Bull. Nat. Inst. Oceanogr. & Fish., A.R.E. 1994. 20 (2): 229 - 248

SHORT AND LONG TERM EFFECTS OF ACID STRESS ON SURVIVAL, BEHAVIOUR, AND SOME CELLULAR BLOOD CONSTITUENTS, IN THE CAT FISH CLARIAS LAZERA.

By

F. I. MOUSSA*; M. B. ABOU SHABANA AND M. Y. EL TOWEISSY.

*** Dept. of Zool., Faculty of Science, Alexandria Univ., Alexandria, Egypt.**
Key Words: Hematology, Cat Fish, Clarias lazera.

ABSTRACT

The fresh water fish Clarias lazera. was exposed to 10 lethal and sublethal concentrations of acid as H_2SO_4. The median tolerance limit (TLm) at different exposure periods 24, 48, 72 and 96 hr appears to be as follows 2.88, 2.94, 3.0 and 3.1 pH respectively. From the chronic tests the maximum acceptable toxicant concentration (MATC) for the fish was between pH 5.4 and pH 4.7 for 12 weeks.

Behavioural changes, as well as a significant increases in haematocrit, haemoglobin levels and total serum protein contents during both duration of experiments were observed. Hyperplasia of epithelium of the primary lamellae and a reduction of the surface area of the secondary lamellae were detected in the gills of Clarias lazera from acidified water at pH 5.4 and 4.7.

INTRODUCTION

The problem of acidification of the natural water and its subsequent effects on survival of resident fish population and there extinction have received intensive studies for over a decade. (Wood, 1988 and Wood et al., 1988a).

The knowledge of variability and dynamics of haematological parameters in fishes, depending on their biological processes in different periods of their life cycle as well as on the effects of external factors is a prerequisite of the application of haematology in various fields of ichthyology (Spinska., 1983).

In this study, we have conducted acute (24h) and chronic (12 weeks) tests in the fish Clarias lazera. to determine the effects of the acid stress on survival, Behavioral changes as well as haematocrit value, haemoglobin content and serum protein as indices of fluid volume disturbance. Histological effects on gills were also documented to assess pathological sequels that may occur due to chronic exposure to acid.

MATERIALS AND METHODS

(1) General

The samples of Clarias lazera. ,Were obtained from lake Maruit, near Alexandria. They ranged from 250 to 300 mm long and from 1500 to 2000gm in weight. They were collected during the summer months June, July and August of the year 1989 and 1990. Fishes were acclimated for at least two weeks under controlled laboratory conditions. They were fed on commercial dry pellets and libitum.

(2) Experimental test solution

The acid used in the present study to reduce pH of the water, was the pure sulphuric acid (H_2SO_4),which is a common acid pollutant in the wild (Beamish and Harvey 1972 and Schofield, 1976). The pH of the water was adjusted using a Titri meter TS_4N (Tacussel electronique pH meter).

(3) Bioassay study:

A- Static acute toxicity tests for TLm's determination:

Groups of 20 active and healthy fish were taken at random and placed in the test as well as in control. Different concentrations were prepared in geometric series using 3.8 - 3.6 - 3.5 - 3.3 - 3.1 - 3.0 - 2.9 - 2.8 - 2.7 and 7.1 (control), the median tolerance limit (TLm) for each time period 24, 48, 72 and 96 hr was estimated according to the method of Sprague (1969).

After 24, 48, 72 and 96 hr of exposure period, survival fish were removed from the containers, samples of blood for haematological study were withdrawn from the caudal peduncle using a heparinized tubes.

B- Chronic toxicity tests:

1-12 weeks experiments were made. Animals were subjected to two different pH_s 5.4 and 4.7 and control (7.1). About 120 fishes were used for each concentration. Tests placed in groups of 15 fishes per aquaria. The behavioral changes of the test organisms were recorded in both acute and chronic experiments.

(4) Hematological study, and serum total protein determination

Samples of blood for hematological study were withdrawn from the caudal peduncle using heparinized tubes after 24, 48, 72 and 96 hr during acute exposure, and were collected every week during chronic exposure. Haematocrits were estimated by Wintrobe, 1974 method, while haemoglobin contents by Sahlis' method, for total serum protein determination, blood was allowed to clot and then centrifuged at 8000 for 5 minutes, and serum total protein was estimated by using Rate Biuvet Reaction (Kingsley, 1972).

(5) Histopathological preparations.

Control and acid - treated specimens of the two chronic concentrations (pH_s 5.4 and 4.7), were dissected alive in fresh water, gills were taken carefully, and fixed with Bouin's fluid. After routine processing, the tissues were embedded in paraffin and sections were cut at 6 um and stained with the double stain haematoxylin - eosin.

Students' t - test was the test used for comparing and explaining the observed results according to the normal control values.

RESULTS

Under the experimental conditions, acid produced a variety of recognizable effects which have been divided into:

Survival patterns:

During the acute effect. The TLm's of acid for the exposed fish at different exposure periods (24, 48, 72 and 96 hr) appeared to be as follows (2.88, 2.94, 3.0, and 3.1 pH_s) respectively Fig (1).

Behavioural changes

Certain integumentary responses represent another aspect of acid stress were noticed during acute exposure (pH 3.1, 96 hrs), notably increased mucous secretion and respiratory distress, usually mucous appeared within the first hours of exposure and continued to accumulate over the exposure interval.

Onset of gill mucification appeared to coincide with the time where a thick film covered the entire integument. Thus mucification appeared to be a generalized response involving the entire surface of fish showing maximal behavioural symptoms of respiratory distress.

Pigmentary changes were also evident during the first weeks of chronic acid exposure (pH5 5.4 and 4.7). By time fish become very dark almost black in comparison to control fish, which remained pale. This reaction persisted throughout the duration of acid exposure.

Haematocrit value and haemoglobin content:

Clarias lazera. exhibited significant increases in haematocrit and haemoglobin levels during both durations.

Acid treatment, at pH 3.1, caused an elevation in haematocrit values from the initial phase of the study. A significant increase from the control level (46.33± 0.88% to reach 56.0 ± 1.04% ($p < 0.005$). The same acid treatment caused an increase of haemoglobin content from the control value (6.63 ± 0.32 g %) to reach 8.40 ± 0.12 g % ($p < 0.01$) Fig (2).

