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Preliminary Observations on the Effects of Lime and Common Salt as Prophylactics and Therapeutics Against Epizootic Ulcerative Syndrome in Fish Culture Ponds

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Abstract

To control epizootic ulcerative syndrome (EUS) in fish, pond treatment using quick lime (CaO) combined with common salt (NaC1) was tested in seven experimental ponds at Gazipur and Faridpur, Bangladesh. Basic water quality parameters were monitored before and during treatments. Therapeutic treatments using lime (CaO) either at 0.005 or 0.01 ppm or lime plus common salt at 0.005 ppm each gave unsatisfactory or delayed results. Simultaneous treatments using both chemicals at 0.01 ppm each improved water quality parameters (pH, acidity, alkalinity, hardness and chloride), and led to healing of lesions of affected fishes after fifteen days of application. The same dosages used as prophylactics were applied as field trials just before the predicted outbreak and no fish with lesion was observed. Follow up field trials in two subsequent seasons have also indicated the effectiveness of the present doses and treatments. These techniques are disseminated as adaptive measures through government extension agents and are being practiced in semi-intensive fish culture ponds in Bangladesh to combat EUS.

Introduction

Epizootic ulcerative syndrome has been a recurring regional fish health problem since 1980 (Tonguthai 1985) and has become a major threat to freshwater fishes in Bangladesh since 1988 (Sanaullah et al. 1997). The overall incidence varies from 15 to 70% with the highest percentage in

Channa and *Puntius* (Das and Das 1993). The primary etiological agent of the disease is the fungus *Aphanomyces invaderis* (Willoughby et al. 1995), forming typical mycotic granulomas in the affected tissues with external lesions in the affected fish (Callinan et al. 1995, Sanaullah et al. 1997). Early events in the pathogenesis and environmental factors which predispose the fish to the disease are largely unknown (Mohan and Shankar 1994) although indications exist that EUS is commonly preceded by decreasing alkalinity, water hardness and chloride concentrations and significant diurnal fluctuations in temperature (Lilley et al. 1992), especially lower water temperature (Chinabut et al. 1995).

Several efforts in different countries of the region have been undertaken for both prophylactic and therapeutic treatments of EUS by applying lime (CaO), common salt (Nac1), potassium permanganate (KMnO4), bleaching powder (HClO), malachite green and antibiotics (Lilley et al. 1992, Das and Das 1993), but with little success (Mohan and Shankar 1994). Longer term environmental studies indicate that stable alkalinity (> 80 mg^{-1}) with increased concentration (> 10 mg^{-1}) of chloride should be maintained simultaneously (Sanaullah unpubl. data). Manipulation of chloride ions in the environment to prevent nitrite toxicity have been practiced elsewhere (Perrone and Meade 1977; Tomasso et al. 1980). In this study lime (CaO), a safe ingredient used in food fishes (Schnick 1991) was chosen to alleviate any alkalinity deficiency, while common salt containing 60% chloride was chosen to remedy low chloride concentrations in the ponds (Boyd 1990). This paper presents the preliminary observations on the experiments conducted in seven earthen ponds at Faridpur, (180 km southwest of capital Dhaka) and at Gazipur (25 km to the north of Dhaka City), Bangladesh from December 1989 to March 1991. The results of two follow up field trials are also included.

Materials and Methods

Pond description, fish stocking and management

Seven earthen ponds ranging from 1.0 to 3.5 m in depth were selected. Ponds P1, P2 and P4 are located at Baitul Aman, Faridpur, while Ponds P3, P5, P6 and P7 are at the Dhaka Fisheries Ltd. (DFL) in Gazipur, Bangladesh. Details are listed in tables 1 and 2.

Ponds P1, P2 and P4 are village rain-fed ponds with sandy-loamy soils. Wild fish are encouraged to enter these ponds during each rainy season by cutting a channel through the embankment. No feed, fertilizer nor management techniques were applied in these ponds. A huge mass of decomposing water hyacinth *Eichornia liguipes* was buried in the tree shaded embankments of P2.

Ponds P3, P5, P6 and P7 were constructed using highly acidic soils and were initially prepared by applying agricultural lime at 200 $kg \cdot ha^{-1}$. The fish in these ponds were stocked at variable proportions of species. The species composition is given in table 3.

