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The effect of water pH on swimming performance in rainbow trout (*Salmo gairdneri*, Richardson)

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Abstract

A Brett-type respirometer was used to measure the effect of water pH on swimming performance of rainbow trout (*Salmo gairdneri*). Variations in water pH between 6 and 9 had no measurable effect on maximum aerobic swimming speed. At water pH 4, 5, and 10, however, the critical velocity was only 55, 67, and 61% respectively of that recorded for fish in water of pH 7. Exposure to acid conditions increased coughing and breathing frequency. Acid exposure resulted in a decrease whereas alkaline exposure resulted in an increase in both whole blood and red blood cell pH. Blood gas and acid-base characteristics showed little change during swimming at ~2.0 BL/second, but exhaustive swimming resulted in a marked and immediate drop in blood pH in fish in acid, alkaline and neutral water. The blood acid-base status was restored to resting levels after exercise in neutral and alkaline water, but the acidosis was maintained following exercise in acid water. Fatigue occurred earlier and blood lactate levels increased to a higher level in fish swum to exhaustion in acid or alkaline water, compared with fish in neutral water.

Introduction

Fish are found in most natural water bodies. The pH of these waters varies, and many have been acidified due to the large scale production of chemical waste. Fish are chronically exposed to, and must often migrate through, waters with marked changes in pH.

There is now an extensive literature on the effect of low pH environments on freshwater fish (see Wood 1989, for review). Many studies have tried to illucidate the physiological mechanisms of acid toxicity to fish. Disturbances to ionoregulation and acid-base state are particularly well documented (Packer and Dunson 1970; Neville 1979; Packer 1979; McDonald *et al.* 1980; Wood and McDonald 1982). In addition, studies of the effects of acid waters on oxygen transport (Vaala and Mitchell 1970; Packer 1979) and exercise (Graham and Wood 1980) have been reported.

The resistance of various freshwater fish to strong alkalies (*i.e.*, sodium, potassium, and calcium, hydroxides) in distilled and natural waters has been reported (see Douderoff and Katz 1958, for review). In general, pH levels above 9 are often lethal to freshwater fish, with only certain species surviving at pH 10, *e.g.*, the tilapia *Oreochromis alcalicus grahami* (Randall *et al.* 1989). Detailed investigations of the physiological effects of alkaline water on fish, however, are more recent. Exposure of rainbow trout to alkaline conditions impaired ammonia excretion and sodium influx (Wright and Wood 1985), causing elevated blood ammonia levels (Randall and Wright 1989).

Little is known about the effect of water pH on the swimming performance of fish. Fish in the wild must swim actively in a variety of normal behaviours (feeding, avoiding predation, migration, spawning etc.); if swimming ability is impaired in acid or alkaline water, the survival of the fish population may be in jeopardy. The purpose of these experiments was to determine the effect of water pH on swimming performance in rainbow trout (*Salmo gairdneri*).

Materials and methods

Experimental animals

Experiments were carried out on rainbow trout (*Salmo gairdneri*), body weight 200–400 g, fork length 20–35 cm, from the same source and maintained as described by Ye *et al.* (1990).

Experiment I: Measurement of swimming velocity

A Brett-type respirometer, previously described by Kiceniuk and Jones (1977), was used to measure the critical swimming velocity (see Beamish 1978; Hoar and Randall 1978) at a water temperature of 16–17°C. The fish were exercised in the respirometer before each experiment in order to accustom them to the swimtube. The fish were then held in small individual chambers for 14h in aerated water of a known pH, before being transferred back to the respirometer. The water from the chamber was recirculated through the respirometer as well as a large reservoir (120l). The water in the reservoir was aerated continuously and pH was adjusted to a given level (pH 4, 5, 6, 7, 9 or 10) by adding concentrated HCl or NaOH, as described by Ye *et al.* (1990).

Fish were exposed for a total of 24h to the experimental pH (14h in the holding chamber + 10h in the respirometer), before the critical velocity of the fish (U_{crit}) was measured, using increments of 0.5 body lengths per second (BL/sec) at 0.5h intervals, until the fish was exhausted, and would not move from the back grid.

