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EFFECTS OF LOW WATER PH ON SOME HEAMATOLOGICAL AND PHYSIOLOGICAL PARAMETERS OF *Oreochromis mossambicus*

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Abstract

In this study tolerance to acid water pollution and the effects of acid water pollution on some haematological and physiological parameters were investigated in *Oreochromis mossambicus*, a species of fish which is well established and widely distributed in the inland waters of Sri Lanka. From the tolerance tests it was found that the maximum tolerance level, with 100% survival occurred at pH 4. Therefore in this study the fish were acclimated to pH (H_2So_4) 4.0 $\pm$ 0.1 for a period of two weeks before the changes in blood parameters were determined. Water in the control tanks had a pH of 7.0-7.2.

Acid exposure caused significant disturbances in haematological parameters. The haematocrit, red blood cell count and haemaglobin concentration increased while the three haematological indices; haemaglobin content of a single erythrocyte, haemaglobin concentration of a single erythrocyte and cell volume of single erythrocyte decreased. The blood pH also decreased. But O_2 capacity, O_2 consumption, metabolic rate and plasma Na- concentration remained unaltered.

This study reveals that *O. mossambicus* can tolerate a water pH down to pH 4 with little or no difficulty for extended periods, and is able to regulate its physiolobical status to compensate the body's O_2 demands. This is in contrast with reports for many other species whose survival is limited at this level of pH.

Key words : Acid tolernace, Haematology, Blood pH, O_2 capacity, O_2 consumption.

1. Introduction

Acidification of fresh water systems have become a major environmental problem in recent years. The extensive use of fossil fuels and the release of various industrial by-products in to the biosphere has created many fresh water systems with unnaturally acidic waters which is harmful to fish life at every stage of its life cycle. Many workers have reported that low ambient pH levels may adversely influence oogenesis (Lee and Gerking, 1980), spermatogenesis (Kossakowski, 1988) and be responsible for high mortality rates in fertilized eggs and larval stages of fish (Kossakowski, 1988). Leivested and Muniz (1976) report that this may also cause anamolic embryonic developments and episodic fish kills which would lead ultimately to a disappearance of fish populations from many lakes and rivers. The main impact of low environmental water pH on adult fish is still unclear. The most obvious factors are hypoxia caused by various effects of acid water on the respiratory functioning of the gills (Packer and Dunson, 1972; Ultsch *et al*, 1980) salt loss (Ultsch *et al*, 1980) and disturbance of acid base balance of blood (Eddy, 1976). These effects vary with ambient pH level, size of fish, time of exposure and mainly on the fish species (Packer, 1979; Ultsch *et al*, 1980; Ultsch *et al*, 1981).

Haematological and physiological responses of fresh water fish to low water pH have been extensively studied in temperate zones, but information on tropical fish species is scant. *Oreochromis mossambicus* is a tropical fresh water fish speices introduced to Sri Lanka in the nineteen fiftees and is now well established and widely distributed in the inland waters throughout the island. By this study we hope to establish tolerance levels for *Oreochromis mossambicus* to low water pH and to study the effects of low water pH on some of its haematological and physiological parameters.

2. Materials And Methods

Fish were maintained in air saturated water at room temperature and were fed on food pellets throughout the experimental period. In the first set of experiments tolerance of *O. mossambicus* to various acid water levels were determined. A range of acid water concentrations of pH 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 were made up with tap water using concentrated sulphuric acid. The pH of aerated tap water was between 7.0—7.2. Groups of six, randomly selected individuals were transferred abruptly to each of the acclimation baths equipped with aeration. The pH of the acid baths were measured twice daily and maintained in the range of $\pm$ 0.1 unit. The survival times of individual fish in the various media were recorded. Complete arrest of opercular beats followed by complete loss of response to external stimuli were the symptoms used to conclude that the fish were dead. The experiments were carried out for a period of two weeks.

In the second set of experiments haematological and physiological properties were studied in acid acclimated and control animals. pH of the water was lowered to pH 4 from the control level of 7.0-7.2, by addition of sufficient amount of conc. H_2So_4. The pH level was maintained within the range of 4.0 $\pm$ 0.1 units, throughout the acclimation period. Determinations were done on haematocrit, erythrocyte counts, haemoglobin concentrations, O_2 capacity, blood pH, plasma Na + concentrations and O_2 consumption for acid acclimated and control fish. When obtaining blood samples a fish was made unconscious by a blow on its head as quickly as possible. Total duration of the procedure was less than 60 sec. Blood was obtained from the renal artery by cutting off the tail peduncle. Heparin was added so that its concentration in blood was approximately 20iu/ml

