

The Relationship of Some Environmental Factors and the Epizootic Ulcerative Syndrome Outbreaks in Beel Mahmoodpur, Faridpur, Bangladesh

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Abstract

A long-term regional monitoring program on the relationship between the environment and the occurrence of epizootic ulcerative syndrome (EUS) in fish was initiated in Beel Mahmoodpur, a freshwater lake at Faridpur, Bangladesh in early 1988. Agriculture management practices and climatic conditions were closely monitored and quantitative samples of fish populations were taken fortnightly over a three year period between March 1988 to March 1991. The occurrence of EUS from the study site was recorded between October to March every year. Of the 23 fish species sampled, 10 were severely affected. The prevalence of infection was highest in snakeheads (30%). Results of ANOVA of all the investigated parameters were not significant except low chloride ($p < 0.045$) and alkalinity ($p < 0.043$) concentrations. Water temperature also significantly ($p < 0.00065$) fluctuated during EUS outbreaks. Lower concentrations of chloride with respect to alkalinity as well as lower proportions of anions (chlorides and alkalinity) with respect to cations (hardness) were closely correlated with EUS outbreaks at declining temperature periods. The role of total ammonia at low chloride concentration was also related to the outbreaks. A direct relationship of chloride concentration between habitat water and fish muscle was also revealed from fish muscle analyses and further implies a close relationship between chloride deficiency and EUS outbreaks.

Introduction

During the Overseas Development Agency regional seminar on EUS held in Bangkok, Thailand (Roberts et al. 1994) EUS in fish was defined as “a seasonal epizootic condition of freshwater and estuarine warm water fish of complex etiology characterized by the presence of invasive *Aphanomyces* infection and necrotizing ulcerative lesions typically leading to

granulomatus response.” It is often associated with rapid high mortalities among fish of all ages, particularly when it first occurs in an area. EUS affects a wide variety of wild and cultured fish species in freshwater areas in Southeast Asia including Pakistan where it occurred in 1996 (Lilley et al. 1997a), and brackishwater fishes in Karnataka, India (Vishwanath et al. 1997). A similar disease known as Red Spot Disease (RSD) occurring in estuarine fishes from Australia and Papua New Guinea since 1972, with a distinctive common fungal pathogen (Lilley and Roberts 1997) might also be EUS. Lilley et al. (1997a) further reported the pathogen as a conspecific single fungal clone, now known as *Aphanomyces invadans* (David and Kirk 1997), causing large scale fish kills in the pan Asia (Lilley et al. 1997b).

In Bangladesh, EUS was first observed in Faridganj in the Chandpur district in February 1988 (Ahmed and Hoque 1998). Since then, repeated outbreaks have occurred annually and the disease has spread to new areas covering the whole country, recurring in the year 2000 in the Thakurgaon areas of Northern Bangladesh. An estimated loss of US \$ 0.96 million in one of 21 affected districts in Bangladesh during the 1988 outbreak was recorded by Hossain et al. (1992).

EUS is generally associated with changing water quality factors and climatic conditions. Decreased temperature and low levels of hardness and chloride were also cited by Ahmed and Hoque (1998). Raman (1992) reported that decreased salinity due to a heavy influx of inland waters, affected the other water quality parameters, and proposed decreased salinity as a precondition for the outbreak of EUS in brackishwater fishes of Chilka Lagoon in India.

Over the last several years, investigations have been carried out on many aspects of EUS such as: bacteriological, virological, parasitological, mycological and immunological. Most of the studies revealed that the *Vibrionace*, particularly *Aeromonas hydrophila*, and a specific fungus *Aphanomyces* sp. have been consistently reported in EUS outbreaks, irrespective of fish species and country of origin (Lilley and Roberts 1997). None of these pathogens has been established as the primary pathogen of EUS (Mohan and Shankar 1994), and no true etiology of the syndrome has yet been established (Cruz-Lacierda and Shariff 1995). However, in agreement with earlier reports, Ram et al. (1994) indicated the involvement of *Myxobolus* spp. and *Thelohanellus pyriformis* with disease episodes, which is yet to be confirmed.

In countries like Burma, Indonesia, Malaysia and Thailand, EUS commonly occurred in natural swamps and paddy fields. Therefore, as part of the regional program, the Network of Aquaculture Centers in the Asia-Pacific consultants in early 1988 selected the natural lake Beel Mahmoodpur, surrounded by swamps and paddy fields, as a vulnerable EUS site at Baitul aman, Faridpur, Bangladesh for long-term monitoring.

