EPIZOOTIC ULCERATIVE SYNDROME IN FISHES

CENTRAL INLAND CAPTURE FISHERIES RESEARCH INSTITUTE BARRACKPORE

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Preface

Fish diseases, in various forms have been tormenting the aquaculturists ever since man learned the art of fish husbandry. However, no fish disease ever known to man has been as virulent and menacing as the recent outbreak of Epizootic Ulcerative Syndrome. Transcending the confines of culture ponds, the EUS has plagued the natural fish populations of the open water resources. The alarming rate of infections and the trail of destruction left behind by the epizootic have already robbed thousands of fish farmers and riparian fisherfolk of their daily bread in Asia and Australia. Unfortunately, many vital clues regarding the causative organisms and factors responsible for the outbreak of the epizootic are yet to be unravelled and this serious problem is still evading a plausible solution, despite hectic global efforts. Under an eleven-nation endeavour to collect the ecological data pertinent to the disease, the CICFRI has been conducting investigations on the subject for the last two years and some of the findings are presented in this bulletin. This document is, by no means, a comprehensive manual to control the epizootic. Nevertheless, an attempt is made here to document the present state of knowledge on the disease with a view to enlightening the research workers, aquaculturists and the public at large about the disease. It is also hoped that this bulletin will help to dispel the misconceptions and popular fallacies that shroud the disease.

Barrackpore
17. 2. 1990

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INTRODUCTION

Research on fish diseases, though a recent development, is gradually acquiring importance in India and its intensification is imperative for successful implementation of various fishery development programmes. The recent outbreak of the dreaded fish disease 'Epizootic Ulcerative Syndrome' in fishes inhabiting rivers, canals, beels, lakes, paddy fields and ponds of North Eastern and Eastern states of India has seriously impeded fishery activities.

This disease with 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 (CICFRI) 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.I). In Queensland, Australia, an epizootic of marine and estuarine fishes characterised by shallow haemorrhagic ulcers occurred in 1972 with recurrence in subsequent years (Rodger and Burke 1977, 1981). 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 (Haines 1983) and north during 1982-88 (Coates *et al.* as quoted by Tonguthai,1985)). Indonesia also reported similar type of disease in Bogor in 1980 (Anon. 1981) 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 (Tonguthai 1985). 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. Large scale fish mortality is still continuing unabated in these areas. Recently in 1989, reports came that Nepal was affected by the disease.