The alterations in the values of haematocrit and haemoglobin content during the chronic exposure of fish (12 weeks) to pH 5.4 and 4.7 respectively were illustrated in Figs 3 & 4 a.

Significant elevation from the control value (45 ± 0.81 %) to 55.5 ± 1.258 % ($p < 0.01$) for pH 5.4, and from control value (45 ± 0.81 %) to 61.833 ± 1.093 % for pH 4.7 were observed in hematocrit values after 12 weeks of exposure.

Similar results were obtained for the haemoglobin content, a highly significant value for pH 5.4 was observed after 2 weeks of exposure with a value of 7.79 ± 0.14 g % as compared to the control value (5.82 ± 0.17 g %). As for the pH 4.7 treated group a significant increase from the control group (6.35 ± 0.36 g %) to 7.53 ± 0.21 g % ($p < 0.05$) was observed one week after treatment, remaining highly significant till the termination of the experiment.

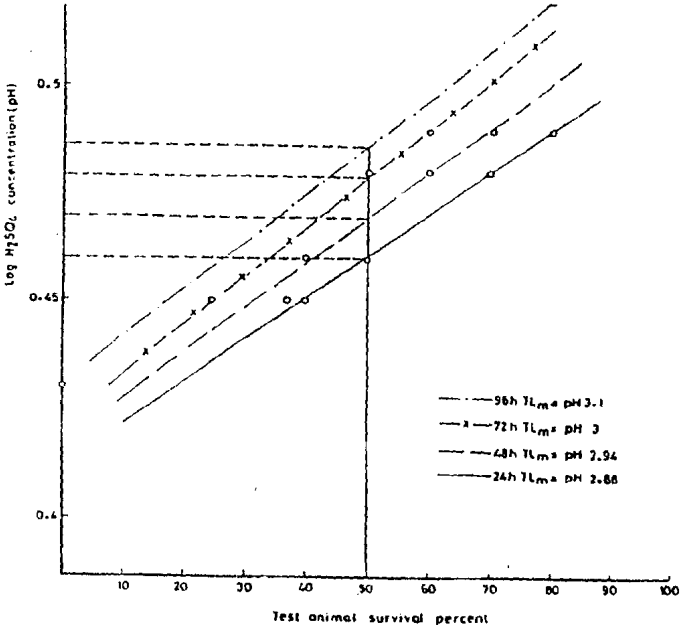


Figure 1: Estimation of median tolerance limit (TL_m) for juvenile Clarias lazera exposed to graded acid concentrations (H_2SO_4) (pH levels).

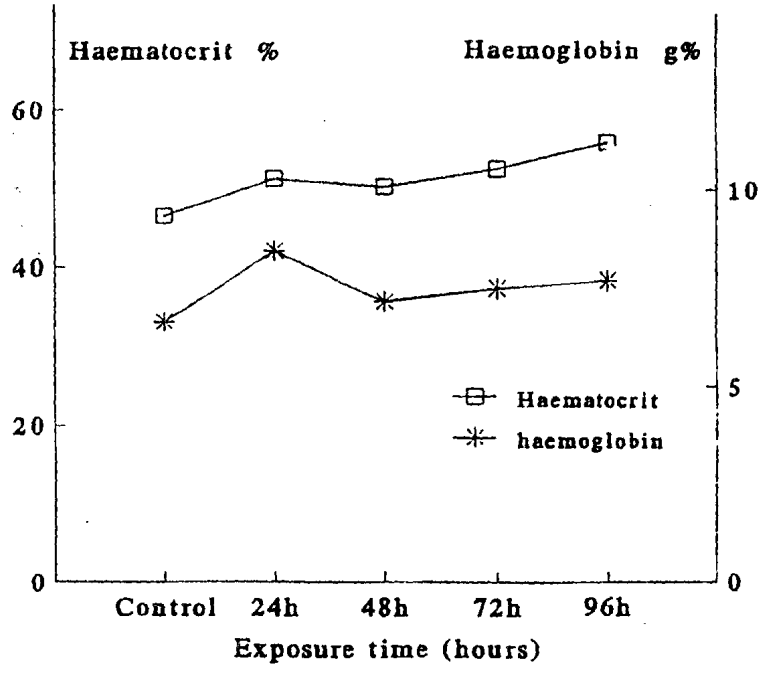


Figure 2: Haematocrit value and haemoglobin content of juvenile Clarias lazera exposed to acid concentration (H_2SO_4) pH 3.1 for 96-h.

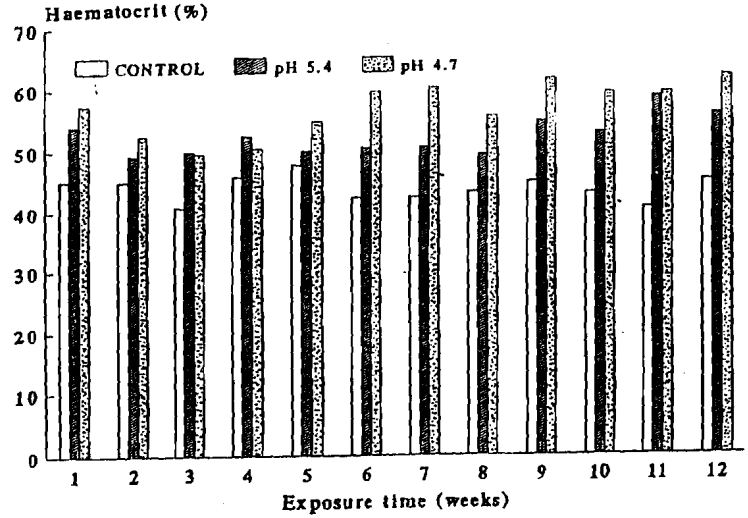


Figure 3: Variation in haematocrit values of juvenile Clarias lazera exposed to different acid concentrations (H_2SO_4) for 12-weeks.