The main objectives were to conduct long-term studies of the environmental parameters in relation to EUS outbreaks under a standardized proforma of data, that include: site description, climatological factors, aquacultural practices, agricultural crops and management practices of the surrounding environment, water quality parameters (including seasonal and diurnal fluctuations), qualitative sampling of fish populations, occurrence and intensity of EUS, and preservation of diseased fish samples.

The present paper correlates environmental parameters such as temperature, rainfall and water quality with EUS outbreaks at Faridpur, Bangladesh over a 3-year period from March 1988 to March 1991.

The impact of rainwater on lake waters and the variations of chloride concentrations between habitat water and fish muscle further supplement the study.

Materials and Methods

Site description

The study site is a 25 ha natural body of water with an average depth of 1 to 2 m. Swampy lands exist at the periphery, further surrounded by approximately 20 ha of irrigated and rainfed paddy fields. Soil condition is sandy to clay-loamy from the periphery to the center of the lake and no acid sulphate soil was traced. It is bordered on the far eastern and northern sides by high land, and on the far southern and western sides by public roads. These roads have two passages connected by bridges for both inflow and outflow to the south and on the western side respectively (Fig. 1). Two to three rice crops with 120 days maturity period are practiced annually in the swamp and in the surrounding paddy fields. During dry season, five different rice crops are practiced and irrigated from 4 to 5 deep scattered tube wells located in the surface water pumped from the lake itself. Limited amount of fertilizers and pesticides were applied to these crops. The local fish are a variety of wild freshwater species under no management practices until 1993, when 110,000 fingerlings of Indian major carp (*C. catla*, *L. rohita*, *L. calbasu*, *C. mrigala*) and Chinese carp (*Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*) were stocked in addition to the wild species. No supplementary feeding or chemotherapy was done. Fish were caught almost every day using traditional gill nets, bamboo traps, hooks and long lines during the rainy season, and bamboo traps and seine nets during the dry season.

About 20 to 30% of the lake is either occupied by submerged vegetation (mainly *Ceratophyllum* spp, *Najas* sp, *Chara* sp and *Hydrilla* sp) or covered with surface vegetation, predominantly water hyacinth (*Eichornia* sp.).

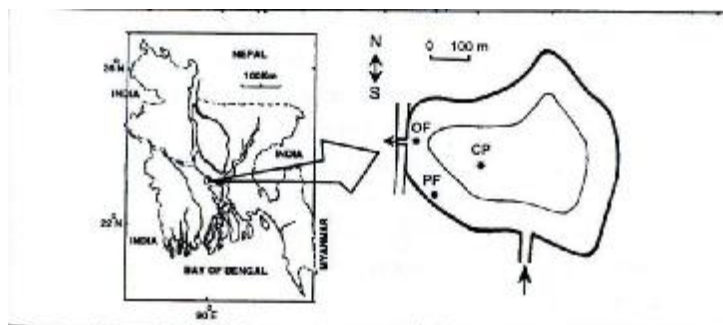


Fig. 1. Map of Bangladesh showing the sampling site and sampling stations: PF, OF, CP; arrows indicate the inflow and outflow of water during the rainy season.

Sampling program

MEASUREMENTS

Three physical parameters (temperature, water depth, secchi disc reading) were recorded and 10 chemical parameters (pH, nitrite, total ammonia nitrogen, unionized ammonia, dissolved oxygen, carbon dioxide, acidity, alkalinity, hardness and chloride) were analyzed using the universal Hach digital titrator Model-16900-01. Water quality parameters were recorded every second week between 700 to 800 h over the 3-year study period. Water samples were taken at about one foot below the surface from two stations: a) in the paddy field (PF); b) in the outflow (OF) during rainy season and for continuation of data during the dry season; c) in the central part (CP) of the lake that was covered when the OF area dried up. (Fig.1). All the water quality and rainfall data were converted to monthly averages (N-35) to test for significant differences (if there is any) over the months relating to the disease outbreaks by ANOVA using the software statistical package, STATISTICA. To monitor diurnal fluctuations of water quality parameters, samples were collected every 4 h for 24 h three times a year starting from April 22, 1988 to March 16, 1991. The values for chloride, alkalinity and hardness from both stations were pooled to see the milli-equivalent ratio of anions to cations. Agricultural management practices, including the application of fertilizer and pesticides were recorded and the climatological parameters, including rainfall and air temperature were collected from an authorized weather station located within 1500 m of the sampling site. Fish samples (live and moribund) were collected from the study site from fishermen every second week. The prevalence of infection was recorded by the presence or absence of gross lesions proportionately according to species sampled at random. Mortalities were recorded by counting dead fish floating on the water surface according to species. Both moribund and apparently healthy fish were preserved in 10% neutral formalin for future reference and some preserved tissue samples were sent to the Aquatic Animal Health Research Institute, Bangkok, Thailand in 1990. From there EUS was confirmed after histological diagnosis. Rahman (1989) was consulted for identification of the fish. The fortnightly water quality data were compiled with monthly averages (N-35), station wise, and pooled for an overview of general water quality.