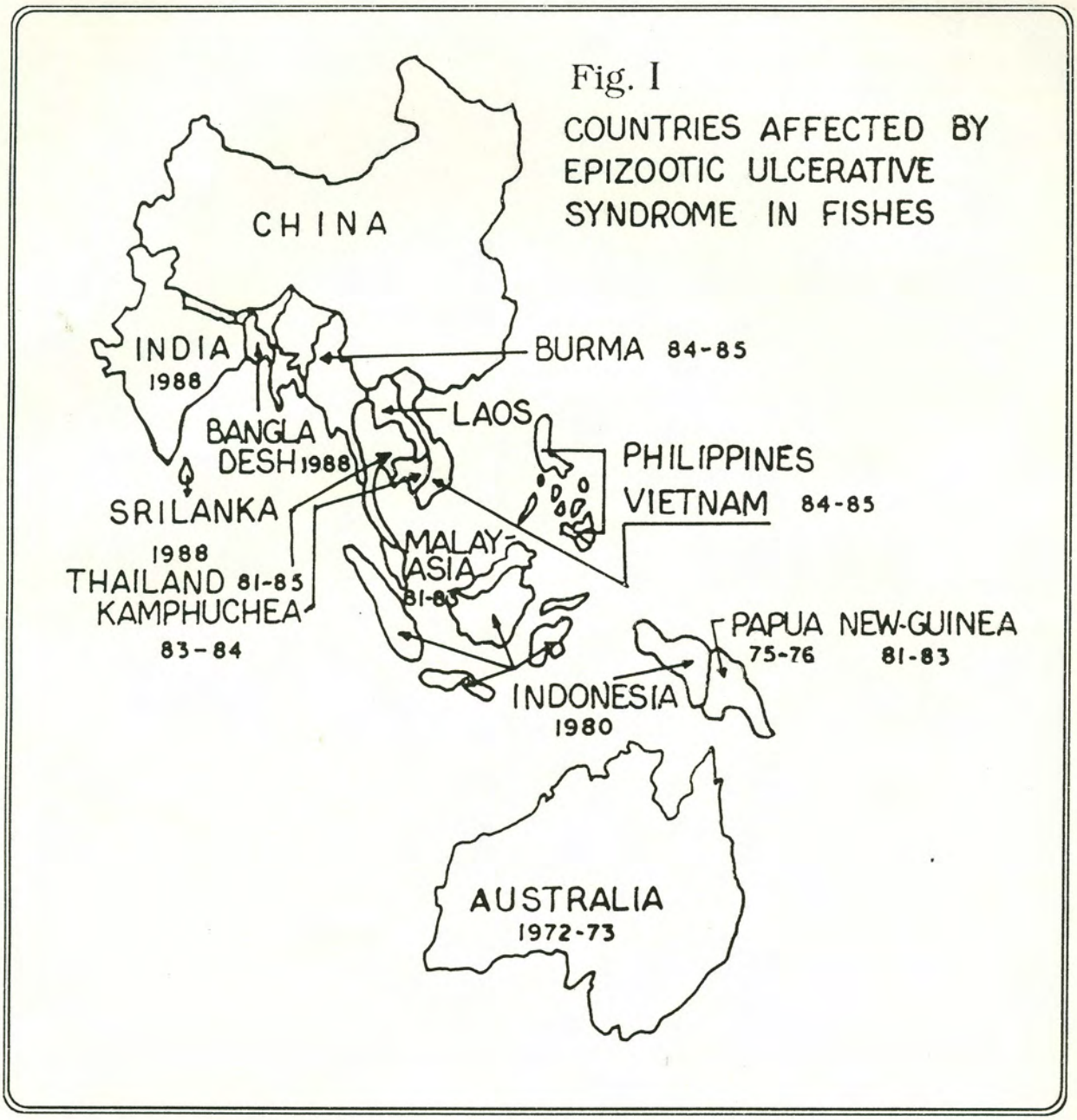


Fig. I
COUNTRIES AFFECTED BY EPIZOOTIC ULCERATIVE SYNDROME IN FISHES
CHINA
INDIA 1988
BURMA 84-85
LAOS
BANGLA DESH 1988
PHILIPPINES
VIETNAM 84-85
SRILANKA 1988
MALAY-ASIA 81-83
THAILAND 81-85
KAMPHUCHEA 83-84
PAPUA NEW-GUINEA 75-76 81-83
INDONESIA 1980
AUSTRALIA 1972-73