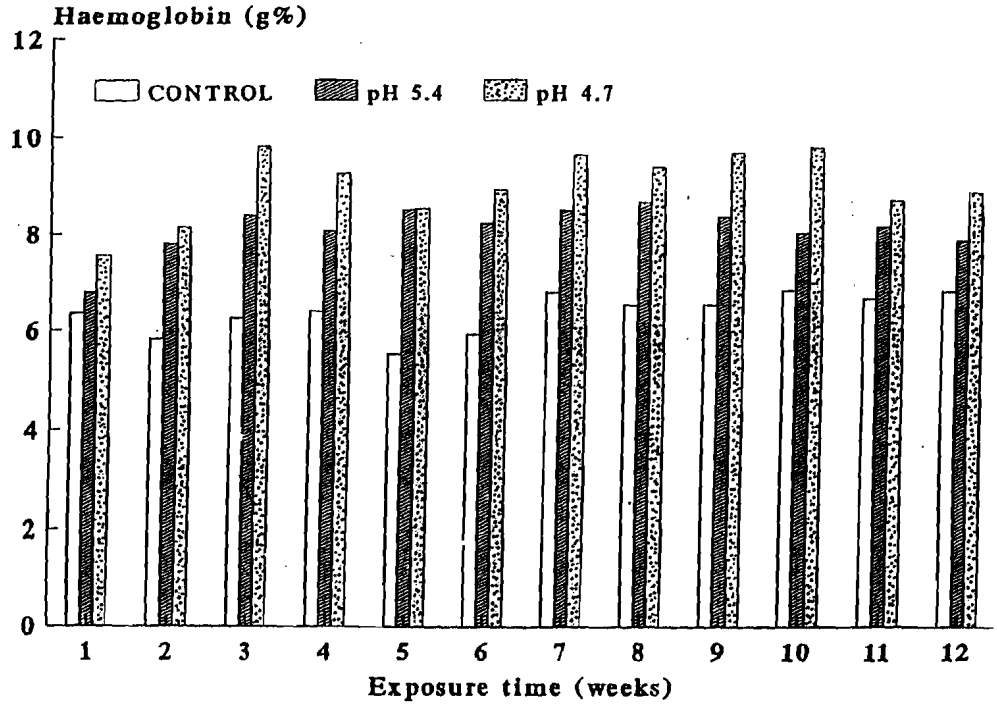


Figure 4: Variation in haemoglobin content of juvenile Clarias lazera exposed to different acid concentrations (H_2SO_4) for 12-weeks.

Fig. (5a): Normal gill structure of a control juvenile Clarias lazera maintained at pH 7.1 (x 100).

primary lamella (pl).
secondary lamella (sl).

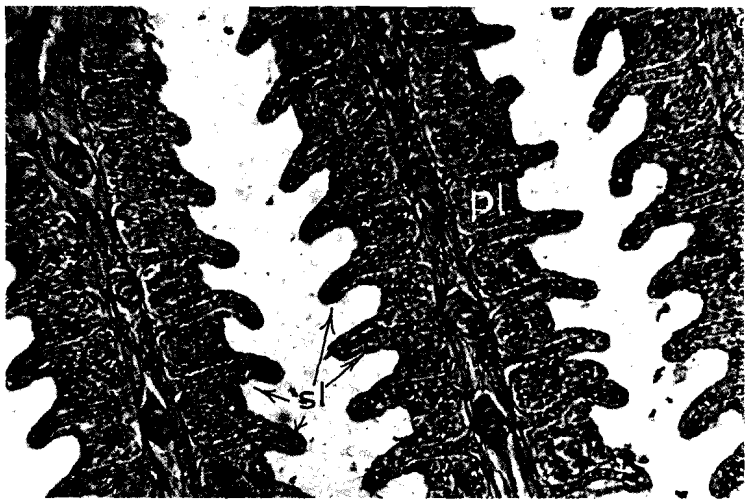
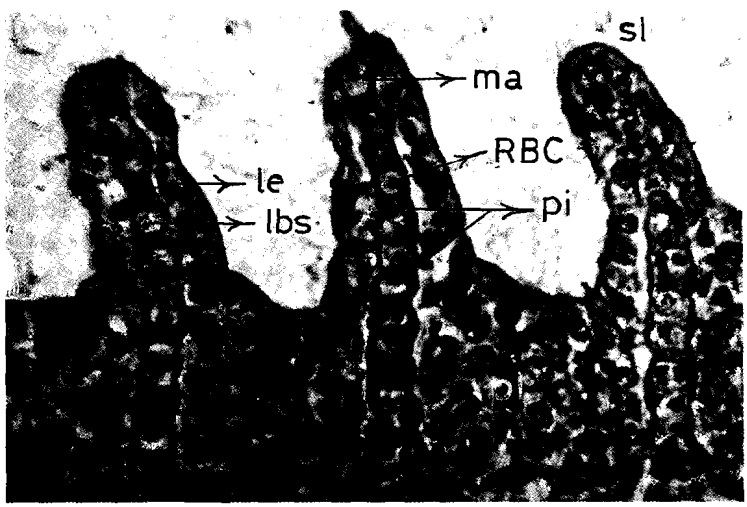


Fig. (5b): A higher magnification of the previous section (x 400).

lamella epithelial cell (le).
lamella blood sinus (lbs).
marginal blood channel (ma).
pillar cells (pi).
red blood cell (RBC).



Fig(6a): Gill filaments of juvenile Clarias lazera exposed to acidified water at pH 4.7 for 4 weeks showing more pronounced hyperplasia of undifferentiated cells in the primary lamellae, resulting in partial blocking of interlamellar spaces (x 100).

primary lamella	(pl).
secondary lamella	(sl).
Hyperplasia	(arrows).