Control pond

Of the seven ponds, only pond P1 at Faridpur was maintained as a control because other than the experimental ponds, the remaining culture ponds at DFL, Gazipur and the surrounding ponds as well as the whole open water areas at Faridpur were all affected by EUS at the same time. Therefore, it was deemed unnecessary to maintain specific control ponds at both experimental sites. This was also the case with prophylactic treatments in field trials FT 1 and FT 2 where the untreated ponds were affected by EUS.

Outbreak seasons

The experiments in ponds P3 and P5 were carried out from 1989 to 1990 (season A). The outbreak period during that season was from 10 November 1989 to 07 February 1990. In ponds P1, P2, P4, P6 and P7 the experiments were conducted in season B (total outbreak period - 10 November 1990 to 15 January 1991). Specific dates are given in tables 1 and 2. Two follow up field trials were conducted during the two subsequent outbreaks; season C (January to February 1992) in Gazipur and season D (December to January 1993) in Jessore. The peak disease period during season B in Faridpur was in the beginning of December while in Gazipur (DFL), it was by the end of December.

Diagnosis of EUS

The disease was diagnosed by the presence of typical mycotic granulomas in the histological sections of the affected tissues. This was also confirmed from AAHRI, Bangkok from 1989 to 1990.

Analyses of water quality parameters

Transparency, water depth, water temperature, and nine chemical parameters (pH, nitrite, total ammonia, dissolved oxygen, carbon dioxide, acidity, alkalinity, hardness and chloride) were analyzed. Water chemistry was analyzed using the universal Hach digital titrator model-16900-01. Water quality was analyzed before and during treatments (Tables 1 and 2).

Application of chemicals

Quick lime was initially mixed with water and was spread all over the pond using a hand jug after cooling, while common salt was applied through broadcast over the whole pond area. Pond P1 was untreated, P2 was treated with 0.005 ppm lime, P3 with 0.01 ppm lime, P4 with 0.005 ppm lime and 0.005 ppm salt. Ponds P5, P6 and P7 were treated with 0.01 ppm lime and 0.01 ppm salt. The chemicals were applied only once in each pond between 0800 to 1000 h. In ponds P2, P3, P4 and P5 the chemicals were applied after the appearance of the disease, while in ponds P6 and P7 the chemicals were applied just before the predicted outbreak period.

Table 1. The experimental ponds, their sizes. seasons A and B, location, doses, dates with water quality parameters, fish stocking density, fertilization, supplemental feeding and the number of grazing ducks, if there is any.

Pond size (ha). location and doses (ppm)	P1-BF (0.14) Untreated		P2-BF (0.08) Lime 0.005		P3-A DFL-P2- (0.17) Lime 0.01		P4-BF (0.26) Lime+Salt 0.005+0.005	
Water analysis	Day 0	Day 60	Day 0	Day 45	Day 0	Day 35	Day 0	Day 35
Dates	30 Sept. 90	01 Dec. 90	02 Nov. 90	17 Dec. 90	22 Dec. 90	26 Jan. 90	13 Nov. 90	27 Dec. 90
Time	0630h	0645h	0630h	0810h	0845h	0745h	0630h	0645h
Transparency (cm)	70	60	10	40	14	15	55	30
Ave. water depth (m)	1.98	1.52	2.44	2.74	1.83	1.71	3.68	3.05
Water temp. (°C)	31	23.33	25.56	25.56	20	28.33	29.44	26.11
Nitrite (mg^{-1})	0.01	0.01	0.01	0.04	0.01	0.01	0.20	0.01
Acidity (mg^{-1})	8.60	8.60	4.00	18.00	3.10	16.60	18.00	9.00
CO_2 (mg^{-1})	7.60	8.00	2.20	9.90	13.60	14.60	9.89	4.95
pH	8.0	7.6	7.5	7.8	8.6	7.2	7.8	7.8
NH_3 (total) (mg^{-1})	0.9	0.7	0.7	1.3	0.4	0.5	0.6	0.8
Hardness (mg^{-1})	73	86	105	92	101	101	91	94
Alkalinity (mg^{-1})	165	74	104	118	140	126	85	96
Chloride (mg^{-1})	6.3	6.9	41.0	48.0	3.1	4.8	10.0	9.0
Stocking density	*		*		12500-15000 ha^{-1}			
Fertilization	None		None		Cow dung 370 $kg \cdot ha^{-1}$		None	
Supplemental feeding	None		None		Oil cake + Wheat bran @1.2-2% body wt. daily		None	
Grazing ducks	6-8		50-60		None		8-10	

*Wild fish

Table 2. The experimental ponds, their sizes seasons A and B, location, doses, dates with water quality parameters, fish stocking density, fertilization, supplemental feeding and the number of grazing ducks, if there is any. A range of desired water quality parameters, that seemed ideal considering Bangladesh perspectives.