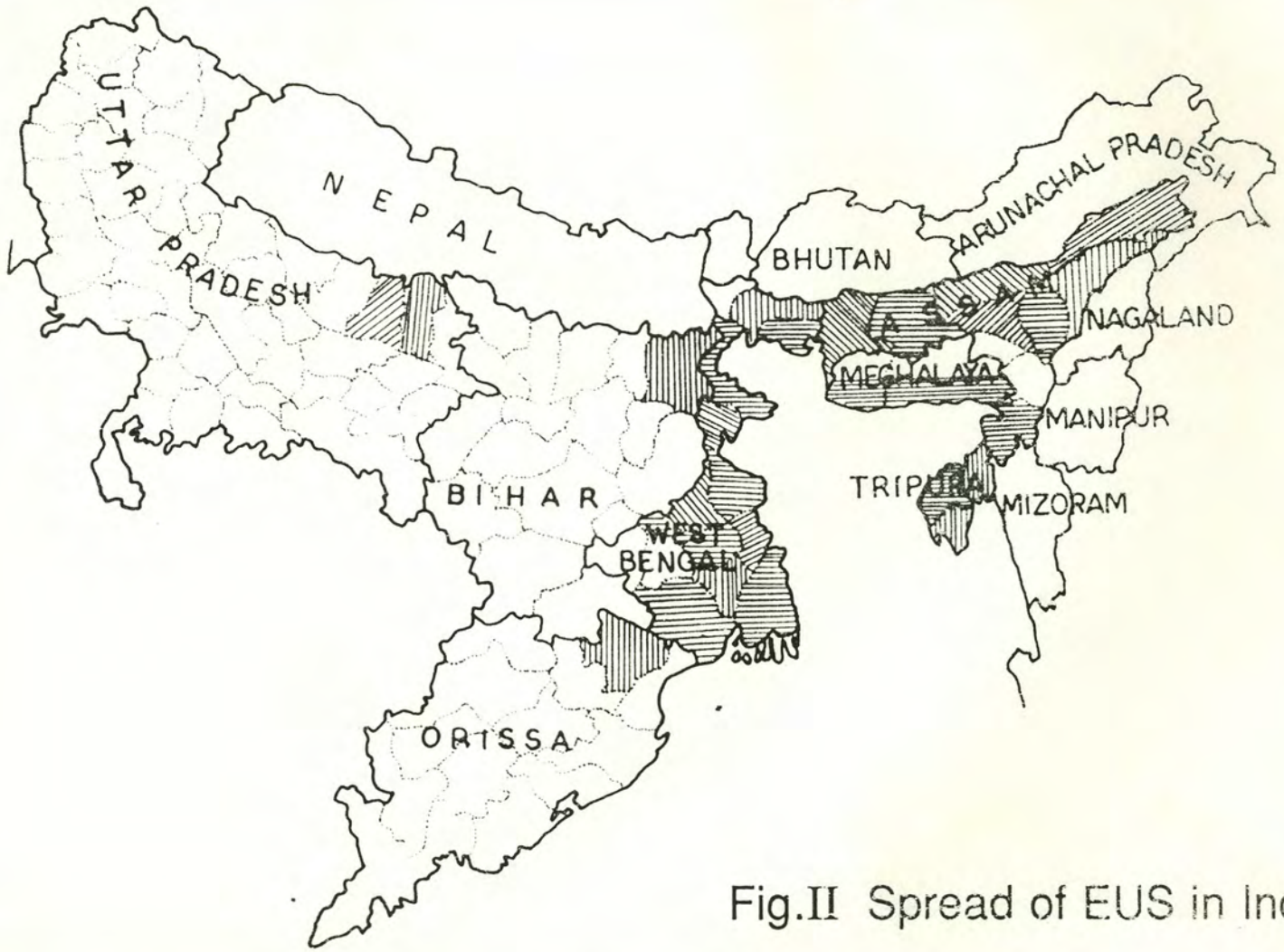


Fig.II Spread of EUS in India

An EUS affected pond surrounded by a paddy field in Tripura. (Below) Initial symptoms of the disease

AREAS AFFECTED BY THE DISEASE IN INDIA

In India, fishes from all types of water bodies are affected by the disease. Initially freshwater fishes in the rivers were affected. Gradually the disease spread to beels, lakes and paddy field areas and finally fishes in culture ponds were also affected. The disease, which occurred first in May 1988 in some districts of Assam, Tripura, Meghalaya and West Bengal bordering Bangladesh, spread to nearly all the districts of these states by 1989. By 1990, the disease spread westwards to other states like Orissa, Bihar and Uttar Pradesh (Fig. II).

SEMIOTICS OF THE DISEASE

Prior to the identification of Epizootic Ulcerative Syndrome in fishes of various countries of Asia-Pacific region, different but less intense ulcerative conditions of varied origin had been reported from fishes. In India, for instance, Gopalakrishnan (1966), Srivastava (1975), Manohar *et al.* (1976) and Pal (1984) reported ulcerative condition in fishes.

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 swiming 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 haemorrohagic 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 .

FISH SPECIES AFFECTED

The disease affects both wild and reared fishes. The wild species are *Channa striatus, C. punctatus, C. gachua, Clarias batrachus, Heteropneustes fossilis, Puntius sophore, P. ticto, Amblypharyngodon mola, Mystus vittatus, M. aor, Mastocembelus pancalus, M. armatus, Ambassis ranga, Nandus nandus, Callichorus pabda* and *Gadusia chapra*. The fishes affected in the culture systems are *Cyprinus carpio, Catla catla, Cirrhinus mrigala, Labeo rohita, Puntius javanicus, Ctenopharyngodon idella and Hypophthalmichthys molitrix*. Fish species most severely affected are predominantly the bottom dwelling fishes of the genera *Channa, Mastocembelus, Clarias, Heteropneustes, Cyprinus* and *Cirrhinus* besides *Puntius* and *Nandus* . The details of fish species affected in India and the Asia-Pacific region are given in Tables 1 and 2.