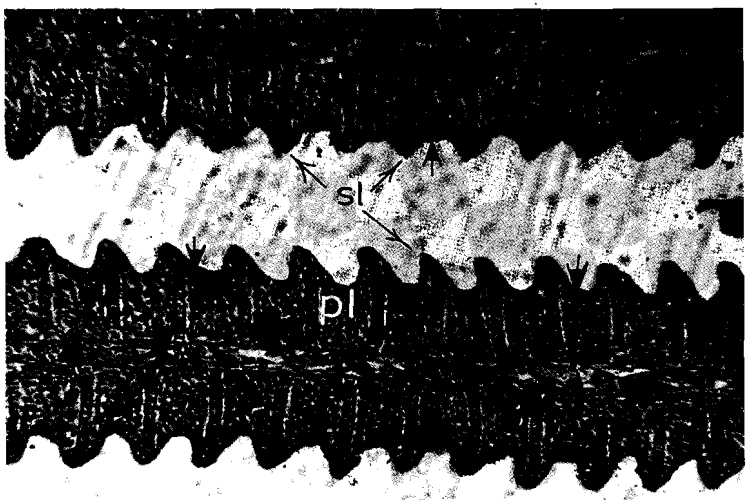


Fig. (6b): A higher magnification of the previous section showing regions of hyperplasia (arrow) in the primary lamellae (pl) (x 400).

primary lamella	(pl).
secondary lamella	(sl).
Hyperplasia	(arrows).

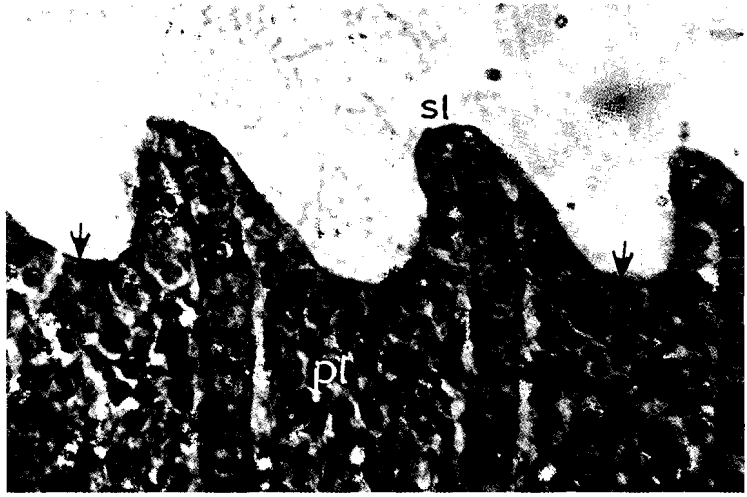


Fig. (7a): A frontal section of gill filaments of juvenile Clarias lazera exposed to acidified water at pH 4.7 for 8 weeks showing severe hyperplasia of undifferentiated cells in the primary lamella, resulting in complete blocking of interlamellar spaces (x 100).

primary lamella	(pl).
secondry lamella	(sl).
Hyperplasia	(arrows).

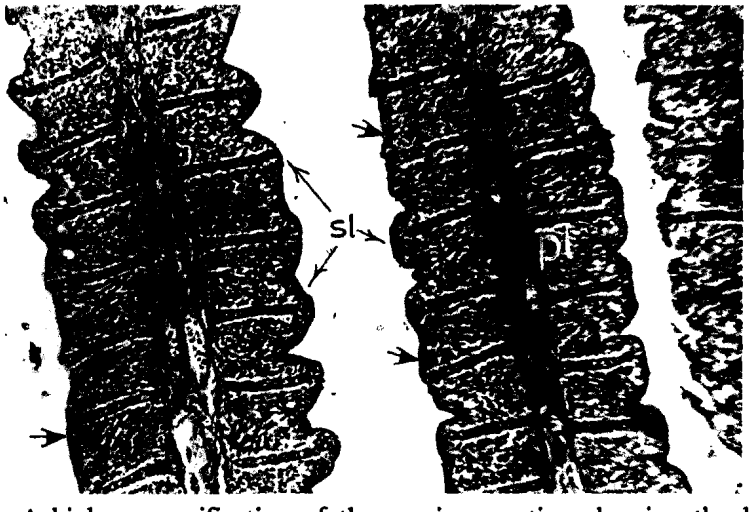
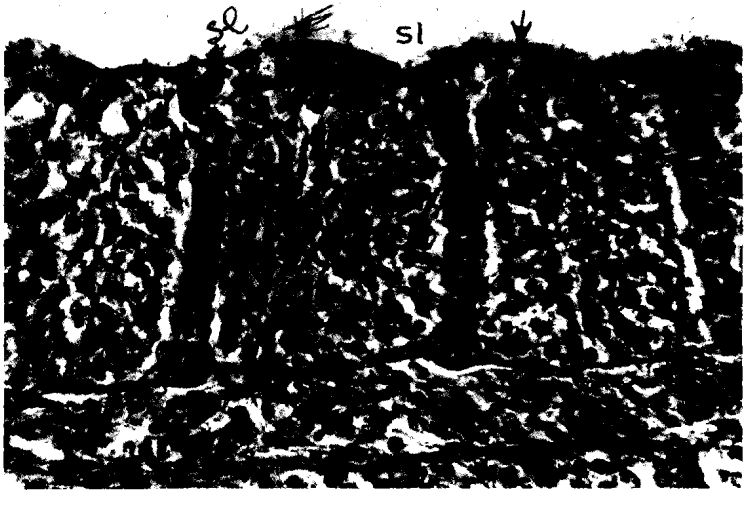


Fig. (7b): A higher magnification of the previous section showing the hyperplasia (arrows) of the undifferentiated cells in the primary lamella (pl) obilterating the secondary lamella (sl) (x 400).

primary lamella	(pl).
secondary lamell	(sl).
Hyperplasia	(arrows).



Serum Protein Content :-

In acute exposure (pH 3.1), Serum protein values were generally greater in fish exposed to H^+ than controls, with peak value being recorded after 24 hour of acidification (Table 1).

Concerning the chronic acid stress, serum protein levels were increased after 1-3 week of acidification (pH_s 5.4 and 4.7) followed by fluctuations during the course of experiment, and finally ending by a second rise to even higher levels (12^{th} week) as shown in Table (2).

Histopathological studies:

Gill exhibited a significant histopathological reaction to chronic acid stress. Gills of Clarias lazera. from water acidified to 4.7 showed a hyperplasia of epithelium of the primary lamellae, and a reduction of the surface area of the secondary lamellae. Fig. 5, 6 & 7.