ANALYSIS OF CHLORIDE IONS AND PHOSPHORUS IN FISH MUSCLES

The fish muscle samples, processed as muscle homogenate, were analyzed for chloride using a direct potentiometric method, Orion model-4074 Specific Ion Analyser meter with chloride ion selective electrode. The chloride concentration in the sample was determined from the calibration curve of a chloride standard solution. Phosphorus was analyzed using a Perkin Elmer (PE) 5000 AAS equipped with a PE 500 graphite furnace. These analyses were done by the Chemistry Division, Bangladesh Atomic Energy Commission Centre, Dhaka.

It was noticed from the general water quality data that fluctuation of chloride concentration was directly related to rainfall. Hence the impact of precipitation was determined by analyzing rainwater collected from a rain gauge, and lake water before and after rainfall in a single day (Table 1).

Results

Gross pathology

Most of the ulcers in affected fishes were found dorso-laterally, over the soft cartilage between the eyes, on the opercular muscles, and in the general caudal region. No ulcers were found on the gills or ventral region.

Behavior of sick fish

Initially, affected fishes displayed red skin lesions. Moderately affected fishes sometimes remained static just beneath the water surface, displaying some trailing fungal hyphae over the gray ulcerations. Severely affected fishes moved aimlessly towards the water surface and later died while floating on the surface upside down.

Occurrence of EUS

The disease was first observed at the site on September 29, 1988 as hemorrhagic lesions in *M. punctata* and *N. nandus*. By early November, 70% of the individuals of affected species were afflicted with initial mortality rates of 55% that gradually declined to 20% by February, although mortalities continued until mid-March (Fig. 2).

In the following season, the disease again started in mid-September, first affecting *Puntius* spp., later the snakeheads and other species. Maximum mortality was 25 to 30%, followed by 17.80% prevalence from mid-November to mid-December and then gradual decline in mortalities to mid-March 1990.

Table 1. Analysis of rainwater and ion proportions.

Parameters	Values	Ion proportions (%)	Lake water chloride concentration		
			Before rainfall	After rainfall	Net increase
Total rainfall duration	58 mm 1200-1330 h				
Temperature	78°F		At 0800 h	At 1600 h	
pH	7		(mg·l)	(mg·l)	(mg·l)
DO	8.25 mg·l				
CO ₂	0 mg·l				
NH ₃ (total)	1.3 mg·l				
Nitrite	0.005 mg·l				
Hardness	4 mg·l	30.76			
Alkalinity	5 mg·l	38.46			
Chloride	4 mg·l	30.76	6	11.8	5.8

From the rain gauge of Faridpur weather station. May 25, 1992

On the third year, lesions were observed from mid-November, with maximum mortality (15%) and prevalence (7%) in the same month, affecting mostly the snakeheads. Later, mortalities declined sharply from 15 to 2% and continued until the end of December.

Fish population

A total of 930 fish comprising 23 species were sampled during the study period (Table 2). Of these, the snakeheads (*C. striatus*, *C. marulius* and *C. punctatus*) were severely affected (30%), followed by *Puntius* spp. (24%), *M. vittatus* (22.22%), *G. giuris* (18%), *X. cancila* (17.14%), *Mastacembalus* spp. (16.30%), *O. pabda* (15.38%), and *A. testudineus* (14.28%). *N. nandus* and *M. cuchia* were severely infected and incurred heavy mortalities during the two initial seasons of the outbreak. Neither *N. nandus* nor *M. cuchia* were found in the third year. *L. calbasu* and *C. mrigala* were rarely affected. The species *C. Catla*, *G. chapra*, *R. daniconus* and *L. guntea* did not display any macroscopic signs of EUS and did not suffer mortalities during the investigation period. Overall, the highest prevalence (30.61%) and mortality were recorded during the first EUS outbreak and declined in subsequent years (17.80% and 7.48% respectively).