PRESENT KNOWLEDGE ON THE SUSPECTED CAUSATIVE AGENTS

A review of the available literature on Epizootic Ulcerative Syndrome (Tonguthai 1985, Anon. 1986) reveals certain factors and agents suspected to be the cause of the disease *viz.*,

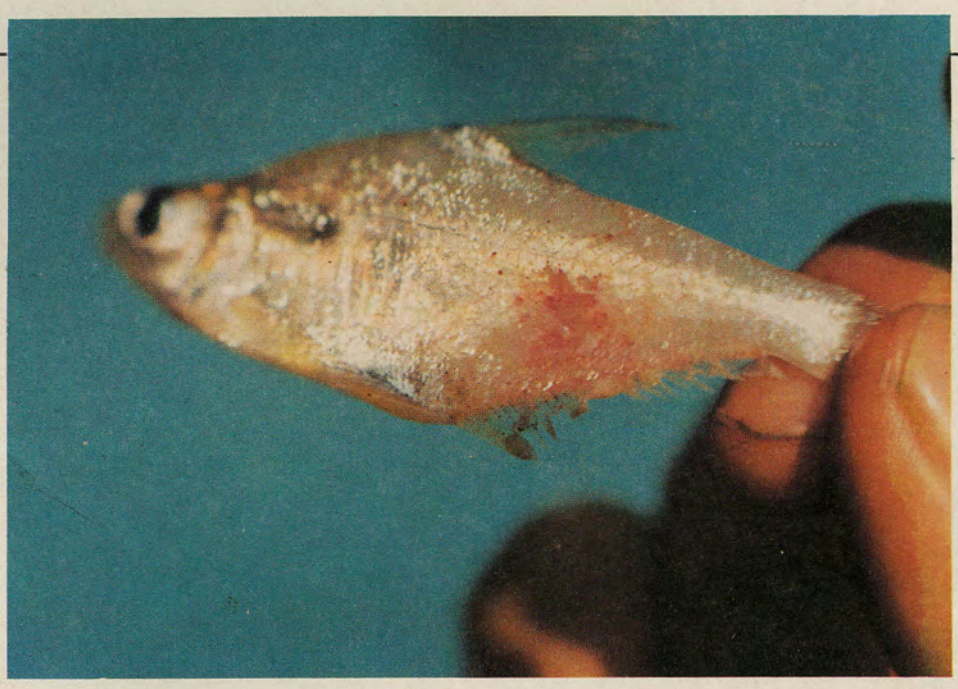
Virus :- Comparatively little virological investigation has been done on the disease, although its occurrence is reported since 1980. Electron microscopic studies of affected fishes in Thailand revealed infestation with virus like particles. They also isolated a birna virus from affected fish. Ulcerative fish disease investigation conducted by an FAO team also isolated identical rhabdovirus from affected snakeheads and swamp eels from five locations and demonstrated partial cytopathic effect suggesting the possibility of a rhabdovirus being responsible for initiating the lesions, followed by secondary infection. The National Inland Fisheries Institute Bangkok is of the view that although virologists in Thailand have found ultrastructural evidence of certain viruses associated with the disease, these cannot be automatically concluded as the major cause of the disease or even responsible for its outbreak. Viruses certainly have serious effect on fish and can cause severe mortality in fish populations but normally they are host- specific. Thus, in order to investigate the full relationship between the presence of virus and occurrence of the disease, further studies are to be conducted on :

TABLE I Fish species affected by EUS in different states

	CULTURED	WILD
Assam	*Cyprinus carpio, Catla catla, Cirrhinus mrigala, Labeo rohita, Puntius javanicus*	*Channa striatus, C. punctatus, C. gachua, Puntius sophore, Puntius ticto, Mastocembelus pancalus, M. armatus, Amblypharyngodonmola, Ambassis ranga, Mystus vittatus, Heteropneustes fossilis, Clarius batrachus, Nandus nandus, Glosso-gobius giuris, Chanda chanda , Gudusia chapra*
West Bengal	*Cyprinus carpio, Catla catla, Cirrhinus mrigala, Puntius sarana, Puntius javanicus*	Same as in Assam & *Callichrous pabda*
Tripura	*Cyprinus carpio, Catla catla, Cirrhinus mrigala, Labeo rohita, Ctenopharyngodon idellus, Puntius javanicus*	Same as in Assam
Meghalaya	Nil	*Puntius sophore, Channa striatus, Channa punctatus, Mastocembelus pancalus, Mastocembelus armatus, Clarias batrachus, Heteropneustes fossilis, Mystus vittatus*
Bihar	*Cirrhinus mrigala, Catla catla*	*Channa striatus, Channa punctatus, Channa gachua,Puntius sophore, Mastocembelus pancalus ,Glossogobius giuris, Nandus nandus, Clarias batrachus, Heteropneustes fossilis, Mystus vittatus, Trichogaster sp.*