Pond size (ha). location and doses (ppm)	P5-A DFLP 22 -(0.07) Lime+Salt 0.01+0.01		P6-B DFLP4 -(0.13) Lime+Salt 0.01+0.01		P7-B DFL-P10 -(0.07) Lime+Salt 0.01+0.01		Conditions that seem ideal
Water analysis	Day 0	Day 35	Day 0	Day 21	Day 0	Day 21	-
Dates	22 Dec. 89	26 Jan. 90	11 Dec. 90	02 Feb. 90	11 Dec. 90	02 Feb. 91	-
Time	0845h	0745h	0745h	0730h	0745h	0730h	-
Transparency (cm)	17	11	20	16	18	14	10-20
Ave. water depth (m)	1.22	1.37	1.07	1.06	1.29	1.80	1.3-2.5
Water temp. (°C)	20	28.33	23.33	26.11	23.33	26.11	29.44
Nitrite (mg^{-1})	0.02	0.10	0.015	0.01	0.01	0.01	> 0.015
Acidity (mg^{-1})	2.5	9.3	0	1.1	0	2.0	< 5
CO_2 (mg^{-1})	10.8	8.2	0	1.8	0	1.9	< 7
pH	7.1	7.9	8.6	8.8	7.6	8.9	8.0-8.5
NH_3 (total) (mg^{-1})	0.6	0.7	0.7	0.6	0.6	0.6	< 0.6
Hardness (mg^{-1})	34	70	54	82	78	95	75-115
Alkalinity (mg^{-1})	48	79	67	115	90	133	85-125
Chloride (mg^{-1})	5.3	13.8	10.0	11.8	8.0	13.2	> 10
Stocking density	12500 - 15000 ha^{-1}		12500 - 15000 ha^{-1}		12500 - 15000 ha^{-1}		
Fertilization	Cow dung 370 kg·ha^{-1}		Cow dung 370 kg·ha^{-1}		Cow dung 370 kg·ha^{-1}		
Supplemental feeding	Oil cake + Wheat bran @1.2-2% body wt. daily		Oil cake + Wheat bran @1.2-2% body wt daily		Oil cake + Wheat bran @1.2-2% body wt. daily		
Grazing ducks	None		None		None		

Fish monitoring

The fish were externally diagnosed according to the appearance of typical EUS lesions. Before treatment, the fish in each pond were sampled using beach seine or cast net and the number of fish with external lesions was noted. On days 15, 35, 45, 55 and 60, fish were sampled. The number and sizes of lesions on each fish and the fish species affected were noted. Detailed fish monitoring data and dosages are given in table 4. Ponds P1, P2 and P4 were finally harvested using seine, while ponds P3, P5 P6 and P7 were drained and harvested at the end of the culture periods.

Table 3. Wild fish species reared mainly in village ponds, cultured species at DFL ponds, Field trials (FT) 1 and 2. The most susceptible species to EUS are marked with asterisks.

Village ponds F P1, P2 and P4	DFL ponds p3, P5, P6 and P7
**Channa stratus*	*Catla catla*
**C. marulius*	*Labeo rohita*
**C. punctatus*	**Cirrhinus mrigala*
**Puntius spp.*	*Hypophthalmichthyes molitrix*
**Mastacembalus armatus*	*Ctenopharyngodon idella*
**M. pancala*	**Puntius gonionotus*
**Nandus nandus*	*Cyprinus carpio*
**Colisa fasciatus,*	
**Glossogobius giuris*	FT-1, DFL P25
Xenentodon cancila	
Mystus aor	FT-2, Private pond, Jessore
M. vittatus	*C. catla*
M. tengra	*L. rohita*
**Anabas testudineus*	*C. mrigala*
Clarias batrachus	*H. molitrix*
Heteropneustes fossilis	**P. gonionotus*
Rasbora daniconus	
Lepidocephalus guntea	

Table 4. The number of EUS affected fish with skin lesions from experimental ponds (P1 to P7) and field trial ponds (Field trials 1 and 2) against post treatment days. The total number of fish sampled are given in parenthesis. Seasons A,B,C and D.

