrok	Beels, ponds, paddy fields
Bihar	1989 to 1992	April to Oct.	29 districts	-do-
U.P.	1989 to 1991	Sept. to Nov.	Gorakhpur, Lucknow, Allahabad, Faizabad, Sultanpur, Gonda, Barabanki, Rai Bareilly, Bahraich, Fatehpur, Varanasi, Unnao, Gazipur, Budaon, Rajgarh, Bilaspur	-do-
M.P.	1990,1991	Nov. to Dec.	Raipur, Durg, Rajnandgaon, Gwalior, Shivpuri, Jabalpur	Irrigation tank, culture ponds
Maharashtra	1990,1991	Sept. to Oct.	Gondia, Bhandra	Beels,culture ponds
Andhra Pradesh	1990,1991	Nov. to Jan.	Eluru	Lakes, canals, drains

contd...

Table 1. contn..

State	Period of outbreak	Duration	District affected	Water areas
Tamil Nadu	1990,1991	Oct. to Feb.	Kancheepuram, Chingleput, MGR Trichy	Lakes, reservoirs
Kerala	1991, 1992	July to Feb.	Wayanad, Kozhikode, Malappuram, Thrissur, Ernakulam, Idukki, Kottayam, Alappuzha, Pathanamthitta and Kollam	Backwaters. lakes, culture ponds
Rajasthan	1991	November	Tonk	Reservoir
Haryana	1991	October	Sonepat	Culture ponds
Karnataka	1990,1991	November	-	Rivers, lakes, ponds

Table 2. Fish species affected by EUS in India

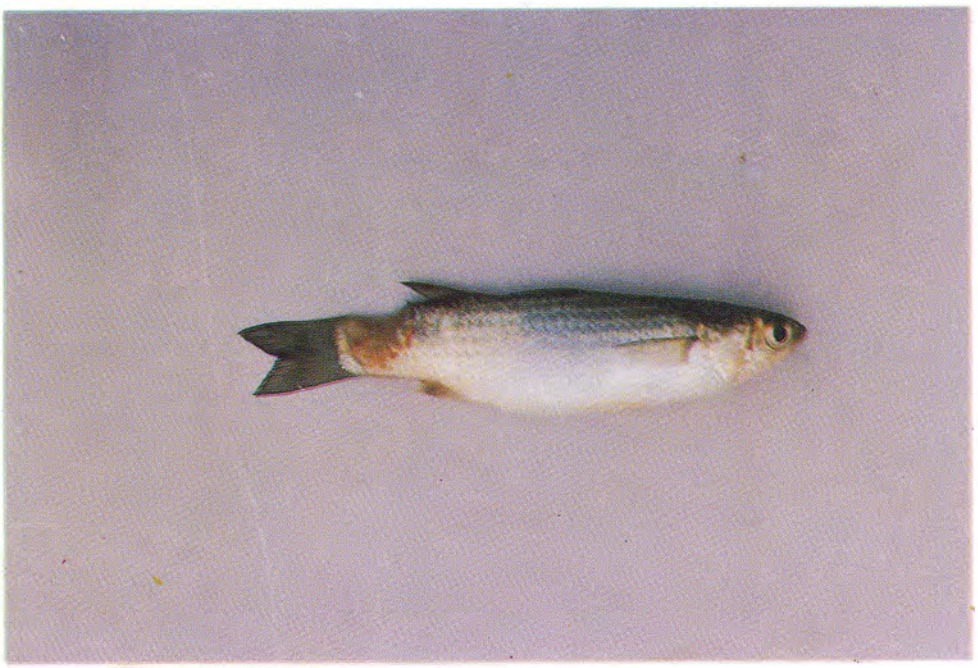
Cultured	Wild
Freshwater	
Catla catla, Cirrhinus mrigala, Labeo rohita, Puntius javanicus, Ctenopharyngodon idella, Hypophthalmichthys molitrix	*Channa striatus, C. punctatus, C. gachua, Clarias batrachus, Heteropneustes fossilis, Puntius sophore, Ambassis ranga, Amblypharyngodon mola. Mystus vittatus, Nandus nandus, Glossogobius giuris, Gadusia chapra, Mastocembelus pancalus, M. armatus, Callichrous pabda, Rhinomugil corsula, Trichogaster* sp., *Acrossocheilus hexagonolepis, Notopterus* sp.
Brackishwater	
Mugil parsia	*Mugil cephalus, Mugil subviridis, Mugil parsia, Etroplus* sp.