Table II : Fish species severely affected by the disease in Asia-Pacific region

Australia	*Liza* spp., *Sillago* spp., *Acanthopagurs australia*, *Arramphus sclerolepis*
Papua New Guinea	*Toxotes chatareus*, *Kurtus gulliveri*, *Oxygleotris* spp., *Glossogobius giuris*, *Scutengraulis seratchlevi*, *Scatophagus* sp.
Indonesia	*Cyprinus carpio*, *Clarias batrachus*, *Puntius javanicus*, *Osphronemus gouramy*
Malaysia	*Channa striatus*, *Trichogaster pectoralis*, *Clarias macrocephalus*, *Anabas testudineus*, *Liza* spp., *Arius* spp.
Burma	*Channa striatus*, *C. punctatus*, *C. marulius*, *C. gachua*, *Mastocembelus* sp., *Symbranchus* sp., *Wallago attu*, *Heteropneustes fossilis*
Vietnam	*Channa striatus*, *C. macrocephalus*, *Ctenopharyngodon idella*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*
Philippines	*Channa striatus*, *Clarias batrachus* *Puntius javanicus*, *Trichogaster pectoralis*
Sri Lanka	*Channa striatus*, *Channa punctatus*, *Puntius dorsalis*, *Puntius sarana*, *Etroplus suratensis*, *Wallago attu*, *Mystus* spp., *Mastocembelus armatus*, *Ompok bimaculatus*
Bangladesh	Most species of snakeheads, catfishes, eels and Indian Major Carps



Infected specimens of Chanda chanda (above),
Puntius sophore and *Gudusia chapra* (below)

(a) optimum isolation procedures,
(b) characterization of virus, its pathogenicity and
(c) species of fish susceptible to infection.

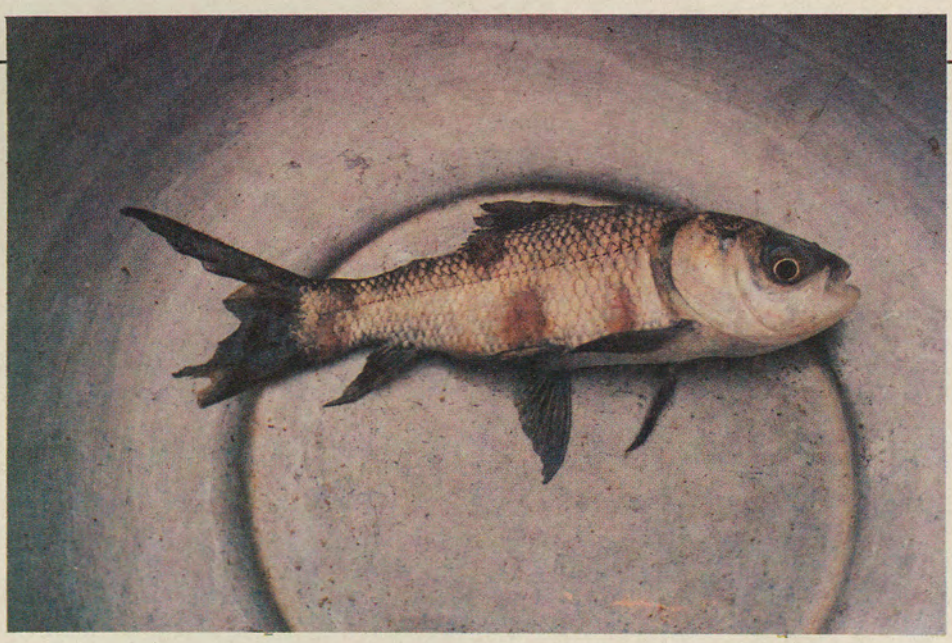
Bacteria : The studies conducted on the affected fishes in different countries recorded a varied bacterial fauna but consistently showed the predominance of *Aeromonas hydrophila* and occasionally *Pseudomonas* sp. from the ulcerated areas of fish. It was inferred that since *A. hydrophila* is ubiquitous in tropical waters, it frequently infects the ulcers as secondary infectants, the primary cause of ulcers being different.