Behavioral changes of the fish were also observed. Blood haematocrit values were determined using a haematocrit centrifuge (Hawksley). About 40 μl of blood was obtained into a heparinized capillary tube and centrifuged at 11000 rpm for 5 min. To determine the erythrocyte counts the blood was diluted by a factor of 200 with 0.9% NaCl solution and counts were made using a haemocytometer. Haemoglobin concentrations were determined by the acid hematin method of Cohen and Smith (Oser, 1965). By using these three parameters the haematological indices; haemoglobin content per single erythrocyte (MCH) haemoglobin concentration per single erythrocyte (MCHC) and volume of a single erythrocyte (MCV) were calculated. O_2 capacity was found by the Haldane method using the Haldane apparatus. Blood pH was measured using a pH meter (WTW,522). Na^+ concentration of plasma was found using a flamephotometer (Corning 410). O_2 consumption rate was measured in vivo using a respirometer. Metabolic rates are expressed as O_2 consumption per unit weight of fish.

Results are presented as mean $\pm$standard error of the mean. Differences between treatments were determined by comparison of means using the Student's 't' test. All statistical differences refer to 0.05 level of significance for a two tailed test.

3. Results

Tolerance of *O mossambicus* **to various levels of acidity:**

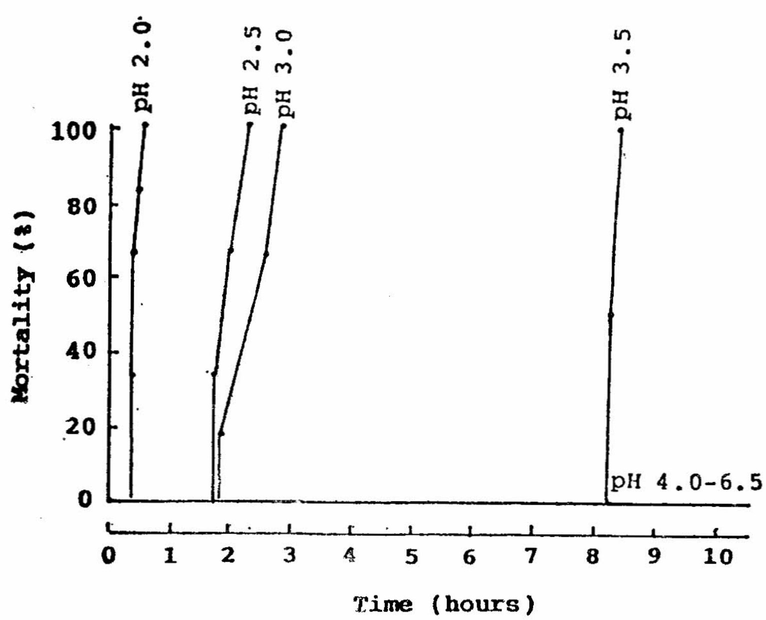

Fig. 1. Tolerance of *Oreochromis mossambicus* to various acid water levels ranging from pH 2.0 to pH 6.5.

Acute acid exposuse of *O. mossambicus* showed high resistance to acid levels from pH 7.0 down to pH 4.0 At water pH levels below 4, the fish did not survive the experimental period. At pH levels of 4 and below they suffered from respiratory distress, markedly shown by the gaping of the mouth, forced expansion of gill opercula and reduction in swimming activity. Poor apetite and increase in pigmentation of body were also apparent. At pH 4 these symptoms lasted for a period of half an hour to one hour, then the fish gradually recovered from the stress, and responded normally throughout the experimental period. No deaths occurred at this level or at higher pH levels. At lower pH levels (ie pH 3.0 and below) they lost their balance, floated with the bodies turned upside down and died. A thick layer of mucus was observed in these fish, covering the head region including the eyes and the gills. At levels below pH 3.0 steep mortality rates were recorded (Fig. 1), and the mean survival times were restricted to 25 min at pH 2.0, 2 hours and 3 min at pH 2.5 and 2 hours and 12 min at pH 3.0. At pH 3.5 the mean survival time was 8 hours and 15 min, a somewhat longer period compared to the previous survival times. At all other pH levels, higher than pH 4.0, the fish responded normally throughout the experimental period.

Effects of low water pH on some haematological and physiological parameters of *O. mossambicus:*

Table 1

Haematological and physiological properties of *O. mossambicus* in fresh water (control fish) and in acid water (acclimated for 2 weeks). All values refer to mean ± standard error.* indicate means significantly different ($P<0.05$) from the control values.

The haematological indices; MCH, MCHC and MCV calculated from the values of haematocrit, RBC count and Hb concentration are also given.