AFFECTED FISHES SHOWING EUS SYMPTOMS

Initial stage of ulceration in *C. catla*

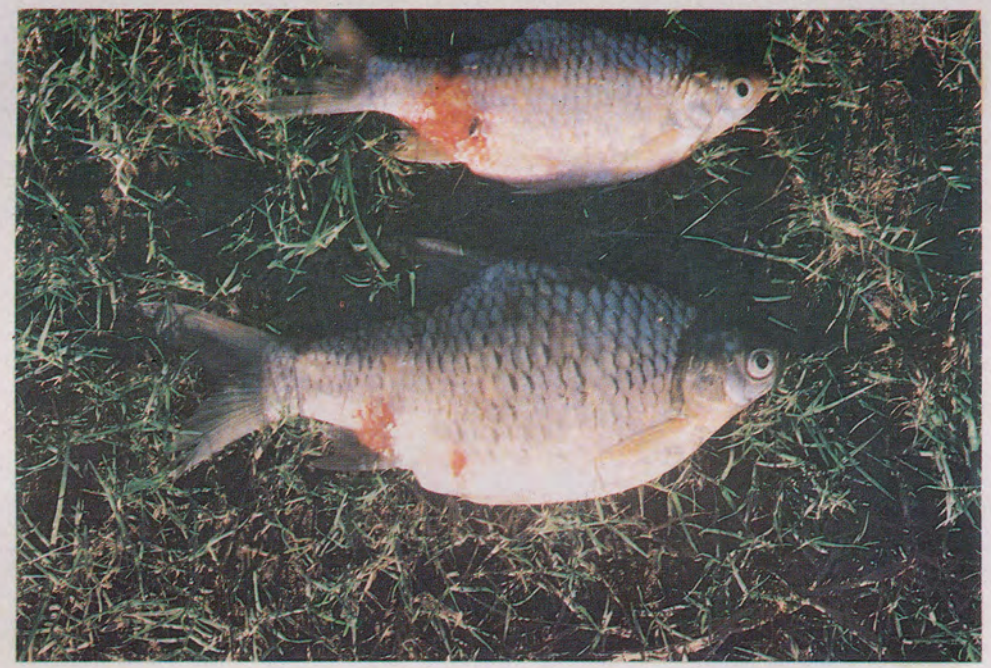
Ulceration increasing in *C. catla*

Ulceration in caudal region of *Rhinomugil corsula*



Ulceration in caudal region of *Liza parsia*

AFFECTED FISHES SHOWING EUS SYMPTOMS



Deep ulcers in *Puntius* spp.

Caudal peduncle degeneration in *Mastocembalus armatus*

AFFECTED FISHES SHOWING EUS SYMPTOMS

Advanced stage of ulceration in *Gadusia chapra*

Assortment of *Channa* sp., *Heteropneustes* sp., *Puntius* sp. and *Mastacembelus* sp. in advanced stage of ulceration

Semiotics of the disease

The symptoms and other characters of Epizootic Ulcerative Syndrome are conspicuously different from the other low level ulcerative conditions reported earlier. It has some distinct manifestations; fishes in the rivers as well as in confined waters exhibit abnormal swimming behaviour with head projected out of water. In the rivers, abnormal swimming behaviour was witnessed with several fishes floating listlessly near the bank.

In the initial stages of the disease, the infection usually commences in the form of multiple inflammatory red spots on the body causing localized haemorrhage. In carps these appear within the scale pockets. In advanced stages of infection, the ulceration covers larger areas with sloughing of scales and degeneration of epidermal tissue. With further advancement of the disease, the ulcers become deep haemorrhagic and necrotic often with black melanistic rim. In advanced stages of the disease, large and deep ulcers are very commonly seen in all parts of the fish, especially the head, abdomen and peduncle. Histopathological studies conducted on ulcerated fishes show identical histopathological manifestations. In heavily ulcerated fishes there is degeneration of epidermis of skin at the ulcerated areas and granulamatous formations. Basically fungal granulomata occur in the dermis and hypodermis[8]. A high degree of inflammatory reactions involving infiltrations by macrophage cells and lymphocytes around some of these granuloma formations were found[9]. Livers of affected fishes did not show any significant change except vacuolization in certain cases[8]. However, Kumar[9] observed most of the sinusoidal space and blood vessels were congested (hyperaemia) and wandering lymphocytes were plenty in liver parenchyma. No changes were observed in kidney of affected fishes. Hematological parameters of affected fishes showed higher counts of phagocytic cells and reflected initiation of defense phagocytosis in blood circulation. The decline in counts of erythrocytes (RBC) followed by drop in hemoglobin (Hb) content and hematocrit (HCT) values indicated anaemia condition[6] (Table 3).

Investigation on the causative factors of EUS in India

The causative agent of this dreaded disease has been baffling the scientist. It is widely suspected that a biological infectious agent is the primary cause of EUS and certain abiotic factors are responsible for creating stress to fish. The suspected biological agents are viral, bacterial, fungal and other animal parasites. The investigations carried out in India uptil now on the different probable causative agents reveal the following points.