Fungus : Some fungal species are found to be commonly associated with ulcerated lesions especially in advanced ulcers. In Thailand a pathogenic fungal species, *Achalya* sp., was mainly encountered with the lesions. It was concluded that fungus was not the primary etiological agent, but infected the fish secondarily.

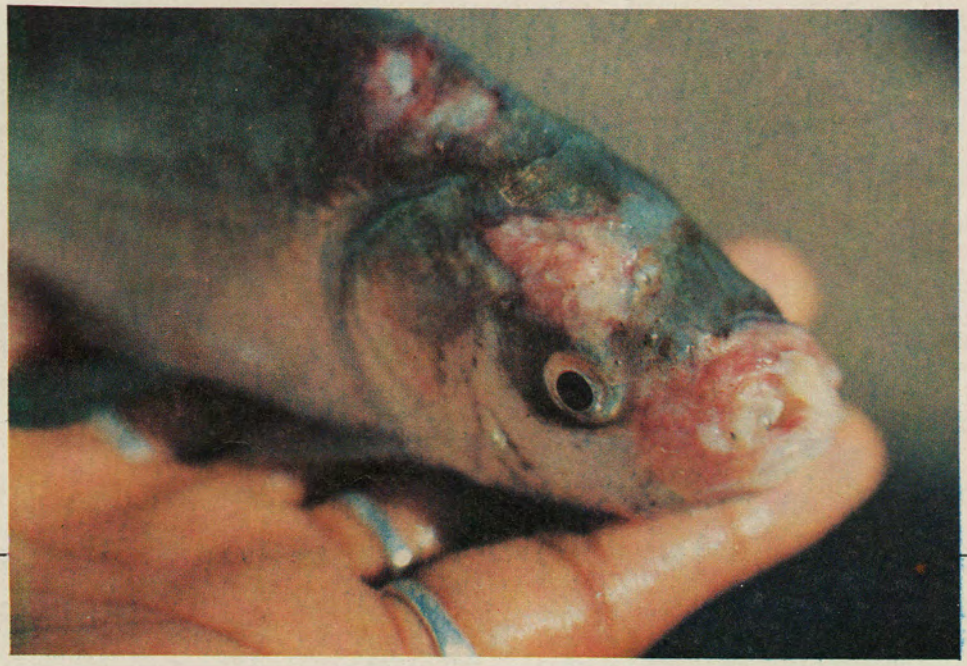
Animal parasites : Certain animal parasites have been recorded to be associated with the disease. The commonly found parasites were the species of *Palisentis, Trianchoratus, Dactylogyrus, Gyrodactylus, Trichodina* and *Epistylis*. However, these parasites could not be definitely identified as the primary cause of ulceration. Most of the parasitic infections found on the sampled fish were at a very low intensity.

Environmental factors : The disease outbreak was common in waters of low alkalinity and hardness i.e. waters closely correlated to acidic, low calcium soils. The ulcerative disease was, of course, not found solely in such waters but was also recorded in alkaline waters associated with periods of heavy rainfall and a concomitant drop in pH, alkalinity and calcium. These environmental factors alone could not be the major factors responsible for initiating the lesions but in all probability, they are a predisposing factor for disease outbreak.

Pesticides : Because the occurrence of the diseased fish in all affected countries was prominent in rice field environments, pesticides were suspected to be associated with ulceration. During the disease outbreak in Malaysia too, pesticides were referred by some workers as the major possible cause of the disease. However, work done in other affected countries showed that the concentrations of pesticides detected in water were below the LC_{50}



Ulcerated specimens of *Catla catla*. The picture below shows a deep ulcer in the head region of the fish



Advanced stages of the disease. Mutilated specimens of *Channa marulius*(above)and *Mastocembelus armatus*(below)

values for several fish species. This dimisses the possibility of pesticide residue acting as triggering agent for the outbreak of the epizootic. Moreover, analyses of pesticide residues in tissues of affected fishes did not show any correlation with occurrence of the disease.

CICFRI's Investigation on causative agents of the disease

Environmental parameters : A wide range of analyses were made on basic water quality parameters like pH, alkalinity, carbon dioxide, hardness, dissolved oxygen, temperature, ammonia, nitrates, and turbidity. None of the parameters transcended the acceptable limits in most of the sites where diseased fish were located. Occasional falls in oxygen concentration and peaks in ammonia were recorded in several organically enriched environments.