Pond number, seasons and dosage (ppm)	Days after initial treatment					
	0	15	35	45	55	60*
P1-B, Control	8(21)	16(36)	3(23)	1(18)	1(31)	0(39)
P2-B, Lime 0.005	12(31)	7(18)	2(13)	3(23)	1(31)	0(36)
P3-A, Lime 0.01	6(41)	2(15)	1(19)	0(38)	-	-
P4-B, Lime+Salt 0.005+0.005	11(42)	7(26)	0(32)	-	-	-
P5-A, Lime+Salt 0.01+0.01	19(164)	0(59)	0(43)	0(39)	-	
P6-B, Lime+Salt 0.01+0.01	0(51)	0(38)	0(53)	0(46)	-	-
P7-B, Lime+Salt 0.01+0.01	0(56)	0(51)	0(38)	0(47)	-	-
FT1-C, Lime+Salt 0.01+0.01	29(87)	0(121)	0(143)	0(167)	-	-
FT2-D, Lime+Salt 0.01+0.01	10(53)	0(51)	0(37)	-	-	-

*Disease period was over.

Follow up field trials

Field trial 1 was conducted between 20 February to 03 March 1992. The pond was initially prepared with lime according to standard farmers' practice (Table 5). During the monoculture of *Clarias gariepinus*, supplementary feeds were provided occasionally as a mixture of livestock blood, wheat/rice bran plus 792 kg soaked mustard oil cake, 4,884 kg poultry litter and 51 kg urea as fertilizer for the entire seven month culture period. After observing skin lesions in netted samples on 20 February 1992, lime and salt at 0.01 ppm each were applied on the same day without prior analysis/assessment of the water quality parameters. Saudi Bangla Fish Feed Ltd. (SBFFL) grower feeds at 2% body weight were given six days in a week as a supplement.

Field trial 2 was conducted in a private pond at Monirampur, Jessore. The pond was used for the polyculture of five species (Table 5). Urea and Triple Super Phosphate were applied at 75 and 50 $kg \cdot ha^{-1}$ respectively, twice a month during summer and once in two months during winter. Fresh cowdung, 10 to 15 kg at a time, from a nearby cowshed was also applied occasionally in the morning. On 17 January 1993 skin lesions were first noticed only on *P. gonionotus*. Lime and salt (at 0.01 ppm each) were applied on 19 January without prior assessment of the water quality parameters. No extra feeding with mustard oil cake plus wheat bran at 2 to 3% body weight was done.

POND 1-B

Skin lesions were noted in this untreated pond on 10 November and continued to coincide with the infection pattern among the wild fish in that area. During the outbreak period there was a trend of reduction in alkalinity (165 to 74 mg^{-1}), static chloride (6.3 to 6.9 mg^{-1}), increase in hardness (73 to 86 mg^{-1}), and lowering of pH (8.0 to 7.6) (Table 1).

POND 2-B

Fifteen days after application of 0.005 ppm lime the fish (*Channa striatus*, *Puntius* spp., *Colisha* sp and *Mastacembalus* spp.) had moderate lesions. After 45 days chloride and alkalinity remained the same (Table 1) with lower hardness and higher NH_3. Disease conditions continued until 15 January 1991.

POND 3-A

After application of quick lime at 0.01 ppm, no notable change in water quality was achieved except for a slight increase in chloride concentration (3.1 to 4.8 mg^{-1}). After 15 days of treatment some of the *C. mrigala* and *P. gonionotus* showed healed lesions but a few lesions persisted on some of the *P. gonionotus* and continued until the end of the disease period (07 February 1990).

Table 5. Fish rearing, pond management and production data of field trial ponds 1 and 2 to control EUS. FT1-C (season), DFL-P.25 Gazipur, Bangladesh.

Pond size (ha)	Species (cm) and stocking density	Pond preparation with lime (CaO)	Application of Lime + Salt with date	Supplemental feeding	No. of fish	Ave. wt. (kg)	Total wt. (kg)	Total rearing period and survival (%)	Production ha^{-1} (ton)
0.12	*Clarias gariepinus* (75-100)	30 kg on dry bottom	@ 0.01 + 0.01 ppm on 20 Feb. 92	SBFFL feed @ 3-5% body wt. 5 days in a week	85	0.223	19	7 months	
				Total 5607 kg	2117	0.546	1156		
	10.000				4048	0.601	2432		
				Grand total	6250	0.577	3607	62.50%	29.70

FT2-D (season), A. Rouf's pond, Nehalpur, Monirampur, Jessore, Bangladesh.