Table 3. Comparative haematological parameters of *Cirrhina mrigala*

	Normal	EUS afflicated
WBC	64 - 7 x 10^3/L	84 - 106 x 10^3/L
RBC	2.4 - 3.6 x 10^6/L	1.2 - 1.8 x 10^6/L
Hb	16.0 - 20.2 g/dL	12.8 - 14.5 g/dL
HCT	42 - 44.5%	24.0 - 28.2%
MCV	198 1	181 1

Environmental factors

It is suspected that the physico-chemical parameters of water and anthropogenic factors such as pesticides, fertilizers and heavy metals play an important role in the outbreak of EUS. As such data was recorded by Das[11] at specific water bodies in EUS disease prone area of West Bengal throughout the year and in various affected water areas in all the affected states on selected physico-chemical parameters having relevance to the EUS outbreak (Table 4). It reveals that the affected water areas in different states where the intensity of disease was severe had low alkalinity and hardness - a characteristic of acidic low calcium soils (Tables 4 and 5). The observation is in agreement with earlier reports from other countries affected by EUS that low alkalinity, hardness, chloride concentration and fluctuating pH showed a link with EUS outbreak. However, in India besides such highly susceptible areas EUS outbreak also occurred in water areas with high alkalinity and hardness but with lesser intensity. Investigation carried out at disease prone site in West Bengal[6,7] shows that EUS outbreak does not commence during the monsoon period. The disease outbreak occurs at the time of waning of rainfall and onset of gradual stagnation from September and fall in water temperature and minimum air temperatures. Sharp fall in the hardness of water from the higher summer values due to dilution during rainy season seems to be another predisposing factor for triggering the disease outbreak.

Heavy metal concentration in water

Though in some affected water areas significantly high values of zinc, copper and mercury were obtained, the data collected[6] so far (Table 6) do not suggest any perceptible role of the heavy metal content in creating stress to fishes and subsequently predisposing it to EUS outbreak.

Table 4. Environmental monitoring in affected water areas in India

State	pH	Alkalinity	Hardness	Chloride	Free CO_2	Ammonia	Salinity
Assam	7.1-7.5	13-74	11-38	4-23	4-10	0-0.4	
Tripura	6.7-7.6	7-49	9-45	3.5-18	2-8	0-0.6	
Meghalaya	6.5-7.5	7-14	10-15	2-12	4-6		
West Bengal	6.7-7.5	10-170	6-180	2.9-13	2-7	0-0.6	
Bihar	6.1-6.8	25-30	13-20	4.7-7	4.0	1.8-2	
Orissa	6.8-7.4	44-138	55-180				1-5
Uttar Pradesh	7.5-8.0	40-217	42-234	0-5.8			
Tamil Nadu	7.8-8.3	103-139	105-158				
Rajasthan	8.0-8.2	140-150	80-90				
Maharashtra	7.5-9.5	30-115	48-140		2.5-3.0		
Kerala	6.3-7.0	0-11	8-17	0-3.4			1.0

Pesticide and other agrochemicals

Since the incidence of EUS is high in rice field environments in India as in case of other countries where EUS occurred pesticides were suspected to be associated with outbreak. Most of the outbreaks of EUS in India occurred after rain fall. This observation is in agreement with reports from other countries leading to suspicion that drainages of agricultural chemicals may have an important role as predisposing factor for EUS outbreak[5].

Analyses of pesticide residue in water, fish and plankton of some specific EUS affected water areas in India were carried out[11,12] to assess the relation between pesticide use and EUS outbreak (Table 7). The studies indicate that although occasionally higher concentrations of organchlorine and organophosphorus pesticides have been found in water and fish samples, no correlation can be made with the presence of pesticide residue and disease outbreak. Studies conducted by Kurup[13] in the Kuttanad aquatic ecosystem in Keralawhere EUS outbreak occurred revealed that indiscriminate

Table 5. Intensity of the EUS outbreak in India

EUS affected fish	Av. % of incidence range	State	Av. % of incidence range	affected water area	Av. % of incidence range
Channa sp.	20-100	Assam	30-60	Rivers	4-15
Puntius sp.	5-100	Tripura	35-70	Confined waters	10-55
Glossogobius sp.	10-60	Meghalaya	10-35		
Mystus sp.	5-75	West Bengal	15-65		
Notopterus sp.	3-25	Bihar	20-30		
Wallago attu	7-20	Orissa	20-45		
Mastacembelus sp.	10-35	Uttar Pradesh	15-20		
Anabas testudineus	10-55	Tamil Nadu	5-25		
Amblypharyngodon mola	5-10	Rajasthan			
Rhinomugil sp.	1-5	Maharashtra	5-10		
Clarias batrachus	10-30	Kerala	30-65		
Heteropneustes fossilis	10-20				
Catla catla	5-15				
Labeo rohita	5-10				
Cirrhinus mrigala	5-20				
Cyprinus carpio	10-25				
Ctenopharyngodon idella	2-5				

