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Impact of water pH on haematology and serum enzyme activities in *Schizothorax richardsonii* (Gray)

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ABSTRACT

Haematological characteristics in fish changes in response to environmental conditions and thus could serve as indicators of sub lethal environmental stress. Exposure of snow trout, *Schizothorax richardsonii* to different water pH (5.0-9.0) for 24 h resulted in alteration in haematological and enzymological parameters. The fishes showed obvious signs of stress at pH 5.0 as indicated by their behavioural changes. Blood haemoglobin (Hb), red blood cells (RBC) and packed cell volume (PCV) were higher at both extremes of pH, but significantly ($p<.05$) higher at lower pH than control group at pH 7.0. Likewise, serum enzymes *viz*. Lactate dehydrogenase (LDH), Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvate transaminase (GPT), Acid phosphatase (ACP) and Alkaline phosphatase (ALP) exhibited elevated levels during acid stress. Conversely, protein concentration fell considerably in fishes exposed to low pH.

Introduction

Environmental stressors both natural and humanly induced could cause changes in cellular function which alter the physiology of organ systems in the fish. Haematology and serum enzyme evaluation of blood provides valuable facts concerning the physiological response of fish to changes in the external environment (temperature, pH, salinity and photoperiod) and thus could serve as indicators of sublethal environmental stress. Though haematological responses to pH changes have been well studied (Nieminen *et. al*., 1982; Dederen *et. al*., 1986; Sexena and Chauhan, 1992), information on serum enzyme response of fish to varying pH is meager.

The snow trout *Schizothorax richardsonii*, is the principal indigenous fish of the Himalayas available in various upland resources. The recent threats posed to the stocks of snow trout due to environmental degradation or human induced changes have made its rehabilitation in depleted waters a priority activity. As attempts have been made in recent past to culture this fish under captivity in order to promote aquaculture in hilly regions, it is imperative to know the optimal conditions for the maintenance, growth, survivability and reproduction of this fish. Hence, during the present investigation an attempt has been made to detect changes in the biochemical and physiological functions of *S. richardsonii* in response to changing water pH.

Materials and methods

The experimental fish, *S. richardsonii* (T. L. 150 mm) were captured by cast nets from river Kosi near Bhowali (Nainital), Uttaranchal and transported to the hatchery at NRCCWF, Bhimtal in well aerated polyethylene bags. Fishes were maintained in flow through hatchery tanks for about a week to acclimatize them under captive conditions and to combat catch and transport stress. The lethal values of extreme limits of pH were determined prior to experiment by exposing the fish to pH of 4-5 and 9-11 for 48 h. In order to reduce the diet induced variabilities in the observations, the fishes were starved for 24 h prior to commencement of experiment.

A set of five well aerated experimental aquaria each containing 18 l of water were maintained at five different pH (5,6,7,8 and 9) adding concentrated HC1 or NaOH pellets. The aquarium containing water of pH 7 was considered as control. Five specimens of *S. richardsonii* were then transferred to each aquarium. The physico-chemical parameters of water were determined before and after the completion of the experiment (Table 1) following APHA (1980). All experiments were carried out at 22°C and repeated thrice. After 24 h, fish were immobilized by giving a blow to head, because prolonged struggling would lead to splenic contraction and changes in haematological parameters. The blood was collected from caudal vein and serum was prepared by centrifuging the whole blood at 3000 rpm for 15 minutes. The various serum enzymes *viz.* Lactate dehydrogenase (LDH-EC 1.1.1.27), Glutamic oxaloacetic transaminase (GOT-EC 2.6.1.1), Glutamic pyruvate transaminase (GPT-EC 2.6.1.2), Acid phosphatase (ACP-EC 3.1.3.2) and Alkaline phosphatase (ALP-EC 3.1.3.1) were analysed. LDH was assayed as per the method described in Plummer (1994). Phosphatases were determined as per the methods of Bergmeyer (1963) and Transaminases by Yatzidis (1960). EDTA was used as anticoagulant for collecting whole blood in order to carry out haematological investigations by the procedures of Dacie and Lewis (1975). Erythrocyte (RBC) counts were determined by Neubauer's improved double ruling chamber (GDR) using Hayem's diluting fluid. Packed cell volume (PCV) was measured by Wintrobe's tubes after filling with double oxalated blood and spinning them at 3000 rpm for 30 minutes. Erythrocyte indices, the so-called 'absolute' values, *viz.* mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) were calculated mathematically from the erythrocyte

TABLE 1: *Water quality parameters during the experiment*

pH	Dissolved O_2 (mg/1)	Free CO_2 (mg/1)	Total alkalinity (mg/1)	Chloride conc. (mg/1)
5.0	6.7+0.37	1.7740.29	17.95+2.35	69.33 41.09
6.0	7.2+0.28	1.60+0.31	34.75+3.82	50.67 + 1.44
7.0	7.6+0.26	1.48+0.30	53.50+2.50	11.00 + 1.06
8.0	7.5+0.29	1.57+0.32	76.35 + 1.60	11.33+1.09
9.0	7.7 40.31	1.60 40.28	92.25 43.57	10.00 40.44

Values are mean ± SEM for six observations at each pH count, haemoglobin concentration and haematocrit. Estimation of protein was done by the method of Lowry *et al.* (1951). The blood parameters and serum enzymes were tested for statistical significance using student 't' test.