One important finding of the environmental survey was that the intensity of the disease outbreak was high in waters of low alkalinity and hardness; characteristics of acidic, low calcium soils . The ulcerative disease was not solely located in such waters. However, it was linked to periods of heavy rainfall in more alkaline environments with consequent drop in pH and alkalinity (Tables 3&4).

Table 3 Values of water quality parameters in areas severely affected by the disease.

	Assam	Tripura	Meghalaya	W. Bengal	Bihar
pH	7.1-7.5	6.7-7.6	6.5-7.5	6.7-7.8	6.1-6.8
Alkalinity	13-25	7.0-49	7-14	10-54	25-30
Hardness	11-38	9-45	10-15	6-64	13-20
Chloride	4-23	3.5-18.2	2-12.0	2.9-13.4	4.7
Ammonia	Nil-0.4	Nil-0.6	--	Nil-0.6	--

Table 4 Values of water quality parameters in areas less severely affected by the disease

	Assam	West Bengal	Bihar
pH	7.2-7.5	7.9-8.1	7.5-7.5
Alkalinity (ppm)	48-76	110-200	170-200
Hardness (ppm)	62-78	92-190	130-135
Chloride (ppm)	4-6	4.7-18	10-48
Ammonia (ppm)	Nil-0.2	Nil-0.3	--
Salinity (ppt)	--	0.3	--

Heavy metals in affected waters : The concentration of various metals in affected areas were analysed (Table 5). The values for zinc ranged from 21.0 to 62.8 $\mu g\ l^{-1}$ in water and 9.13 to 21.6 $\mu g\ l^{-1}$ in fish, whereas the respective values for copper ranged from 1.2 to 3.92 $\mu g\ l^{-1}$ and 2.39 to 2.47 $\mu g\ l^{-1}$. The data obtained so far does not suggest any perceptible role of heavy metal content in creating stress to the fishes leading to outbreak of the disease.

Table 5 Values of heavy metal concentration in affected water bodies ($\mu g\ l^{-1}$)

Site	Fe	Zn	Cu	Cr	Cd	Pb	Hg
Mayapur	280	107	80	8.0	9.0	16.5	0.12
Cooch Behar	200	21	7.0	nd	nd	nd	nd
Maldah	130	32	3.0	nd	nd	3.8	nd
Jorhat	7800	62.8	3.9	nd	nd	5.75	nd
Jhalukbari	-	22.8	1.2	nd	nd	nd	nd
Meghalaya	4840	53.2	2.12	nd	nd	3.68	0.03

nd : not detectable

Table 6 Values of Heavy metal analyses in infected fishes ($\mu g\ g^{-1}$)

Species	Fe	Zn	Cu	Cr	Cd	Pb	Hg
P. sophore	93.09	21.6	1.39	-	nd	-	-
M. pancalus	83.46	9.13	2.47		-	-	-

INVESTIGATION ON PATHOGENS

Bacteria : Study of the specimens from various affected States showed the domination of *Micrococcus* sp. in association with other bacteria *viz., Aeromonas hydrophila, Pseudomonas flourescens* and *Eschirichia coli.* However, *Micrococcus* sp. were consistently isolated from lesions and other haematopoetic tissues of the affected fishes as well as their environments.

The bacterium *Micrococcus* is spherical and gram positive in nature, measuring 1.0-2.0 µm. It generally occurs singly or sometimes in pairs when dividing. It is nonmotile and aerobic in nature. Metabolism is strictly respiratory and oxidises sugars, namely raffinose, sucrose, mannose, adenositol, sorbitol and salicin. Dulcitol is not oxidised. Arginine is usually not hydrolysed though ornithine and lysine are hydrolysed. Generally, the optimum growth is at 30 °C but multiplies slowly at 20 °C.

Transmission of *Micrococcus* sp. was tested *in vitro* on healthy murrels and manifestation of ulcers took place within 72 hrs., both through inoculation and when kept in association with the bacteria.

A survey of the literature reveals that gram positive bacteria were considered as less important fish pathogens, with the exception of streptococciosis which was reported among framed rainbow trout of Japan (Hoshina *et al.*, 1958). During 1976-77 *Staphylococcus epidermidis* was also recognised as a fish pathogen,

causing severe mortality among farmed yellow tail and sea bream. However *Micrococcus* sp. was never considered as a virulent pathogen though Conroy (1966) encountered Micrococcosis among farmed rainbow trout of Argentina. In the recent outbreak of the epizootic ulcerative syndrome in India, the *Micrococcus* sp. was found to be highly virulent.