Table 6. Heavy metal analysis in affected water areas (μg/b) (1) Levels were not detected (nd)

Site	Fe	Zn	Cu	Chr	Cd	Pb	Hg
Mayapur	280	107	80	8.0	9.0	16.5	0.12
Cooch Behar	200	21	7.0	nd1	nd	nd	nd
Maldah	130	32	3.0	nd	nd	3.8	nd
Jorhat	7,800	62.8	3.9	nd	nd	5.75	nd
Jhalukbari		22.8	1.2	nd	nd	nd	nd
Meghalaya	4,810	53.2	2.12	nd	nd	3.68	0.03

pesticide application for paddy cultivation have aggravated water pollution problem. Analysis of water and sediment revealed the active ingredient of lindane Y-HCH and X-HCH concentration in water samples vary between 0-400 mg/litre. In sediment the concentration varies between 0-20,000 mg/litre. High concentration of DDT and its metabolites DDE and DDD were present in samples, the range in some stations being 12,000-22,000 mg/litre. The range of endosulfan values registered is 66-1,114 mg/litre. The sublethal values of DDT and for fish was 10,000-10,000 ng/litre. Sublethal of X and Y-HCH for fish and crustacean are between 10-80 ppb and LC_{50} values for endosulfan was found to be considerably above toxic levels for fish at some places of the 27 stations monitored.

The study indicated that the extent of pollution may create a stressed condition for aquatic life and may be the predisposing factor for EUS outbreak.

Virus

Virological studies conducted on the EUS affected fishes in India by Sitdhi[14] from samples of EUS affected fishes, namely, *C. idella, Colisa* sp., *P. javanicus, H. molitrix* and *P. sophore* from Assam, *C. catla* and *C. carpio* from Tripura, *C. punctatus, M. armatas, N. nandus, P. sophore* from West Bengal, showed no cytopathic effects on snakehead cell line upto 14 days when tissue extracts (spleen, liver, gills, and ulcerated parts) were inoculated. The monolayer of snakehead cells in the control and inoculated flasks were the same. The electron microscopy studies for occurrence of viral agents in the kidney and liver showed negative results.

However, investigations conducted by Kumar[9] on samples of EUS affected fish genera of *Channa, Puntius* and *Mastocembelus* showed initial positive indications. Inoculum from these affected fishes when injected in confluent cultures of BB, FHH, EPC cell lines showed CPE within three to seven days in culture. In all cases spherical virus like particles were visualized which await detailed characterisation. Though a primary viral aetiology has been considered a likely possibility given the rapid and uncontrollable spread of EUS and its distinct clinical sign[2,5]. However, from the extensive study conducted on viral aetiology of EUS in different countries, Frerichs[9] opined that although seemingly frequent isolation of rhabdovirus might at first sight present an attractive proposal for casual agent, it should be realized that the virus has never been isolated from more than 5% of diseased fish examined. It is still not known what role any of the viruses so far isolated or visualized play in the pathogenesis or spread of the disease. Seemingly successful transmission of the disease has been achieved on a few occasions in snakehead fish by different investigators by co-habiting infected and clinically healthy fish but attempts of the experimental induction of disease with an isolated and identified virus have failed so far.

Bacteria

Investigations on the bacterial pathogens from EUS affected fishes have been conducted by several workers[8,9,14,16,17,18,19,20] . These workers isolated a wide variety of pathogenic bacterial forms from lesions and other internal organs such as gills, kidney and liver. Table 7 depicts the variety of bacterial fauna isolated from EUS affected fishes in different states of India. Further, there is no significant relationship between the forms of bacteria isolated and a particular species of diseased fish or a location of disease outbreak. In India as in other countries the predominant bacterial form isolated is *Aeromonas hydrophila.* However, it is not considered to be the primary causative agent. Lilley[21] is of the opinion that the absence of any hemorrhagic septicaemia characteristic of *Aeromonas* infections in all but the most ulcerated fish suggest that *A. hydrophila* is unlikely to have any primary infective role. Indeed *A. hydrophila* is usually not isolated at all from fish in the early stage of the disease.