Paddy crops management

Generally, there were two paddy crops annually (Aus-April to July, and Aman-August to November) in the surrounding paddy fields. The paddy fields (PF) normally flood during the rainy season between mid-April to mid-October. Only a portion of the plots was placed under the Bangladesh Rice Research Institute (BRRI) BR-4 cultivation (December to March) by irrigating with water from the lake during 1988 and 1989. From November, 1990 almost all of the surrounding lands were brought under BR-4 cultivation. By sinking several deep tube wells around the lake, the farmers were able to plant three paddy crops annually.

Fertilizers and pesticides

The farmers used chemical fertilizers N-P-K in ratios of 10-20-10, two to three times in one crop cycle in the PF and on very few occasions applied Busodin (diazinon) @ 2.47 kg·ha Furadon, (carbofuran) @ 7.41 kg·ha, and Ripcord (cypermethrin) @ 7.41 kg·ha, in small areas of the PF. Ninety

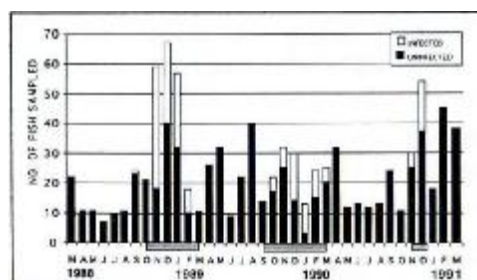


Fig. 2. Prevalence of infection and the outbreak of EUS over the years.

five percent of fertilizers were applied at the BR-4 cultivation, starting from mid-December, and pesticides (if needed) were used from mid-January to mid-February. Applications of these fertilizers and pesticides were done almost after the disease period every year.

Weather parameters

TEMPERATURE

The disease occurred at the onset of declining temperature every year. ANOVA indicated that water temperature was a highly significant ($p < 0.00065$) parameter, inversely correlated with disease outbreaks.

RAINFALL

Rainfall also showed seasonality (Fig. 3). Disease occurred when rainfall waned with the onset of gradual stagnation. ANOVA indicated that rainfall was significantly ($p < 0.021$) less during months of disease outbreaks. The annual precipitation in 1988 to 1990 was 2176, 1635 and 1928 mm, respectively.

Table 2. Fish species sampled and prevalence of EUS (%) over the years.

Fish species	March 1988 to April 1989		May 1989 to April 1990		May 1990 to April 1991	
	Fish sampled	Prevalence (%)	Fish sampled	Prevalence (%)	Fish sampled	Prevalence (%)
<i>*Monopterusuchia</i>	2	100	1	100	0	0
<i>*Nandus nandus</i>	14	86	3	67	0	0
<i>C. punctatus</i>	42	43	27	33	31	16
<i>Channa striatus</i>	35	40	30	30	25	16
<i>Puntius spp</i>	68	34	57	21	37	11
<i>Mystus vittatus</i>	29	31	18	17	16	12
<i>C. marulius</i>	3	33	5	20	7	14
<i>Glossogobius giuris</i>	15	27	9	22	15	67
<i>Xenentodon cancila</i>	12	25	18	11	5	20
<i>Mastacembelus spp</i>	40	18	29	21	23	9
<i>Ompok pabda</i>	4	25	2	50	7	0
<i>Anabas testudineus</i>	9	33	7	14	12	0
<i>Labeo calbasu</i>	3	0	1	0	5	20
<i>Clarias batrachus</i>	3	33	8	13	12	0
<i>Heteropneustes fossilis</i>	6	17	8	13	9	0
<i>Mystus tengara</i>	17	12	9	0	11	9
<i>Cirrhinus mrigala</i>	3	33	2	0	8	0
<i>Mystus aor</i>	5	20	2	0	7	0
<i>Colisa fasciatus</i>	11	18	21	5	10	0
<i>Rasbora daniconius</i>	7	0	11	0	18	0
<i>Lepidocephalus guntea</i>	10	0	12	0	19	0
<i>Gadusia chapra</i>	3	0	8	0	6	0
<i>Catla catla</i>	2	0	4	0	11	0
Total	343	31	292	18	294	7

Fish species are listed in accordance with the level of infection.