Fungus : Fungi are invariably associated with the lesions which, in all probability, are the secondary infections . *Saprolegnia* sp. was identified as the major fungus.

Animal parasites : Trichodinids of the genus *Tripartiella*, several myzozoans and *Dactylogyrus* spp. were encountered from the diseased fishes but they could not be ascertained to be the cause of the disease because of their low intensity of infection.

Emergence of the disease in India

Emergence of the disease in India from May, 1988 can be traced to the diseased fishes entering along with the flood waters from Bangladesh where severe outbreak of the disease started from February/March 1988. This aspect raises the important question of transmission of serious disease through contiguous water areas and exotic fishes.

PROPHYLACTIC AND THERAPEUTIC MEASURES TO CONTROL THE DISEASE

Generally, any severe outbreak of fish disease occurs when the balance among fish population pathogens and the environment gets disturbed. When the environmental qualities conducive to fish health deteriorates, fishes experience tremendous stress making them vulnerable to various pathogens. According to Snieszko (1974), disease manifestation is a function of three parameters viz., the host, the pathogen and the environment. Thus, prophylactic measures assume importance. It has been observed in many cases where manageable water areas were limed adequately and other measures mentioned in succeeding paragraphs were taken, the disease outbreak did not occur, or the severity was less.

The remedial measures in manageable water areas are primarily based on the following three chemicals :

(i) Lime, (ii) K MnO_4 and
(iii) Antibiotic theraphy .

Lime : Lime application @ 200-600 kg per hectare has given encouraging results in various affected water areas. The favourable actions of liming are that it-

i) raises pH of acidic water to neutral or slightly alkaline value,

ii) increases the alkaline reserve in water and mud preventing extreme changes in pH,

iii) promotes biological productivity by enhancing the breakdown of organic substances by bacteria, creating optimum levels of oxygen and carbon reserves,

iv) precipitates suspended or soluble organic materials, decreases biological oxygen demand, and increases light penetration, and

v) kills most of the undesirable microrganisms especially pathogens due to its caustic reaction.

Potassium permanganate : Application @ 0.5 - 2 ppm in water is also very helpful in curing the ulcers.

Antibiotic therapy: Pending the availability of a suitable freshwater fish vaccine, use of antimicrobial compounds is still invaluable for reducing losses arising out of the epizootics of fish. Although several antimicrobial agents may prove to be useful in controlling the disease, stringent precautions are necessary to prevent misuse of antibiotics Based on the experiments on antibiotics therapy conducted at CICFRI, a micro- encapsulated feed containing 30% protein fortified with nalidixic acid and erythromycin (to check the bacterial population of both the gram negative and gram positive bacteria) along with vitamins A & C has been formulated. Trials with the pelleted feed on diseased fishes along with selected antibiotic bath (chloramphenicol) at the dosage of 15 ppm has resulted in recovery.

In general, it is recommended to use the antibiotics erythromycin/ nalidixic acid/ oxytetracyline/ terramycin @ of 60-100 mg per kg of feed for 7 days to cure the ulcers. Besides the above therapeutic measures, a close vigil on the occurrence of disease in the contiguous areas should be maintained. Utmost care should be taken to prevent the flow of water from the disease infected waters to the healthy ponds.

CONCLUSIONS

1 The rivers, lakes, swamps, rice fields and other open water systems constitute the major source of inland fish production in India, Bangladesh and most of the South East Asian countries. As a good number of the diseased fishes belong to open water bodies, it is pertinent to look into such natural environments to know the factors responsible for the disease.

2 Question arises as to how the disease reappear in paddy fields after a season when the fields dry up. It may be that the disease pathogens at that time are harboured in the reservoirs or swamps where fishes congregate during dry season. It is quite possible that the crowded condition and consequent stress to the fishes in these water areas cause the spread of the disease to other fishes.

3 Chances of spreading and emergence of the disease are more when irrigation is the source of water for agriculture and fisheries as it carries pathogens to large areas of water use. A similar situation arises during flood time too. Thus, if causative organisms of the disease are present in such waters, the disease problem gets aggravated, unlike rainfed or ground water.

4 A serious disease like EUS poses a challenging situation to the fishery scientists. However, as opined by Tonguthai (1985), no assumption should be made about the common identity and causative organisms of the ulcerative conditions described in different countries, unless complete clinical, pathological and microbiological evidences are obtained.

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