DISCUSSION

Dramatic changes in the biota of lakes and streams coincident with the declining pH takes place. The most visible and costly effect is the loss of entire fish population (Hendrey et al., 1976 and Pfeiffer and Festa, 1980).

The effect of reduced environmental pH on fresh-water fish have been extensively studied. Rahel and Magnuson, 1983; Somers and Harvey, 1984; Jones et al, 1985 a α b; Witters, 1986; Audet et al. 1988; Booth et al, 1988; wood, 1988; wood et al, 1988 a; b - c; Playle et al, 1989 and Witters et al, 1991) Toxicity data available in the literature showed wide differences is susceptibility to acids not only in different fish species but also within the same species (Mount, et al, 1988 a).

In broad over view, there have been many studies on the toxic effects of acute exposure of fish with a duration of one to four days (Barton et al, 1985; Hohze and Hutchinson, 1989. and Witters et al, 1991). On the other hand, the effects of chronic sublethal acid exposure are not so well known, and no clear pattern has yet emerged (Audet et al, 1988).

The time course of acid stress was followed in juvenile Clarias lazera. exposed to graded pH levels (H_2SO_4) for 96 hours, The effect of this toxicant on survival time was measured quantitatively, and the median tolerance limit (TLm) for 24, 48, 72

Table (1): Changes in serum total protein of juvenile Clarias lazera during exposure to acidified water (pH 3.1) over a 96-h period.

	Exposure period (hours)	Range	Mean± S.E.	S.D.	% Coefficient of variation	Level of significance
Total protein (g/dl)	Control	4.00-5.00	4.533±0.290	0.503	11.103	
	24 h	7.96-8.71	8.223±0.244	0.422	5.131	P < 0.001
	48 h	7.00-7.54	7.283±0.156	0.271	3.721	P < 0.005
	72 h	6.66-7.00	6.867±0.105	0.181	2.643	P < 0.005
	96 h	6.17-6.89	6.483±0.213	0.369	5.691	P < 0.01

Table (2): Changes in serum total protein concentration (expressed as g/dl) of juvenile Clarias lazera exposed to different treatment with H_2SO_4, pH 7.1(control), 5.4 and 4.7 over a 12-week period.

Exposure period (weeks)	Range	Mean±S.E.	S.D.	%Coefficient of variation	Level of Significance
First week	4.00-4.30	4.150±0.122	0.212	5.112	
	4.90-5.20	5.033±0.089	0.153	3.035	P<0.005
	6.00-6.59	6.207±0.192	0.332	5.354	P<0.001
Second week	4.00-4.50	4.250±0.204	0.353	8.319	
	6.32-6.73	6.517±0.119	0.205	3.153	P<0.001
	6.64-6.97	6.833±0.099	0.172	2.519	P<0.001
Third week	4.00-4.40	4.200±0.163	0.283	6.734	
	7.89-8.08	7.990±0.055	0.095	1.194	P<0.001
	7.54-7.78	7.643±0.071	0.123	1.615	P<0.001
Fourth week	4.20-4.34	4.270±0.057	0.099	2.318	
	5.20-5.90	5.567±0.203	0.351	6.309	P<0.005
	6.00-6.99	6.517±0.287	0.496	7.618	P<0.005
Fifth week	4.30-4.54	4.420±0.097	0.169	3.839	
	5.90-6.54	6.153±0.196	0.340	5.529	P<0.005
	5.04-5.96	5.613±0.289	0.500	8.909	P<0.025
Sixth week	4.60-5.00	4.800±0.163	0.283	5.892	
	7.01-7.56	7.333±0.166	0.287	3.919	P<0.001
	5.09-5.99	5.650±0.301	0.521	9.224	N.S.
Seventh week	4.70-4.90	4.800±0.081	0.141	2.946	
	6.00-6.89	6.517±0.267	0.462	7.089	P<0.005
	7.00-8.00	7.537±0.291	0.504	6.687	P<0.001
Eighth week	4.83-4.87	4.850±0.016	0.028	0.583	
	6.71-7.09	6.893±0.109	0.190	2.761	P<0.001
	7.38-7.54	7.470±0.047	0.082	1.096	P<0.001
Ninth week	4.75-4.80	4.775±0.204	0.353	0.740	
	5.84-6.00	5.923±0.046	0.080	1.354	P<0.01
	7.81-7.96	7.877±0.044	0.076	0.969	P<0.001
Tenth week	4.80-4.89	4.845±0.037	0.064	1.313	
	6.13-6.61	6.430±0.151	0.261	4.067	P<0.001
	6.19-6.53	6.337±0.101	0.175	2.757	P<0.001
Eleventh week	4.70-4.90	4.800±0.081	0.141	2.946	
	7.99-8.23	8.083±0.074	0.128	1.591	P<0.001
	8.10-8.42	8.233±0.096	0.166	2.023	P<0.001
Twelfth week	4.80-4.90	4.850±0.041	0.071	1.458	
	6.89-7.31	7.067±0.126	0.218	3.082	P<0.001
	7.84-8.21	8.070±0.116	0.201	2.487	P<0.001

- Each mean result corresponds to a mean value of the three experiments.
S.E. = Standard error.
S.D. = Standard deviation.

and 96 hours were, 2.88, 2.94, 3 and 3.1 respectively Since results of acute toxicity tests can be used for planning chronic toxicity studies. The chronic exposure system in two different pH levels (5.4 and 4.7) for 12 weeks was designed.

Among the many behavioural patterns in fish, Locomotion and activity behavior are of particular significance. Abnormal behavior is an indicator of stress. Changes in activity during acid stress have been frequently reported (Jones et al, 1985 a; Thommesen; 1983; Lemly and smith; 1985 and 1987; Dively et al, 1977; Mac Farlane and Livingston, 1983.

Data observed in the present study lend support to this relation. Experimental Clarias lazera. showed behavioral manifestation of physiological stress almost immediately after the beginning of each exposure period. Rapid swimming and opercular movements, surfacing and gulping of air were commonly observed during the acute exposure system. Decreased activity was generally exhibited by all fish throughout the time course of chronic exposure experiments.