*Species not found in the last sampling season.

The Secchi disc was visible through the water column at both stations at all times. The nitrite ranged from 0.005 to 0.2 mg⁻¹ throughout the study period. The unionized ammonia ranged from 0.72 to 0.89 mg⁻¹. On one occasion it reached up to 1.32 mg⁻¹.

The pH fluctuated between 6.8 and 10 throughout the study period with greater fluctuations at the outflow (OF) than in the PF. The OF also had higher acidity (> 30 mg⁻¹) during the dry season than the PF. The DO and CO₂ from both stations were recorded with variable ranges (1 to 7.8 mg⁻¹ and 0 to 37 mg⁻¹ respectively).

Chloride concentration dropped below 5 mg⁻¹ from June to September 1988; July and August 1990; and around 6 mg⁻¹ from July to October 1989 (Fig. 4). All of these decreases coincided with subsequent EUS outbreaks and were inversely correlated with the length of the outbreak period, as well as the severity of disease (mortalities).

The ANOVA indicated that chloride concentrations were significantly ($p < 0.0451$) different during the months relating to the disease outbreaks. Total ammonia fluctuated more in the PF than at the OF, with a maximum of 1.2 mg⁻¹ in August 1990. The data also indicate that during the period of low chloride concentrations, proportionately higher total ammonia persist.

At both stations (Fig. 5) beginning March 1988 hardness (67 to 124 mg⁻¹) was usually greater than alkalinity (69 to 90 mg⁻¹) until March 1989. At PF, both parameters fluctuated similarly with usually higher hardness from April 1989 up to the end of the study period except June to September 1989 and January to February 1990. At OF from April 1989 until the end of the study period, both parameters fluctuated similarly with alkalinity (82 to 204 mg⁻¹) usually higher than hardness (83 to 173 mg⁻¹). ANOVA indicated that alkalinity significantly ($p < 0.043$) fluctuated monthly, and was inversely correlated with the disease outbreaks at PF.

At the beginning of the study period an unusually high flood inundated the lake. The addition of excess ground water soaked the surrounding paddy fields at the end period. When the milliequivalents of total anions (alkalinity + chloride) and hardness were compared, cations (hardness) predominated over the anions (alkalinity + chloride) before the EUS outbreaks in every season (year) at both stations (Fig. 6).

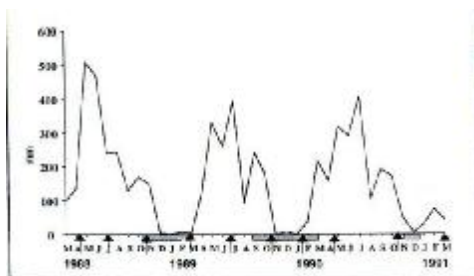


Fig. 3. Monthly rainfall pattern over the years and EUS outbreaks, arrows indicate 24h sampling dates (1-10).

DIURNAL WATER QUALITY PARAMETERS

Altogether, ten 24h samplings were carried out. The first sampling was confined to PF and the rest were done at both stations.

Low to moderate acidity with almost normal trends and concentrations of pH were recorded from both stations. Oxygen was generally above 3 mg⁻¹, except in the predawn hours in April, while CO₂ was generally below 5 mg⁻¹. A moderate concentration of total ammonia was also recorded from both stations except in April. DO and pH were more or less normal throughout the whole study period. Chloride levels were below 5 mg⁻¹ in July 1988 and in November 1990. By contrast, chloride levels were between 10 to 30 mg⁻¹ in March 1991 after tube well irrigation of the paddies. Alkalinity and hardness fluctuated similarly throughout

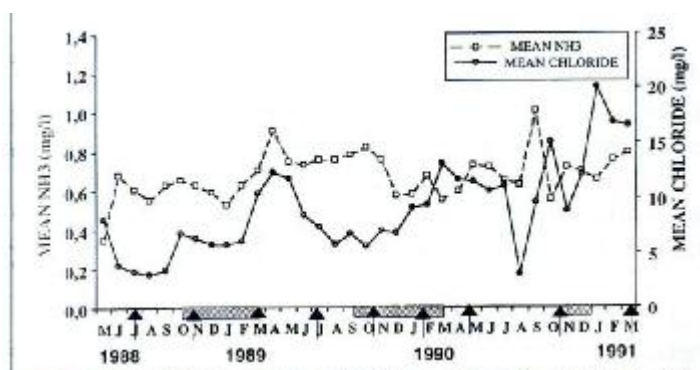


Fig. 4. Mean values of chloride and NH₃ from both stations and the occurrence of EUS over the years, arrows indicate 24h sampling dates (2-10).