Pond size (ha)	Species (cm) and stocking density	Application of Lime + Salt with date	Supplemental feeding	No. of fish harvested with (Ave. wt. in kg)		Total no. of fish and ave. wt. (kg)	Total rearing period and survival (%)	Production ha^{-1} (ton)
0.15	2600	@ 0.01+0.01 ppm on 19 Jan. 1993	Mustard oil cake + wheat bran 2:1 @ 3% body wt. 5-6 days in a week	Year 1	Year 2		19 mos	19 mos
				14 March to 27 April 93	22 April 1994			
	C. catla (45-65)			23 (0.78)	135 (1.35)	158 (1.27)		
	L. rohita (40-55)			57 (0.47)	197 (1.04)	254 (0.91)		
	C. mrigala (35-45)			45 (0.35)	153 (0.90)	198 (0.78)	0.577	3607
	H. molitrix (60-75)			84 (0.68)	259 (1.40)	343 (0.93)		
	P. gonionotus (35-50)			409 (0.20)	137 (0.31)	546 (0.23)	54.60	
					Grand total	1499 1029 kg	57.65	6.70

POND 4-B

Lime and salt at (0.005 and 0.005 ppm) were applied at a time when fish (*Channa* spp., *Puntius* spp., *Mastacembalus* spp. and *Glossogobius giuris*) were already affected. After 15 days, healing of lesions was observed in some of the snakeheads, particularly those with small lesions. *Puntius* spp., *G. giuris*, *Mystus* sp and some snakeheads still displayed lesions, but the color changed from reddish to pinkish. By day 35, no fish with lesions was found, but some had conspicuous healing traces (Fig. 1A). A slight increase in alkalinity (85 to 90 mg^{-1}) with more or less unchanged hardness and chloride concentration was recorded.

POND 5-A

Seven days after treatment with lime and salt at 0.01 ppm each, the lesion sizes were reduced in *Labeo rohita* (2.5 to 2.0 cm), *C. mrigala* (3.5 to 2.5 cm) and in *P. gonionotus* (3.5 to 2.5 cm) (Fig. 1B). After fifteen days, most of the fish showed traces of healing and no fish had extensive lesions. After 35 days, alkalinity had increased to 79 mg^{-1}, chloride to 13.8 mg^{-1} and hardness to 70 mg^{-1} (Table 2).

POND 6-B AND POND 7-B

Lime and salt 0.01 ppm each were applied before the outbreak period. There was no fish with skin lesions during the usual EUS outbreak period (Table 4). Improvements in water quality parameters including pH, were noted (Table 2).

Field trial 1

Seven days after treatment, the initial lesions (Fig. 1C) were reduced (2.25 to 0.75 mc) while some were healed (Fig. 1D). After 15 days when the whole pond was seined, not a single fish with skin lesions was observed, although healing traces were noted on a few specimens. Total production during the seven month period was 360 kg and survival rate was 62.50%.

Field trial 2

Fifteen days after treatment, the whole pond was seined. No infected fish was observed, although a few healing traces were noted on some of the previously infected *P. gonionotus*. Total production in this pond was 6.7 $ton \cdot ha^{-1}$ after 19 months with 54.60% survival of the EUS affected species *P. gonionotus*.

Results

During the study, a combination of quick lime and common salt at 0.01 ppm each seemed effective in correcting the water quality in pond situation.

Lime alone at 0.005 or 0.01 ppm provided only a little improvement in the alkalinity, possibly due to acid soil characteristics. From their extensive survey, Bhaumik et al. (1991) reported that treating EUS-affected ponds only with lime could give a maximum of 68% positive results. Low doses of a combination of lime and salt resulted to a slow rate in healing skin lesions. We found that treatment with 0.01 ppm of lime and salt resulted to a 100% healing rate by 15 days of application. This also improved the water quality as observed in pond P5. There was no infected fish at Day 0 in ponds P6 and P7 that were treated with lime and salt at 0.01 ppm each. Schnick (1991) reported that sodium chloride and calcium chloride salts at 0.5 to 1% can be applied for an indefinite period as osmoregulatory enhancers for fish. These data indicate that higher anions (> 55) in comparison to the cations as hardness (< 45%), enhance fish health as previously observed (Sanaullah unpubl. data).