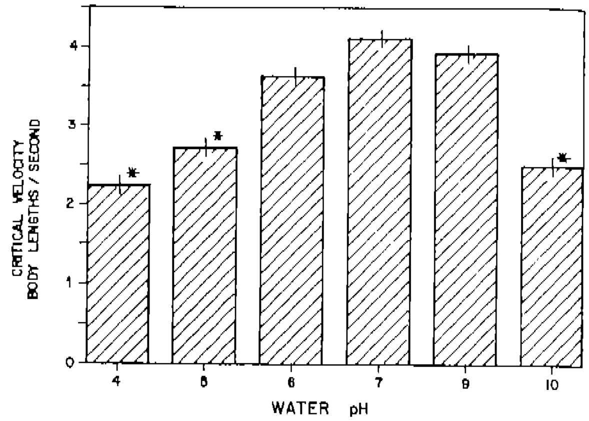

Fig. 1. The effect of water pH on the critical swimming velocity of rainbow trout; * = significantly different from neutral control ($p < 0.05$).

Swimming speed was corrected for the blocking effect of the maximal cross-sectional area of the fish, which causes a narrowing of the available water channel, accelerating water flow over the body (see Beamish 1978).

At pH 4 and pH 7, the breathing and cough frequency of fish were visually determined before exercise.

Experiment II: Swimming cannulated fish

The experiments were repeated on the second group of fish in water of either pH 4 ($n = 6$), pH 7 ($n = 6$) or pH 10 ($n = 3$). The fish were implanted with chronic dorsal aortic cannulae and allowed to recover as described by Ye *et al.* (1990). The fish were placed in the respirometer and exercised. Blood was sampled under three conditions: (1) resting (after 10h in the respirometer swimming at 1.0 BL/sec); (2) fatigue (when the fish was exhausted and would not move off the back grid); (3) recovery (after 2h recovery in the respirometer at 1.0 BL/sec). At each sampling time, 500 μl blood was removed from the fish and replaced with heparinized (10 iμ/ml) Cortland saline. Whole blood pH (pH_e), blood total CO_2 ($CaCO_2$), plasma lactate (La^-) and erythrocyte pH (pH_i) was measured as described by Ye *et al.* (1990).

All data are given as arithmetic means ± 1 SEM. The significance of value changes from the control

Table 1. The effect of acid water on whole blood pH (pH_e), red cell pH (pH_i), arterial blood total CO_2 ($CaCO_2$), blood lactate (La^-) concentration in rainbow trout following exhaustive exercise

	Resting	Fatigue	2h recovery
pH_e			
neutral	7.89 ± 0.02 (6)	7.69 ± 0.04 (5)*	7.85 ± 0.02 (5)
acid	7.82 ± 0.02 (6)**	7.69 ± 0.01 (5)*	7.59 ± 0.02 (4)***
pH_i			
neutral	7.46 ± 0.03 (6)	7.46 ± 0.03 (5)	7.49 ± 0.03 (5)
acid	7.33 ± 0.02 (6)**	7.37 ± 0.04 (5)***	7.42 ± 0.02 (4)***
$CaCO_2$ (mM)			
neutral	7.65 ± 0.03 (6)	7.05 ± 0.03 (5)	8.52 ± 1.19 (4)
acid	6.10 ± 0.53 (6)	4.55 ± 0.26 (5)***	4.54 ± 0.44 (4)***
La^- (mM)			
neutral	0.73 ± 0.28 (6)	3.75 ± 1.25 (5)*	5.94 ± 1.25 (4)*
acid	1.43 ± 0.52 (6)	3.79 ± 0.67 (5)*	6.70 ± 0.94 (4)*

Data expressed as mean ± SEM (n); * = significantly different from resting level; ** = significantly different from neutral water level ($p < 0.05$); *** = * and **.

conditions (*) ($p < 0.05$) were determined using the paired (or unpaired as appropriate) Student's t-test.