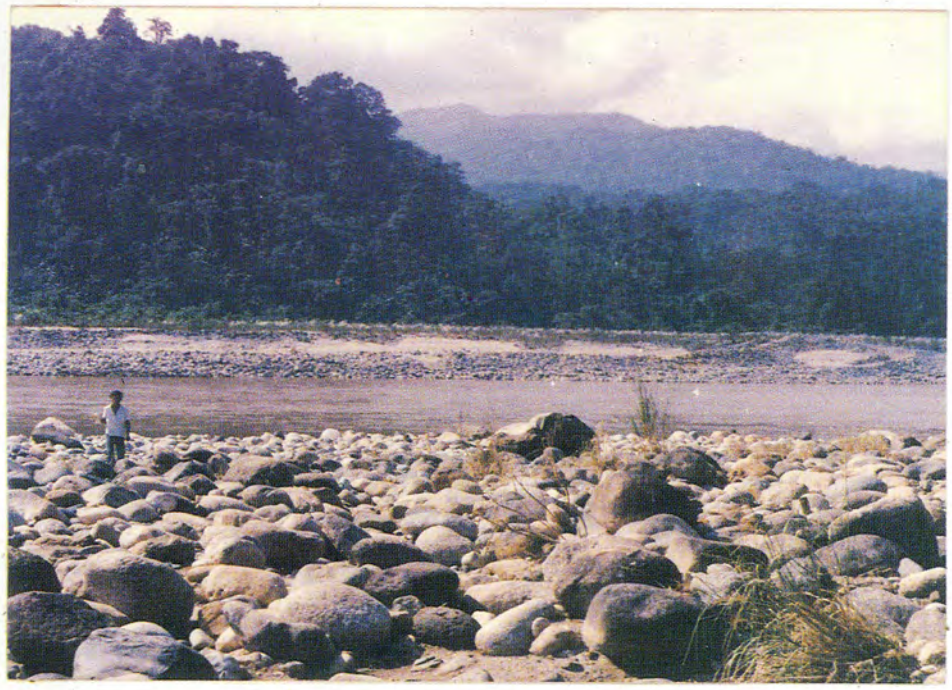
Investigators in India have tried to reproduce the disease symptoms inoculating pure bacterial isolates from EUS affected fishes with mixed success. Jhingran and Das[22] reported transmission of isolated *Micrococcus* sp. in vitro on healthy murrels and manifestation of superficial ulcers took place within 72 hours both through inoculation and when kept in association with the bacteria. Pradhan and Pal[23] and Pal and Pradhan[24] isolated *Pseudomonas flourescens, P. aeroginosa, Aeromonas hydrophila anaerogens* and *Micrococcus varians* from ulcer tissue of EUS affected air breathing fishes. Mixed cultures of four bacteria induced severe ulcer in

WATER AREAS AFFECTED BY EUS IN FISH

An irrigation canal

A pond in Tripura

WATER AREAS AFFECTED BY EUS IN FISH



Shella river in Meghalaya

A beel in Assam

Table 7. Pesticide residues in two EUS affected water bodies near paddy field areas

Pesticide	Balda pond (Antpur) Water (µg/g)	Fish flesh (µg/g)	Ganrapota beel (Bongaon) Water	Fish flesh
		1988		
α - endosulfan	0.00035	1.25		
ß - endosulfan	0.008	1.14		
Total endosulfan	0.0088	2.39		
Methyl parathion	0.085	10.85		
Monocrotophos	0.538	523.5		
		1989		
BHC	0.032	1.9	0.108	27.6
BHC	0.011	0.39		3.3
DDT				
OP'DDE		0.97		0.065
PP'DDE		1.82	0.009	2.73
OP'DDD	0.103	12.73	0.019	7.2
OP'DDD	0.25	46.28	0.063	193.1
OP'DDT	0.011	2.07		0.5
PP'DDT	0.023		0.005	11.2

healthy *Anabas testudineus*, cultures of two flourescent Pseudomonads and Aeromonads induced superficial ulcers in *C. punctatus*. Mixed culture also induced severe ulcer in *C. punctatu,s* aeromonads induced ulcer not so severe as caused by the mixed culture, the two pseudomonads induced superficial ulcers formation and coccus had no effect. Ali and Tamuli[25] isolated *Vibrio* sp., *Aeromonas* sp. and *Micrococcus* sp. from ulcers of diseased *L. rohita, Clarias batrachus, Channa punctatus* and *Anabas testudineus*. In reinfection test, pure culture of *Vibrio* induced simila r disease symptoms; *Aeromonas* sp. produced only mild infection and Micrococcus sp. failed to induce any disease

Table 8. Bacteria isolated from different organs of EUS affected fish specimens in India