The heavy decomposition of water hyacinth, and addition of large amounts of duck manure daily directly into the pond water, could have caused the higher ammonia level. The reason for higher chloride in pond P2 was unknown. Such factors together or individually at a certain level of parameter may further worsen the EUS condition in freshwater pond conditions. The presence of higher ammonia during the disease outbreak was obvious, particularly when the chloride level decreased below 7 mg^{-1} as observed in ponds P1 and P3. At the same time with higher chloride, normal alkalinity with low concentrations of hardness may not be solely compatible for a normal fish if higher ammonia persists there as observed in pond P2, which requires further study. In the untreated pond the changes in important water quality

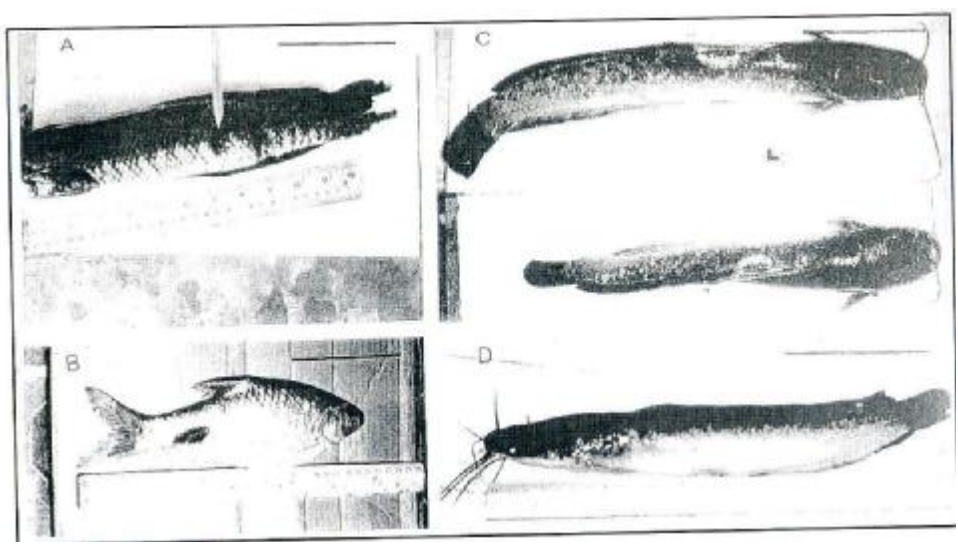

Fig. 1. (A) Concave traces of healed lesions at the caudal region of *Channa striatus* from pond P4-B on 27 Dec. 1990. (B) Reduced lesion with pinkish color on the lateral side of *Pontius gonionotus* from Pond P5-A on 27 Dec. 1989. (C) Infected *Clarias gariepinus* from DFL, P-25 before treatment from FT-1-C on 20 Feb. 1992. (D) Reduced lesion with pinkish color on the dorso lateral side of *C. gariepinus* from pond FT-1-C on 28 Feb. 1992 (Bar = 10 cm for figs. A, B, C and D).

parameters seemed to be due to seasonal phenomena (rain/evaporation). So far we have observed that the variable fish species composition in different pond trials could have very little influence on the chemicals that have been applied.

Conclusion

Results from the field trials indicate good efficacy of the dosages. In field trial pond 1 production and survival rates were quite satisfactory in comparison to previous production records. The other affected untreated ponds incurred severe production losses in the same catfish farm. The eradication of the disease from pond field trial 2 also indicated efficacy of the dosages in comparison to the surrounding EUS affected ponds adopted with no such treatments in that locality. Survival of the affected fish *P. gonionotus* was good although total pond production was found fairly normal in comparison to previous production records of the farmers.

The obvious limitations of our data on replications and controls at both sites over the outbreak seasons could be considered inconclusive at this stage. However, the present data suggest that in a pond situation both the alkalinity and chloride concentrations must be simultaneously improved. The ammonia concentration should also be reduced to such level that it does not have a deteriorating effect on the overall water quality when lower chloride concentrations prevail. Our observations further imply that predisposing conditions of EUS occurrence might be associated with lower chloride concentrations and alkalinity, as well as temperature fluctuation (Lelley et al. 1992). Treatment with lime and salt may have positively influenced the water chemistry. How these chemicals may affect the pathogens involved in EUS or simultaneously improve the humoral capacity of the host (van-Muiswinkel 1995) also warrant further studies.

Whatever primary causes are involved or the efficacy levels of the doses applied, control should be based on an understanding of all the factors (Snieszko 1983). Our data further point to the possibility of combating EUS by improving the water quality parameters, at least in pond situations, using low cost ingredients easily available to farmers in villages.

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