Clarias lazera. exhibited significant increases in hematocrit and haemoglobin levels during both durations several attempts were offered to explain the mechanisms involved in these elevations. From (1980) suggested that the elevation of hematocrit due to acid exposure is a hemopoietic as erythrocyte mobilization response to hypoxemia induced by acid stress. Milligan and Wood (1982) indicated that a decrease in arterial Po_2 nor increase in lactate content of the blood which would be indicative of tissue hypoxia have been reported in acid exposed (pH 4.0 - 4.5) brook trout (Vaala and Mitchell, 1970 and Dively et al; 1977) and rainbow trout (Neville, 1979 a α c and McDonald et al, 1980). Milligan and Wood (1982) attributed this increase to redistribution of body water resulting in decreased plasma volume with no change in total body water. It has been suggested by Powell and McKeown (1986) that since elevated haematocrits caused by erythrocyte Swelling was though to be related to possible impairment of oxygen uptake in acid - stressed fish, and since increased erythrocyte swelling in acid exposed fish was also thought to be a symptom of blood acidosis (Packer and Dunson, 1970). Acidosis of blood can cause a decrease in the oxygen causing capacity of the blood via Bohr and Root effects (Packer and Dunson, 1972 and Milligan and Wood, 1982).

Swelling of erythrocytes due to osmatic shifts and or adrenergic stimulation of the Na^+ / H exchange (Nikinmaa, 1986 and Witters et al, 1987), mobilization of erythrocytes from the spleen, manifested as increases in the number of circulating erythrocytes and reticulocytes due to splenic contraction in response to increase circulating catecholamines, as well as reduction in spleen haemoglobin (Milligan and Wood, 1982; Yamamato et al, 1985, Fryer et al, 1988 and Witters et al. 1991) and

finally, utilization of some tissues involved in osmoregulation and electrolyte balance such as the endocrines to more oxygen under acid stress conditions (Notter et al, 1976), were other reasons offered to explain this hematocrit elevation.

In the present study, haemoglobin contents were observed to follow a similar increasing trend as that of hematocrit during acute and chronic acid exposure experiments. studies by Neville (1979 b), Giles et al, (1984) and Audet et al, (1988) support this observations.

Audet et al, (1988) demonstrated that rainbow trout Salmo gairdneri had increased haemoglobin concentration in the blood in the blood under chronic acid exposure (3 months, pH 4.8). Exposure of the same species for 22 days to water pH < 5.5 had also increased haemoglobin concentration progressively.

This increase was indicated either as a decrease in mean cellular haemoglobin of newly released erythrocytes, an increase in volume (swelling) of circulating erythrocytes or a combination of both factors (Giles et al; 1984).

The increase of haemoglobin level and maintenance of other factors (arterial Po_2 and blood lactate) were clues used by Neville (1979 c) to document that no hypoxic stress occurred to fish exposed for 12 days to pH 4.0 despite the acidemia - Rather surprisingly wood et al., (1988 b) showed that haemoglobin content of about brook trout exposed for 10 weeks to sublethal acid pH 5.2 was unaffected and no explanation was given.

Increased serum protein concentration have been almost observed in the previous studies on acute and chronic acid stress, this was in agreement with the results of Milligan and wood, 1982 and Hobe et al., 1983 on acute acid exposure, and with the results of Wood and Mc Donald., 1988a; Brown et al., 1986b and Jones et al., 1987, on chronic acid exposure. Increases in plasma protein are though to provide an idex of decreased plasma volume during exposure to low pH (Spry et al., 1981, Wood and Mc Donald, 1988a and Brown et al., 1986b). Milligan and Wood (1982) found that 3 days of acid exposure (pH 4.0 - 4.5) reduced the plasma volume by 27 % and increased protein by 90 % in rainbow trout. The reduced plasma volume results from lass of blood ions to the environment and subsequant osmotic movement of extracellular fluid to the intracellular compartment. (Milligan and Wood, 1982).

The normal morphology of **Clarias lazera**. gills examined under controlled laboratory conditions revealed that it is similar to that of other species described (Morgan and Tovell, 1973; Laurent and Dunel, 1980 and Lewis and Potter, 1982).

Gill hyperplasia of undifferentiated cells seemingly originating from interlamellar region (primary lamella) as judged by massive cell proliferation resulting in obliteration of the space between lamellae was the most consistent lesion that occurred in Clarias lazera. under chronic acid exposure experiments (8 weeks, pH_5 at pH 4.7). This morphological change has been reportedly occurring in brook trout, Salvelinus fontinalis (pH5 5.5 and 6.6) (chevalier et al., 1985) and in pearl dice Semotilus margarita, pH 5.2 (Leino et al., 1987)

High H' ion concentration was shown to produce also severe gill changes: lifting of lamellar epithelium, frank separation of epithelial layers in respiratory lamellae, fusion of adjacent respiratory lamellae, necrosis, chloride cell hyperplasia, excess secretion of mucus and general epithelial damage. (Tuurala and Soivio, 1982; Stromberg et al., 1983; Leino and McCormick, 1984; Chevalier et al., 1985 and Freda et al., 1991).

REFERENCES

Audet, C., Munger, R. S., and Wood, C. M., 1988. Long-term sublethal acid exposure in rainbow trout (Salmo gairdneri) in soft water: effects on ion exchanges and blood chemistry. Can. J. Fish. Aquat. Sci. 45: 1387-1398.

Audet, C., and Wood, C. M., 1988. Do rainbow trout (Salmo gairdneri) acclimate to low pH ? can. J. Fish. Aquat. Sci. 45: 1399-1405.

Barton, B. A., Weiner, G. S., and Schreck, C. B., 1985. Effect of prior acid exposure on physiological responses of juvenile rainbow trout (Salmo gairdneri) to acute handling stress. Can. J. Fish. Aquat. Sci. 42: 710-717.

Beamish, R. J., and Harvey, H. H., 1972. Acidification of the La Cloche mountain lakes, Ontario, and resulting fish mortalities. J. Fish Res. Board Can. 29: 1131-1143.

Booth, C. E., McDonald, D. G., Simon, B. P., and Wood, C. M., 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (Salvelinus fontinalis). Can. J. Fish. Aquat. Sci. 45: 1563-1574.