State	Host fish	Predominant bacterial form
Tripura	*Channa* sp.	*Salmonella* sp., *Klebsiella* sp.
	Mastacembelus sp.	*A. hydropila, Shigella* sp.
	Puntius sp.	*Staphylococcus* sp., *Bacillus* sp.
	C. catla	*Micrococcus* sp.
Assam	*Channa* sp.	*Pseudomonas mattophila, Shigella* sp.
	Mastacembelus sp.	*Klebssiella ozaenae, Staphylococcus* sp.
	Puntius sp.	*A. hydropila, E. coli*
	C. catla	*Vibrio* sp.
Meghalaya	*Channa* sp.	*P. mattophila, Bacillus* sp., *Micrococcus* sp.
	Puntius sp.	*A. hydrophila*
	Clarias sp.	
West Bengal	*Clarias* sp.	*Corynbacterium hoffmani, Klebsiella aeruginusa*
	Puntius sp.	*A. hydropila*, Acid fast Nocardioform CAN)
	Cyprinus sp.	
	Anabas testudineus	
	Mugil parsia	
Orissa	*Channa* sp.	*Shigella* sp., A *hydrophila, Staphylococcus* sp.,
	Puntius sp.	*Enterobacter agglomerans, Arthobacter* sp., *Micrococus* sp., *E.*
	Clarias sp.	*coli, Pseudomonas mattophila*
Tamil Nadu	*Channa* sp.	*Citrobacter intermedica*
Kerala	*Channa* sp.	*Micrococcus lutens, Staphylococcus* sp., *Citrobacter freundi, NAG Vibrio A. hydropila, Acinetobacter* sp., *Streptococcus* sp.

symptom. Incidence of *Vibrio* sp. was found to be cent percent in experimentally infected fishes. Recently Singh *et al.*[18] isolated *A. hydrophila, Acinetobacter* sp. and *Streptococcus* sp. from ulcerated tissue and *A. hydrophila* and *Streptococcus* sp. from blood, kidney and liver of EUS affected fishes in Kerala. Inoculation of pure cultures of *A. hydrophila* and *Acinetobacter* sp. together caused formation of characteristic ulcers and intra muscular inoculation of *Streptococcus* lead to *oedema*, inflammation and death.

Evidently, though reinoculation of pathogenic bacterial forms isolated from EUS affected fishes could produce ulcers in apparently healthy fishes, further experiments under different environmental conditions are required to produce the disease of similar clinical symptoms.

Fungus

Fungal species is consistently isolated from the lesions of EUS affected fishes especially in an advanced stage of ulceration. The species most frequently isolated in India is *Saprolegnia* sp.[8]. *Aspergilus* sp. was also recorded in the liver parenchymatous tissue from severely affected fishes[9]. It is inferred that these fungal species secondarily infect the fish.

Animal parasites

Urecolariid ciliates of the genus *Tripartiella* and *Trichodina*, myxozoans of the genus *Thelohanellus* and *Myxobolus*, monogenetic trematodes of the genus *Dactylogyrus* and less frequently parasitic copepods *Ergasilus* sp; were encountered predominantly from the gills of EUS affected fishes[9]. These parasites could not be attributed to be the primary cause of ulceration. Most of the parasitic infestations recorded on the sampled fish were at a low intensity. However, Ran[26] reported myxozoans *Myxobolus* and *Thelohanellus* species as primary causative agents of EUS outbreak in Haryana.

Present state of knowledge on EUS

Till now, investigators throughout the world have put in a great deal of effort for ascertaining the aetiology of epizootic ulcerative syndrome in fishes, but to date no firm conclusions have been reached regarding the cause of the disease. During January 1994 in the Regional seminar on Epizootic ulcerative syndrome organised by ODA at AAHRI, Bangkok, scientists from affected countries presented upto date findings on EUS and its relationship to Red spot disease in Australia, Menhaden disease in USA and Piscida disease in Japan.

The conclusions and recommendations that emanated from the deliberations reveal the latest state of knowledge on Epizootic ulcerative syndrome (EUS).

Definition of EUS

A seasonal epizootic condition of freshwater and estuarine warm water fish of complex infectious aetiology characterised by the presence of invasive *Aphanomyces* infection and necrotizing ulcerative lesions typically leading to a granulomatous response.

Similar diseases

Recent pathological and epizootiological evidence has indicated that the condition known as *Red Spot Disease* in Australia is indistinguishable from EUS.

Similarly all available evidence suggests that the condition known in Japan as mycotic granulomatosis is indistinguishable from EUS.

Extension of range

EUS is endemic in many countries and is still extending its geographical range even into sub tropical, sub temperate and temperate climates. Experimental evidence indicates that the *Aphanomyces* involved is capable of causing disease in temperate species.

Human significance

All available evidence suggests that consumption of EUS infected fish poses no proven specific health problems to humans provided that they are properly prepared in sanitary conditions.

Recommendations for future work

In view of the importance and continuing extension of this serious disease, the meeting recommended the following research areas as being of high priority in terms of future work.