Parameter	Freshwater (pH 7.0—7.2)	Acid water (pH 4.0±0.1)
Haematocrit (%)	17.68±1.13	29.52±0.91*
RBC Count (no. cells/mm^3 blood)	$0.62\times10^6 \pm 4.8\times10^4$	$1.73\times10^6 \pm 5\times10^4$*
Hb concenration (g Hb/100ml blood)	5.49±0.36	7.19±0.23*
O_2 capacity (O_2 ml/100ml blood)	7.26±0.39	7.72±0.61
Blood pH	7.46±0.06	6.96±0.09*
Na^+ concentration (mequiv/l)	139.06±6.54	139.8±9.3
O_2 consumption (O_2 ml/h)	5.08±0.48	5.86±0.55
Metabolic rate (O_2 ml/h/g body wt)	0.136±0.007	0.158±0.016
Haematological Indices		
MCH ($\mu\mu$g)	88.5	41.56
MCHC (%)	31.05	24.35
MCV (μ3)	285	171

Acid acclimation caused significant increases in haematocrit, rbc count and haemoglobin concentration. O_2 capacity elevated from 7.26 to 7.72 mlo_2/100 ml blood in acid acclimated fish, but this was not a significant increase. Blood pH decreased significantly by 0.5 units. O_2 consumption, metabolic rate and plasma Na+ concentration did not change significantly. The three haematological indices were lower in acid acclimated fish than in control fish (table I).

4. Discussion

The test for survival of *O. mossambicus* in various acid levels show that its tolerance level was considerably high in comparison to most other species (brook trout-Packer, 1979; sockeye salmon-Jonas and Tomlinson, 1962). From water of pH 7.0 down to pH 4.5 it did not show any behavioral changes, but at this point it begins to experience difficulties. At pH 4.0, *O. mossambicus* experienced disturbances in its physiological status accompanied by behavioral changes but gradually it compensated these difficulties and adapted to maintain the normal metabolic rates which showed the ability of the fish to fulfil its body's O_2 demands.

The severerity of the effects of low water pH on blood physiological properties may depend on several factors such as water temperature (Cameron, 1978) CO_2 concentration of the water (Eddy and Morgan, 1969), chemical composition of water (Freda and McDonald 1988), seasonal changes (Robinson *et al*, 1976) size and maturity level of fish (Robinson *et al*, 1976) and interspecies variations (Freda and McDonald, 1988). Fish deaths at low environmental pH has been attributed to several factors, such as suffocation due to coagulation of mucus on the gills (Plonka and Neff, 1969; as quoted by Ultsch *et al*, 1981) decreased partial pressure of O_2 in blood (Dheer *et al*, 1987) and decreased blood O_2 carrying capacity as blood pH is lowered (Green and Root, 1933). Packer and Dunson (1972) found that exposure of brook trout *Salvelinus fontinalis* to low pH (below 3.5) caused a significant loss of body Na+, lowering of blood pH and O_2 consumption rate. In this study we found that exposure of *O. mossambicus* to pH 4 caused a significant decrease in blood pH, but plasma Na+ level, O_2 consumption and metabolic rate remained unaltered. Packer (1979) has reported that fish deaths occurred once blood pH fell below 6.97 in brook trout. Jonas and Tomlinson (1962) reported fish deaths at a blood pH of 6.8 in sockeye salmon. In the present study 100% survival was observed in *O. mossambicus* at a blood pH of 6.96. Green and Root (1933) found that at blood pH values below 7.0, O_2 carrying capacity was severely affected and this had lethal effects on the lives of fish. Townsend and Cheyne (1944) have shown a decreased ability of fish to extract O_2 from water of low pH. In *O. mossambicus* the lowering of O_2 carrying capacity due to low bood pH was probably compensated by changes in haematological parameters. The values of haematocrit, rbc count and haemoglobin concentration showed significantly higher values than for control fish. These elevations most probably compensated the drop in O_2 carrying capacity caused by the fall of blood pH, so that the overall O_2 capacity did not change significantly. By this mechanism of respiratory regulation, these fish have been able to carry out their metabolic processes successfully. This was apparent by the unaltered O_2 consumption and metabolic rates.

The pattern of changes in haematological indices; MCH, MCHC and MVC in acid acclimated *O. mossambicus* was very significant. This is more or less in agreement with the findings of other authors. The MCH and MCV values of acid acclimated fish showed that the size and haemoglobin content of a single erythrocyte had reduced to approximately half of its normal value. Dheer *et al* (1987) studied a population of different developing stages of red blood cells in haematopoietic tissues and observed a distinct decrease in mature erythrocytes with increasing acidity and exposure time in *Channa punctatus* This type of phenomena may explain the reduction in the haemoglobin content, concentration and volume of a single erythrocyte observed in *O. mossambicus* in this study.

Plasma Na^+ concentration of *O. mossambicus* did not change at this level of pH showing that this fish is able to overcome the increased H^+ concentration and to maintain ionic regulations of the body.

This study reveals that *Oreochromis mossambicss* is a hardy fish compared to other temperate species which have been studied in respect of their tolerance to acid water pollution. This is hardly true for other species of fish, found naturally in Sri Lankan waters, which are probably more sensitive to acidity and have lower thresholds to acid tolerance.

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