Brown, S.B., Evans, R.E., and Hara, T.J., 1986b. Internal, thyrodial, Carbohydrate and electrophyte responses in rain bow trout (Salmo gaiddneri) during recovery from the effect of acidification. Can. J. Fish. Aquat. Sci. 43: 714-718.

Chevalier, G. Gauthier, L. and Moreau, G., 1985. Histopathological and electron microscopic studies of gills of brook trout (Salvelinus fontinalis) from acidified lakes. Can. J. Zool. 63: 2062-2070.

Dively, J. L., Mudge, J. E., Neff, W. H., and Anthony, A., 1977. Blood Po_2, Pco_2, and pH changes in brook trout (Salvelinus fontinalis) exposure to sublethal levels of acidity. Comp. Biochem. Physiol. 57A: 347-351.

Freda, J., Sanchez, D.A., and Bergman, H.L., 1991. shortening of brachial tight junctions in acid-exposed rainbow trout (Oncorhynchus mykiss). Can. J. Fish. Aquat. Sci.: 48: 2028-2033.

Fryer, J. N., Tam, W. H., Valentine, B. and Tikkala, R. E., 1988. Prolactin cell cytology, plasma electrolytes, and whole-body sodium efflux in acid stressed brook trout (Salvelinus fontinalis). Can. J. Fish. Aquat. Sci. 45: 1212-1221.

Fromm, P.O., 1980. Areview of some physiological and toxicological responses of freshwater fish to acid stress. Environ. Biol. fish. 5: 79-93.

Giles, M. A., Majewski, H. S., and Horden, B., 1984. Osmoregulatory and hematological responses of rainbow trout (Salmo gairdneri) to extended environmental acidifications. Can. J. Fish. Aquat. Sci. 41: 1686-1694.

Hendrey, G. R., Baalsrud, K., Traaen, T. S., Laake, M., and Raddum, G., 1976. Acid precipitation: some hydrobiological changes. Ambio, 5: 224-227.Holtze, K.

Hohze, and Hutchinson, N. J., 1989. Lethality of low pH and Al to early life stages of six fish species inhabiting PreCambrian Shield waters in Ontario. Can. J. Fish. Aquat. Sci. 46: 1188-1202.

Hobe, H., Wilkes, P.R.H., Walker, P.L., Wood, C.M., and McMahon, B.R., 1983. Acid-base balance, ionic stalus and renal function in resting and acid-exposed white suckers (Ctostomus commersoni). Can. J. Zool. 61: 2660-2668.

Jones, K. A., Hara, T. J., and Scherer, E., 1985a. Behavioral modification in Arctic char (Salvelinus alpinus) chronically exposed to sublethal pH. Physiol. Zool. 58: 400-412.

Jones, K. A., Hara, T. J., and Scherer, E., 1985b. Locomotor response by Arctic char (Salvelinus alpinus) to gradients of H^+ and CO_2. Physiol. Zool. 58: 413-420.

Kingsley, G.R., 1972. Standard methods of clinical chemistry, G.R. Cooper, ed., New York, Academic Press, Inc, Vol. 7.

Laurent, P., and Dunel, S., 1980. Morphology of gill epithelia in fish. Am. J. Physiol. 238: 47-59.

Leino, R. l., and McCormick, J. H., 1984. Morphological and morphometrical changes in chloride cells of the gills of (Pimephales promelas) after chronic exposure to acid water. Cell Tissue Res. 236: 121-128.

Leino, R. L., Wilkinson, P., and Anderson, J. G., 1987. Histopathological changes in the gills of pearl dace (Samotilus margarita) and fathead minnows (Pimephales promleas) from experimentally acidified Canadian lakes. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 126-134.

Lemly, A. D., and Smith, R. J. F., 1985. Effects of acute exposure to acidified water on the behavioral response of fathead minnows (Pimephales promelas) to chemical feeding stimuli. Aquat. Toxicol. 6: 25-36.

Lemly, A. D., and Smith, R. J. F., 1987. Effects of chronic exposure to acidified water chemoreception of feeding stimuli by fathead minnows (Pimephales promelas): Mechanisms and ecological implications. Environ. Toxicol. Chem. 6: 225-238.

Lewis, S. V., and Potter, I. C., 1982. A light and electron microscope study of the gills of larval lampreys (Geotria australis) with particular reference to the water-blood pathway. J. Zool. (Lond.) 198: 157-176.

MacFarlane, R. B., and Livingston, R. J., 1983. Effects of acidified water on the locomotor behavior of the gulf killifish (Fundulus grandis) a time series approach. Arch. Environ. Contam. Toxicol. 12: 163-168.

McDonald, D. G., Hobe, H., and Wood, C. M., 1980. The influence of calcium on the physiological responses of the rainbow trout (Salmo gairdneri) to low environmental pH. J. Exp. Biol. 88: 109-131.

McKeown, A.B., Geen, G.H., Watson, T.A. Powell, J.F., and Parker, D.B., 1985. The effects of pH on plasma electrolytes, carbonic anhydrase and ATPase activities in rain bow trout (Salmo gairdeneri) and Largescale suckers (Catostomus macrocheilus). Comp. Biochem. Physiol., 80A: 507-514.

Milligan, C. L., and Wood, C. M., 1982. Disturbances in hematology, fluid volume distribution, and circulatory function associated with low environmental pH in the rainbow trout (Salmo gairdneri). J. Exp. Biol. 99: 397-415.

Morgan, M., and Tovell, P. W. A., 1973. The structure of the gill of the trout (Salmo gairdneri). Z. Zellforch. Mikrosk. Anat. 142: 147-162.

Mount, D.R., Hockett, J.R., and Gern, W.A., 1988a. Effect of long-term exposure to acid, aluminum, and low calcium n adult brook trout (Salvelinus fontinalis). 2. Vitellogenesis and osmoregulation. Can. J. Fish. Aquat. Sci. 45: 1633-1642.

Neville, C.M., 1979a. Influence of mild hypercapnia on the effects of environmental acidification onrainbow trout (Salmo gairdneri). J. Exp. Biol. 83: 345-349.