Results

Behavioural observations

The lower and upper lethal limits of pH for *S. richardsonii* were observed to be those concentrations of HCI and NaOH which caused a pH of 4.0 and 11.0 respectively. The mortality of fishes were recorded within two hours of their exposure to these pH levels. The skin and gills of fish were abundantly covered with mucous; the respiratory epithelium of gills was destroyed at both extremes of pH. Under acute acidic environment, the fish assumed a diagonal position with its head towards the water surface, became sluggish, at times jerking convulsively followed by death. Fishes exposed to lethal alkaline pH exhibited restlessness, swam rapidly with lashing movements of their tail region. Further, it was noticed that fish which were exposed to pH 5.0 showed initial disturbed swimming movements and appeared pale in colour after approximately 15 minutes followed by mortality of about 50% specimens between 24 to 48 h of exposure. On the other hand, about 70% fishes died at pH 10 within 48 h of experiment. The snow trout kept at other pH levels did not show any conspicuous behavioural changes.

Haematological observations

Variations in RBC, Hb and PCV values and related absolute indices (MCH, MCHC and MCV) are presented in Table 2. A significant increase ($p<0.05$) in the values of RBC, Hb and PCV was observed in acidic condition (pH 5.0). The respective values of RBC, Hb and PCV increased by 26, 20 and 16% in fishes maintained at pH 5.0 in comparison to the fishes of control group, whereas a significant increase was noticed at alkaline pH of 9.0. MCV showed significant ($p<0.05$) decline by 11% at pH 5.0. while MCH and MCHC varied non significantly at this acidic condition. On the other hand, no significant variations in all haematological parameters were observed at water pH 6.0 and 8.0 as compared to control. A considerable reduction ($p<0.05$) in the values of serum protein were also observed at low pH of 5 without any remarkable changes in fish groups exposed to other pH levels.

Enzymatic observations

Serum enzymes *viz.* LDH, GOT, GPT, ACP and ALP exhibited significant ($p<0.05$) elevated levels at pH 5.0 (Table 3). Activities of GPT and ALP increased substantially by 147.9 and 158.3% respectively. LDH, GOT and ACP exhibited 101.6, 74.6 and 75.2% increased activities respectively in serum at pH 5.0 as compared to control group maintained at pH 7.0. Similar increase in activities of all the five enzymes were visualised at higher water pH of 9.0, but only the rise in levels of ACP was noticed to be statistically significant ($p<0.05$).

Discussion

Dederen *et al.* (1986) reported that acute exposure of fishes to water of pH between 3.5 and 5.0 is lethal for most species which supports the present findings. The exposure of snow trout to acidic pH of 4.0 appeared to be lethal and exhibited severe stress at pH 5.0 during 24 h of experiment leading to 50% mortality in 48 h. The present results are also in agreement with the observations of Metelev *et al.* (1983) who reported that a reduction in pH below 5.0 is critical for carps and trouts, which led to mortality after one day. Likewise, threshold pH

TABLE 2: *Haematological variations in* Schizothorax richardsonii *on exposure to different pH*

pH	TEC X10^6/cmm	Hb (g%)	PCV (%)	MCH (pg)	MCHC (%)	MCV (nm^3)	Protein (mg/ml)
(Control)							
7.0	1.99+0.18	9.36+0.62	42.95+2.37	47.42 + 1.23	21.78+0.60	218.57+8.99	33.13 + 1.02
5.0	2.51+0.07*	11.20+0.37*	49.82 + 1.09*	44.57+0.63	22.85 40.54	195.36+3.61*	26.35+2.20*
6.0	2.24+0.11	10.90+0.59	46.12+2.07	45.66+2.23	22.20+1.02	205.04+2.46	36.40 + 1.49
8.0	2.13+0.16	10.32+0.69	44.12+2.87	48.55 + 1.03	23.40+0.60	207.81+4.73	36.50+2.18
9.0	2.15+0.15	10.52+0.69	45.22+3.23	48.95+0.42	23.31+0.81	210.11+1.70	34.60 + 1.71

* 0.05 level of significance

Values are mean ± SEM for five observations at each pH

TABLE 3: *Serum enzyme activities in* Schizothorax richardsonii *on exposure to different pH*

pH	LDH (units/ml)[1]	GOT (units/ml)[2]	GPT (units/ml)[3]	ACP (units/ml)[4]	ALP (units/ml)[5]
(Control)					
7.0	0.596+0.120	0.138+0.018	0.048+0.012	0.359+0.087	0.012+0.002
5.0	1.202+0.212*	0.241+0.032*	0.119 40.019*	0.629+0.397*	0.031+0.008*
6.0	0.641+0.109	0.158+0.010	0.066+0.013	0.448+0.055	0.014+0.003
8.0	0.462+0.065	0.159 40.014	0.051+0.006	0.249+0.068	0.015+0.007
9.0	0.708+0.180	0.157+0.011	0.069+0.012	0.660+0.052	0.016+0.004