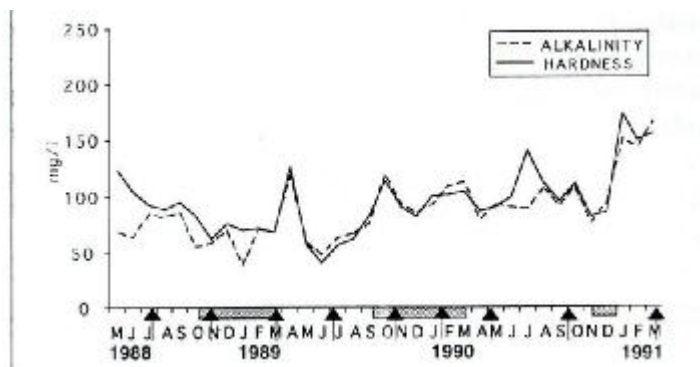


Fig. 5. Variations of alkalinity and hardness at PF and the occurrence of EUS over the years, arrows indicate 24h sampling dates (2-10).

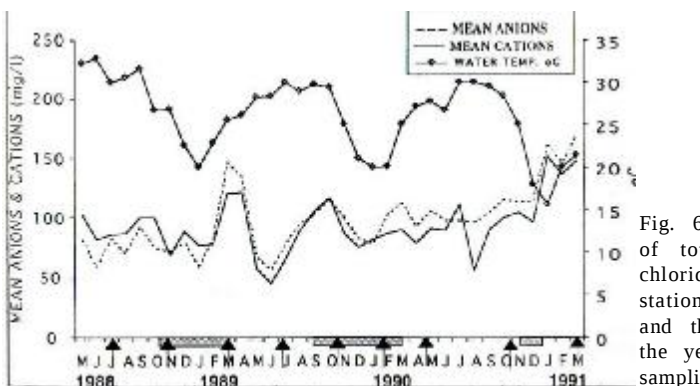


Fig. 6. Variations of mean values of total anions (alkalinity + chloride) and cations from both stations, the water temperature, and the occurrence of EUS over the years. Arrows indicate 24h sampling dates (2-10).

the sampling except in April and November 1988, March and July 1989. Overall, the diurnal data showed clear fluctuations with marked variations of proportions only among the ions (alkalinity, chloride and hardness).

ANALYSIS OF IONIC PROPORTIONS

The ionic proportions data indicate lower chloride concentration in April (4%) and July (2%) 1988; and November (4%) 1989 and 1990 from PF (Fig. 7a). This coincides with the subsequent EUS outbreaks every year. At the same time, a declining trend of alkalinity in 1988, and in July and May 1989 and 1990, as revealed from the mean ionic proportions, also significantly correlated with the outbreaks (Fig. 7b).

ANALYSIS OF RAINWATER

Rainwater chloride was only 4 mg⁻¹ (30.76% of the ions) while in the lake water chloride contributed a maximum of 7% of the ions throughout the study period (Table 3). The total anions were 69.22% compared to the total cations (30.76%). It indicates that every 10 mm rainfall added 1 mg⁻¹ of chloride to the lake water. If there had been sufficient cations, then these free chloride ions could have been lost in the form of CaCl₂, MgCl₂ and so on.

ANALYSIS OF MUSCLE CHLORIDE AND PHOSPHORUS

The analysis revealed that during disease outbreaks a considerable depletion of chloride occurred both in the water as well as within the fish muscle (Table 3). Healthy fishes had higher muscle chloride (46.3 mg·kg) than infected fishes (343 mg·kg).

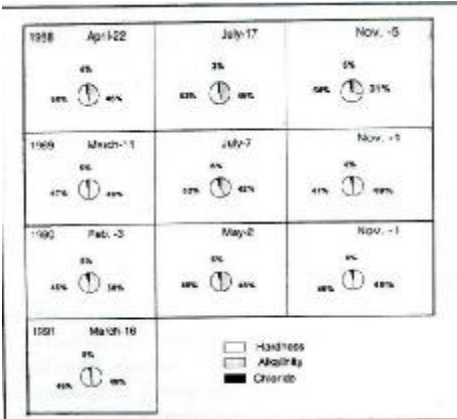


Fig. 7a. The ion variations (proportions) over the seasons (years) at PF.