Neville, C.M., 1979b. Sublethal effects of environmental acidification on rainbow trout (Salmo gairdneri). J. Fish Res. Board Can. 36: 84-87.

Neville, C.M., 1979c. Ventilatory response of rainbow trout (Salmo gairdneri) to increased H^+ ion concentration in Blood and water. Comp. Biochem. Physiol. 63A: 373-376.

Nikinmaa, M., 1986. Control of red cell pH in teleost fishes. Ann. Zool. Fenn. 23: 223-235.

Notter, M.F.D., Mudge, J. E., Neff, W. H., and Anthony, A., 1976. Cytophotometric analysis of RNA changes in prolactin and stannius corpuscle cells of acidistressed brook trout. Gen. Comp. Endocrinol. 30: 273-284

Packer, R. K., and Dunson, W. A., 1970. Effects of low environmental pH on blood pH and sodium balance of brook trout. J. Exp. Zool. 174: 65-72.

Packer, R.K., and Dunson, W. A., 1972. Anoxia and sodium loss associated with the death of brook trout at low pH. Comp. Biochem. Physiol. 41A: 17-26.

Pfeiffer, M. H., and Festa, P. J., 1980. Acidity status of lakes in the Adirondack region of New York in relation to fish resources. N. Y. State Dep. Environ. Conserv. Rep. FW-P168, Albany, New York.

Playle, R.C., Goss, G.G., and Wood, C. M., 1989. Physiological distubances in rainbow trout (Salmo gairdneri) during acid and aluminum exposures in soft water of two calcium concentrations. Can. J. Zool. 67: 314-324.

Powell, J. F. F., and McKeown, B. A., 1986. The effects of acid exposure on the ion regulation and seawater adaptation of coho salmon (Oncorhynchus kisutch) parrs and smolts. Comp. Biochem. Physiol. 38A: 45-52.

Rahel, F.J., and Magnuson, J J., 1983 Low pH and the absence of fish species in naturally acidic Wisconsin lakes onferences for cultural acidification. Can J Fish Aquat Sci. 40: 3-9

Schofield, C L., 1976 Acid precipitation: effects on fish. Ambio, 5 228-230

Somers, K M., and Harvey, H. H., 1984 Alteration of fish communities in lakes stressed by acid deposition and heavy metals near Wawa, Ontario Can J.Fish Aquat Sci. 41 20-29

Sprague, J B., 1969. Measurement of pollutant toxicity of fish. I Bioassay methods for acute toxicity Water Resour. 3: 793-821

Spry. D.J Wood, C.M. and Hodson, P V., 1981 The effect of environmental acid on freshwater fish with particular reference to the soft water lakes in Ontaris and modifying effects of heavy metals. Aleterature review Can. Tech. Rep. Fish Aquat. Sci. 999: 145 p.

Spinska A., 1983. Effect of physiological factors, stress and disease on haematological parameters of Carp, with a particular reference to leukocyte pattern. Variability of haematological indices of carp in relation to age and gonad maturity stage. Acta Ichthyol. piscat. 13, 59-81

Stromberg, P C., Ferrante, J. G., and Carter, S., 1983. Patho;ogy of lethal and sublethal exposure of fathead minnows (Pimephales promelas) to cadminm a model for aquatic toxicity assessment. J. Toxicol. Environ. Health 11 247-259

Thommesen, G., 1983. Detection of a blocking effect of low pH in the trout olfactory organ. In K.B. Doving, ed., Chemoreception Studies of Marine Pollution. University of Oslo, Norway, pp. 44-48.

Tuurala, H., and Soivio, A., 1982. Structural and circulatory changes in the secondary lamellae of (Salmo gairdneri) gills after sublethal exposures to dehydroabietic acid and zinc. Aquat. Toxicol. 2: 21-29.

Vaala, S. S., and Mitchell, R. B., 1970. Blood oxygen-tension changes in acid exposed brook trout. Proc. Pa. Acad. Sci. 44: 41-44.

Wintrobe, M.M., 1974. Clinical Haematology. By M.M. Wintrobe and other 7 edition Lea and Feblger, philadelphia. 1896 p.

Witters, H. E., 1986. Acute acid exposure of rainbow trout (Salmo gairdneri) [Richardson]: effects of aluminum and calcium on ion balance and haematology. Aquat. Toxicol. 8: 197: 210.

Witters, H. E., and Vandenborght, O. (ED)., 1987. Ecophysiology of acid stress in aquatic organisms. Anals. Soc. R. Zool. Belg. 117 (Suppl.1) 472p.

Witters, H. E., Van Puymbroeck, S., and Vandenborght, O. L. J., 1991. Adrenergic response to physiological disturbances in rainbow trout (Oncorhynchusmykiss) exposed to aluminum at acid pH. Can. J. Fish. Aquat. Sci. 48: 414-420.

Wood, C. M., 1988. The physiological problems of fish in acid waters. In R. Morris, D. J. A. BBrown, E.W. Taylor and J. A. Brown [ed]. Acid toxicity and aquatic animals. Society for Experimental Biology Seminar Series, Cambridge University Press, Cambridge.

Wood, C. M., McDonald, D. G., Booth, C. E., Simons, B. P., Ingersoll, C. G., and Bergman, H. L., 1988a. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (Salvelinus fontinalis). 1. Blood composition and net sodium fluxes. Can. J. Fish. Aquat. Sci. 45: 1587-1596.

Wood, C. M., Playle, R. C., Simons, B. P., Goss, G. G., and McDonald, D. G., 1988b) Blood gases, acid-base status, ions and haematology in adult brook trout (Salvelinus fontinalis) under acid/aluminum exposure. Can. J. Fish. Aquat. Sci. 45: 1575-1586.

Wood, C. M., Simons, B. P., Mount, D. R., and Bergman, H. L., 1988c. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (Sal;velinus fontinalis). 2. Blood parameters by cannulation. Can. J. Fish. Aquat. Sci. 45: 1597-160.

Yamamato, K. I., Itazawa, Y., and Kobayashi, H., 1985. Direct observation of fish spleen by an abdominal window method and its application to exercised and hypoxic yellow-tail. Jpn. J. Ichthyol. 31: 427-433.