*0.05 level of significance

Values are mean ± SHM for five observation at each pH

1 A unit of enzyme is the amount that caused the conservation of 1 mmol of pyruvate to lactate per min at 37°C

2,3 A unit of enzyme is the amount that cuased the release of 1 mmol of pyruvate per min at 37°C

4,5 A unit of enzyme is the amount that cuased the release of 1 mmol of p-nitrophenol from p-nitrophenyl phosphate per min at 37°C level in an alkaline medium for brown trout, perch, roach, pike and carps were reported to be 9.2, 9.2, 10.4, 10.7 and 10.8 respectively which further confirms the present findings of lethal impact of pH 10-11 on *S. richardsonii*, a member of family cyprinidae. The mortality of the fishes at extreme limits appeared to occur due to the progressive damage to the respiratory epithelium of gills and fish may die due to suffocation and accumulation of carbon-di-oxide in the body. Wood and McDonald (1982) and McDonald (1983) also sugges-ted that stress caused by acidic circumstances led to impede oxygen uptake and trans-port due to deterioration of the ionic and acid base regulation. Oxygen diffusion is inhibited at high pH also (Igram and Wares, 1979).

The elevated levels of Hb, RBC and PCV of fishes exposed to acidic environment recorded in the present study would help to combat decreased oxygen uptake which appeared to arise from extra blood cells supplied by spleen (Milligan and Wood, 1982; Yamamato *et al.*, 1980) under the effect of hypoxia. The present observations are also in agreement with the findings of Nieminen *et al.* (1982) and Dederen *et al.* (1986) who documented higher value ot Hb and PCV in whitefish *(Coregonus peled)*, trout *(Salmo trutta)* and in *Umbra pygmaea* at low pH due to hypoxia. Higher number of RBC but lower concentration of Hb during acid stress was reported in *Clarius batrachus* (Sexena and Chauhan, 1992). The higher Hb concentration may also be attributed to the Bohr effect which involves a decreased affinity of haemoglobin for oxygen at low pH due to altered configuration of the Hb molecule by the increased hydrogen ion concentration. This effect appears to be prominent in fishes like *S. richardsonii* that are adapted to habitats with high oxygen content and low CO_2 of hill streams. Increased haematocrit due to haemopoiesis was not a likely mechanism because the test were only 24 h long. Milligan and Wood (1982) suggested another reason for increase in RBC and PCV. Haemoconcentration due to loss of vascular fluid to the interstitium leads to decrease in blood volume during acid exposure and this could be the reason for decreased MCV at low pH. The nonsignificant rise observed in the values of Hb, RBC and PCV in *S. richardsonii* at higher pH of 9.0 are contrary to the observations made in rainbow trout by Nieminen *et al.* (1982). Rainbow trout belonging to family salmonidae is probably more sensitive to alkaline pH than the members of cyprinidae to which the test fish belongs (Metelev *et. al.*, 1983). Reduction in protein level in 24 h of experiment is in contrast to the observations of Milligan and Wood (1982) who reported increase in plasma protein in 72 h on exposure to low water pH in rainbow trout. The suppression of protein levels of blood at pH 5.0 may be attributed to spontaneous utilisation of amino acids in various catabolic reactions that arise to combat stress during initial period (24 h) of exposure to acidic environment. This may be followed by increase in protein concentration due to higher rate of protein synthesis at later stages of exposure to stress conditions as detected in rainbow trout.

Serum enzymes have close relationship to a particular organ or tissue and so have high degree of specificity. These are intracellular and present in small amounts in the blood of healthy animals. But, when there is an increase of cell breakdown due to damage of tissues caused either by disease or pollutants, they escape in greater quantities in the blood with consequent increase in their activities in serum.

Heart, liver, gills, muscles, kidney, small intestine and red blood cells are rich in LDH, GOT, GPT, ALP and ACP enzymes and their measurement in plasma or serum could often be related to cell damage to specific organs (McDonald and Milligan, 1992). Thus the present study clearly depicts the damage accruing to various organs of the test fishes at pH 5.0 as indicated by their elevated levels in serum. No impact on the activities of GOT and GPT in whitefish and trout was reported on exposure to varying water pH, which contradicts our findings (Nieminen *et. al.* 1982). On the other hand, considerable increase in levels of acid phosphatase in serum at pH 9.0 may be due to the leakage of this enzyme from RBC caused by change in cell permeability at this pH.

Changes in pH interfere with a number of physiological processes in freshwater fish either by reducing oxygen uptake by the gills or due to changes in the structure of haemoglobin which are accompanied by acid base imbalance. Thus the haematological and enzymological studies carried out under present investigation showed that *S. richardsonii* can thrive well in pH range of 6.0-9.0 but cannot tolerate high acidic and alkaline conditions.

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