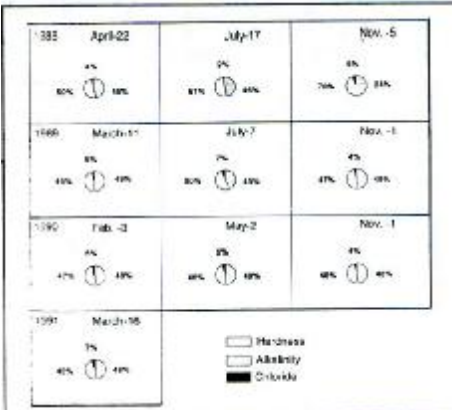


Fig. 7b. The ion variations (proportions) over the seasons (years) as a whole in the lake water.

Discussion

Nineteen of the 35 indigenous species in the lake were affected by EUS over three seasons. During the first season, the disease affected a wide variety of species of all ages, incurring higher prevalences and mortality than in the subsequent seasons. Among the carps, *L. rohita* and *C. mrigala* were infected only at peak periods, while *C. catla* and three other species were never affected. Similar observations on major carps have been reported from southern India (Vishwanath et al. 1997). The variations of fish species susceptibility to EUS pathogen *A. invadans* was further studied by Khan et al.1998.

At the site, significant EUS infections among the middle and bottom dwelling carnivorous fishes were noted. Llobrera and Gacutan (1987) also reported EUS infection among the bottom dwelling fishes (mud fish/snakeheads *O. striatus*, *C. batrachus*, *G. giuris* etc.) in the Laguna de Bay, Philippines. These variations may be due to variation in susceptibility and resistance of the species or the affected fish species might congregate near the pathogenic agents. Boyd (1990) reported higher salinity tolerance in *C. catla* (12,000 mg⁻¹) and *L. rohita* (9,000 mg⁻¹), which may explain the low rate of infection in these species.

In the present study EUS occurred between October to March, with peaks from November to-December every year. Such seasonality is also in agreement with other reports (Vishwanath et al. 1997). There was no correlation between agrochemicals (fertilizer and pesticides) and EUS outbreak.

Our data indicate that a few weeks of rainfall and the onset of declining seasonal temperature from mid-September with maximum daily fluctuations from October to February each year were closely correlated with disease outbreaks. ANOVA showed that both rainfall ($p < 0.0214$) and temperature ($p < 0.00065$) were highly significant, and inversely related to EUS outbreaks. The fluctuations in water temperature (10°C in a single day) could have contributed to the overall environmental stress. Salinity and temperature changes have also been suggested as predisposing conditions of RSD in estuarine fishes of Australia. It was reported that 72% of EUS outbreaks occur during the dry season and normally stopped at the onset of the next rainy season.

Table 3. Analysis of fish muscle chloride, its relationship to the simultaneous collection of habitat water, and the variation in phosphorus both before and during outbreaks.

Fish species	Sampling date-25/8/91 Non diseased				Sampling date-19/1/92 Diseased period						
	Healthy fish				Infected fish				Uninfected fish		
	Chloride		Phosphorus		Chloride		Phosphorus		Chloride	Fish muscle	Phosphorus
	Habitat water	Fish muscle	gm·kg		Habitat water	Fish muscle	gm·kg		Habitat water	Fish muscle	gm·kg
	mg·l	gm·kg	Tail	Head	mg·l	gm·kg	Tail	Head	mg·l	gm·kg	Tail
<i>C. punctata</i>	12	1.16	0.72	1.39	6	0.53	1.45	7.29	-	-	5.75
<i>Puntius</i> sp	12	0.73	-	-	6	0.34	-	-	6	0.46	-