Investigation of early stage

It was clear from the information presented to the seminar that there is a distinct and critical early pre-mycotic stage of the pathogenesis of the disease and it is essential that detailed multi-disciplinary research is carried out on this stage.

Virology

As studies have shown the presence of a wide range of viral agents in fish affected with EUS, it is recommended that further extended work be undertaken to determine more accurately the incidence and distribution of tropical food fish viruses throughout the region, in addition to specific EUS-related investigations.

Epidemiology

As proposed studies are likely to produce large bodies of complex data relating to EUS outbreaks in fish populations it is recommended that epidemiological expertise be developed within the region to enable these data to be effectively utilised.

Environment

The evidence presented at the seminar strongly points to a relation between the initiation of EUS and environmental factors. It was recommended that further studies on environmental conditions, including physical, chemical and biological factors, be carried out to better understand their role in outbreaks of EUS. The seminar expressed concern over the limited understanding of the relationship between fish health and environmental conditions, in general, and recommended expansion of research effort in this important subject area.

Speciation of fungus

It is essential given the fundamental importance of fungal organisms of Aphanomyces sp. to this disease that the detailed mycology and molecular genetics of these strains be compared in detail. The isolates should be fully characterised and their relationship defined.

Diagnosis

Currently diagnosis is of necessity based upon a number of clinical and pathological features of the disease. It is important that a rapid, specific, accurate, low cost diagnostic test capable of being used under field conditions is developed.

Recommendations for treatment

It would appear from information presented at the seminar that although some measure of control may be achieved for instance, liming, options for the treatment of the disease are currently limited to empirical management of pond situations. There is a need to understand the current inadequate control methods in order to improve them.

Development of resistance

Evidence from some countries suggests that after the initial outbreak, an element of resistance to the disease may develop in the fish. This resistance may be ecological, genetic or associated with some acquired immunity. It is important that the mechanism for this is now investigated.

Mode of transmission

The evidence suggests that although the extension of the disease in Asian countries was largely via river systems and natural waterways, there was, nevertheless, evidence of distinct transfer over marine barriers. It is important that an understanding be gained as to the mechanism of transfer between water bodies and it is essential that attention be given to the development of quarantine measures to prevent the transfer of these via live fish transportation or infected material.

Socio-economic impact of EUS

As in other countries, the outbreak of EUS in India created panic in the affected areas with sizeable loss of valuable edible fish. This unprecendented appearance of the disease caused grave bioecological and socio-economic consequences. The rivers and large water bodies were affected most in the initial stages, with heavy mortality of valuable stock of fish. As a result, depletion of fisheries is evident, with the consequent impact on fisherman development. This is obvious from a case study conducted during 1987 to 1991 at Jorhat Fish Assembly centre in Assam, to evaluate the damage caused by the disease in fisheries of Brahmaputra river system[7] (Table 9).

Social effect

Investigations carried out in five districts of West Bengal[28] reveal that 73% aquaculture operation units were adversely affected by EUS. The outbreak of the disease depressed the fish consumption rate by 28.7%, 23.3% and 20.5% in urban, sub-urban and rural sectors respectively. Consequently the fish trade was also affected seriously. Owing to consumer resistance, the traders did not accept such fish for selling. In rural markets diseased fishes were sold at a very low price.

About 42.19% of the aquaculturists suffered 31 to 40% loss of fish in their culture ponds followed by 21 to 30% by 25.05%. The pecuniary loss faced by 50% aquaculturists was in the range of Rs. 1,001 to Rs. 5,000/- while 19.73% culturists suffered a greater loss ranging from Rs. 5,001 to 10,000. A section of the farmers had to search for alternate jobs. 88.9% fish traders also suffered losses to some extent during the affected period.

Another study undertaken in 5 Districts of Kerala[29] revealed that the spread of EUS completely paralysed the inland fish market and threw the fishermen out of their occupation and women fish vendors were particularly subject to severe hardship. They had to seek alternative employment as agricultural labourers, head-load and quarry workers, etc. without much success.

Remedial measures

The remedial measures both prophylactic and therapeutic so far tried in India for either controlling or containing EUS are applicable only in manageable water areas. In large open waters such as rivers, reservoirs, lakes and big beels above 30 hectares and backwaters where EUS outbreak occurred remedial measures developed so far are not applicable.

The difficulty encountered in countering the disease outbreak at present is primarily lack of knowledge on the primary causative agent, occurrence of the disease in large water bodies affecting wild population.

The chemicals used for therapeutic and prophylactic treatments in manageable water areas are lime, $KMnO_4$, NaCl, bleaching powder, and antibiotics. The chemical treatment is primarily aimed at controlling the external pathogens observed such as bacteria and fungus.