Immediately after the flood in 1988, there was a considerable decline in chloride concentrations in addition to declining alkalinity. Raman (1992) also suggested declining salinity as one of the major causes of EUS outbreaks in Chilka lagoon, India, when it received a heavy influx of inland waters. The increased concentrations of acidity and CO_2 , particularly during the winter and during late night and early morning hours in other periods might be due to the heavy growth and decomposition of aquatic weeds in the littoral zone of the lake. Variations in rates of photosynthesis might have also contributed to higher CO_2 and DO (Boyd 1990). However, most fish species are assumed to be able to survive in waters containing $60 \text{ mg}^{-1} \text{ CO}_2$ or more, provided DO concentrations are high (Hart 1944). Higher ammonia in decreased DO level late at night and early in the morning could have been more toxic to fish (Alabaster et al. 1979), causing the fish to be stressed and osmotically imbalanced. Under such conditions, with low chloride levels in the water, fish might be more vulnerable to EUS. High rainfall correlates with decreased alkalinity and increased hardness. Schindler (1991) also reported that precipitation of acids can have two effects on surface waters: a) depletion of the acid neutralizing capacity and b) increasing $\text{Ca}^{2+} + \text{Mg}^{+}$ quantity. Chloride ($p < 0.045$) and alkalinity ($p < 0.043$) were the only factors that significantly correlated with EUS outbreaks in each season. Immediately after a few weeks of rain, hardness predominated over alkalinity and simultaneously a decrease in chloride concentration in the water. Increased noncarbonate hardness and depletion of chloride and alkalinity might have caused a concomitant decline in chloride concentration within the fish. If there had been sufficient free chloride in the water, this could compensate in the form of CaCl_2 , MgCl_2 before affecting the fish physiologically (Bonga 1997). Therefore, with lower cation concentration, free chlorides could play a significant role in controlling or preventing EUS.

Our preliminary data on fish muscle chloride as well as that of the habitat waters may indicate that prolonged water quality changes eventually affect the range of metabolic and physiological compensation processes in the fish (Bonga 1997), or through changes in osmoregulatory capacity. It has been reported that at low temperature the cellular and humoral defenses of fish are reduced, allowing bacteria to multiply. However, in the present study chloride and alkalinity both fell below 50% with respect to hardness. The onset of declining temperature with large daily fluctuations was correlated with EUS outbreaks. In July 1988, when chloride and alkalinity were low, there was no EUS outbreak. This may be due to the persistently higher temperature at that time. Later, as the temperature declined from mid-September 1988, EUS occurred within 2 to 3 weeks. A similar syndrome of chronic dermatitis lesions occurs under almost similar environmental conditions (lower salinity and temperature) in the estuarine fish *M. cephalus* infected with RSD, as has been reported by Callinan and Kepp (1989).

Our data and simultaneous field observations of fish catches indicated that when hardness was less than 50% with respect to chloride and alkalinity (together) and chloride concentration increased more than 4%, such prevailing

water quality was free from EUS and seemed to be favorable for fish growth. Most likely because of these reasons when infected fishes were transferred to tap water having chloride concentration $> 10 \text{ mg}^{-1}$ with normal alkalinity (90 mg^{-1}) and hardness (95 mg^{-1}), they were healed of ulcers within two weeks (Barua, pers. comm.). A similar quick recovery of EUS infected fish, when put in cleaner water, was also reported by Balasuriya et al. (1990). The annual rainfall data indicates that there were no major variations in total precipitation during EUS outbreak seasons. However, the only sources of chloride ions in the natural waters are rain or ground water. Boyd (1990) also reported that chloride ions were likely of atmospheric origin, and ground water generally contains more ions than surface waters. Schindler (1988) reported that the ratio of acid neutralizing capacity- $\text{ANC}/(\text{Ca}+\text{Mg})$ declines under the influence of acid precipitation, and instead of normal values near 1, ratios of 0.6 or less are reported in areas of high acidic precipitation. The pathogens consistently involved in EUS are: *A. hydrophila*, *A. sobria*, chemoautotrophic nocardioform bacteria, *Aphanomyces* fungus and rarely rhabdovirus, birnaviruses or reovirus. None of them are reported to be the primary pathogens of EUS in the entire region (Mohan and Shankar 1994).

Conclusion

The occurrence and seasonality of EUS implies some common factors in all regions. Our study indicates that aquatic ion ratios may be involved. The susceptibility or resistance of some species to EUS may be related to their physiological capacity to cope with environmental changes.

We speculate that rapidly declining seasonal temperature and changing water quality, particularly lower chloride and alkalinity with respect to hardness, might cause severe stress, depress immunity, reduce resistance to pathogens and eventually cause a breach of the skin. This provides a pocket for opportunistic and secondary pathogens like viruses, bacteria, fungi and protozoa and may result to EUS.

Further field studies have indicated therapeutic benefits in healing skin lesions provided by lime and salt. This should be followed up by controlled experiments. A study (Lilley and Inglis 1997) further points to this idea.

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