Liming

Depending upon the pH quick lime at 100-600 kg per hectare has been found effective in manageable water areas. In areas having alkalinity below 40 ppm (Table 4) the higher doses of lime is applied and in areas with higher alkalinity the lower dose of lime is applied at an interval of 1 month during the outbreak period. It is observed that CaO applied at 50 kg/hectare in the disease prone water area in the post monsoon period just prior to the outbreak of disease have either arrested the occurrence of the disease or if outbreak occurred the intensity is mild[1]. The information collected from the different states of India through questionaire developed by CICFRI, Barrackpore and distributed to all the states it is gathered that lime treatment has given encouraging results in checking the intensity and spread of the disease. A study conducted in West Bengal revealed that as remedial measure the clientele adopted different remedial measures[28]. The study revealed maximum respondents (358) applied lime to control the disease followed by application of $KMnO_4$ (227). Only limited number of farmers[28] applied antiobiotics. About 68% of the respondents obtained positive result from the treatments.

Potassium permanganate

Application of this chemical as a deterent for EUS is quite widespread in India. An application rate ranging from 1 ppm to 10 ppm has given fairly encouraging result in the different states. While the application rate for bath treatment of fish is 1-6 ppm the pond treatment rate is 5-10 ppm. This rate has been found effective in containing EUS and healing up of initial stage of ulceration of fish.

Bleaching powder

Bleaching powder at 1 mg/litre or 5-10 kg/hectare was reported to be useful in healing up of initial lesion of EUS affected fishes. Das[27] recommended use of bleaching powder at 3-5 ppm for desinfecting all fishery equipments used fr fishery activities in EUS affected areas. Investigations at CICFRI showed that EUS can be contained in manageable water areas, by applying a prophylactic dose of 50 kg/ha CaO and after one week bleaching powder @ 0.5 ppm in disease prone water areas.

Therapeutic dose of 100 kg/ha CaO and after one week bleaching powder @ 1 ppm when initial symptoms of EUS is seen.

Salt (NaCl)

Application at a concentration of 3-4% dip treatment of affected fishes have given fairly effective result in healing up of ulcers at the initial stage of the disease[8]

Antibiotics

Pending knowledge of the definitive primary causative agent of the disease what is apparent is that the EUS affected fishes are afflicted by a wide variety of bacteria and in acute cases fungus. A microencapsulated feed containing 30% protein, nalidixic acid, erythromycin along with vitamin A and C has been formulated by CICFRI. Trial with the pelleted feed to diseased fishes showed the fishes recovering. In general it was found that antibiotics either erythromycin or oxytetracycline or terramycin at 60-100 mg/kg of feed for 7 days cured the ulcers of EUS affected fishes[22].

CIFAX

A drug formulated by CIFA for application in EUS affected captive waters is reported to show encouraging result in controlling EUS. The durg applied at 1 litre/hectare metre of water area with the notice of the symptoms of EUS in the pond is reported to cure affected fishes within 7 days.

Table 9. Species-wise landing (kg) EUS affected fish during 1987-91 and percentage increase/decrease* through years with 1987-88 as base

Species group	1987-88	1988-89	1989-90	1990-91
Puntius spp.	34804	12696 (-63.5)	3623 (-89.6)	10401 (-70.1)
Amblypharyngodon mola	22616	7712 (-65.9)	14153 (-37.4)	6601 (-70.8)
Labeo rohita	17316	6350 (-63.3)	8170 (-52.8)	8823 (-49.0)
Catla catla	13046	8434 (-35.4)	8029 (-38.5)	8151 (-37.5)
Puntius sarana	5100	9359 (+83.5)	4072 (-20.2)	5097 (-0.1)
Cirrhinus mrigala	2089	702 (-66.4)	1499 (-28.2)	908 (-56.5)
Labeo bata	1701	783 (-54.0)	219 (-87.1)	651 (-61.7)
Heteropneustes fossilis	13848	13816 (-0.2)	22333 (+61.3)	18291 (+32.1)
Mystus sp.	7006	5219 (-25.4)	3228 (-53.1)	5067 (-27.7)
Ompok spp.	5009	3683 (-26.5)	1926 (-61.5)	2065 (-58.8)
Channa punctatus	30091	4649 (-84.6)	1829 (-93.9)	2622 (-91.3)
Channa striatus	22332	2777 (-87.6)	3941 (-82.4)	3406 (-84.7)
Channa marulius	8079	4309 (-46.7)	7992 (-1.1)	7419 (-8.2)
Anabas testudineus	10189	5963 (-41.5)	15555 (+52.7)	13142 (+29.4)
Colisa spp.	1888	805 (-57.4)	871 (-53.9)	2095 (+11.0)
Nandus nandus	2816	500 (-82.2)	62 (-97.8)	150 (-94.7)
Gadusia chapra	100	883 (+783.0)	339 (+239.0)	376 (+276.0)

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