Results

Experiment I: Swimming uncannulated fish

The effects of water pH on the critical velocity of rainbow trout are shown in Fig. 1. The reduction in swimming speed for fish in water of pH 6 and 9 was not significant compared with that recorded for fish in pH 7 water. The U_{crit} for fish in water of pH 4, 5, and 10, however, was only 55%, 67% and 61% respectively of that recorded for fish in water of pH 7. The reduction in swimming speed was, in these cases, significantly different ($p < 0.05$) from that at pH 7.

Exposure to acid conditions (pH 4) increased coughing and breathing frequency in resting fish. Coughing rate increased significantly from a mean of 0.7/min to 9.8/min, and breathing rate also increased significantly from a mean of 81/min to 104/min when fish was transferred from water at pH 7 to water at pH 4.

Experiment II: Swimming cannulated fish

As in experiment 1, the fish in either acid or alkaline water had a reduced critical velocity and fatigued more quickly than fish in neutral water. The effect of acid water on blood characteristics during swimming is shown in Table 1. The effect of acid and alkaline water on pH_e and pH_i during swimming is shown in Fig. 2. In neutral water, pH_e was decreased significantly at the time of fatigue but was restored to resting levels after 2h recovery. Erythrocytic pH was maintained throughout the activity and then was slightly increased above resting levels after 2h recovery. Exposure of resting fish to acidic water induced a small but significant fall in both plasma and red blood cell pH. In addition, pH_e was decreased significantly at the time of fatigue and was reduced even more after 2h recovery. pH_i was not affected by exercise-induced extracellular acidosis during active swimming in acid water and was even significantly increased after 2h recovery. Thus, acid water induced a significant fall in both pH_e and pH_i, but pH_i was not affected by an exercise induced extracellular acidosis.

There was a marked increase in pH_e in resting fish in alkaline water. During swimming there was

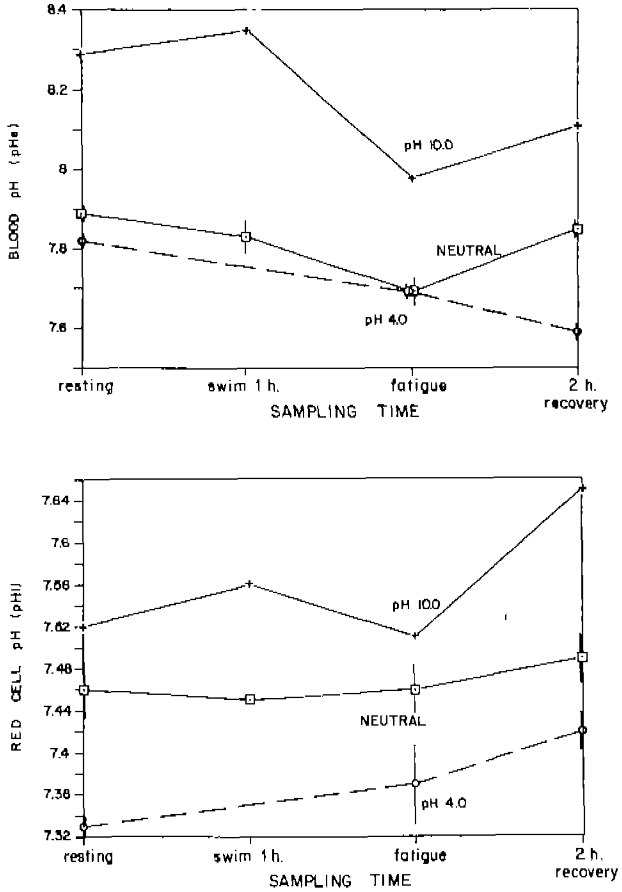

Fig. 2. The effect of water pH on (A) whole blood pH (pH_e) and (B) red cell pH (pH_i) in rainbow trout before, during and after being swum to fatigue. Data expressed as mean ± SEM, alkaline exposure n = 2; n for acid exposure, see Table 1; * = significantly different from resting level and ** = significantly different from neutral control ($p < 0.05$).

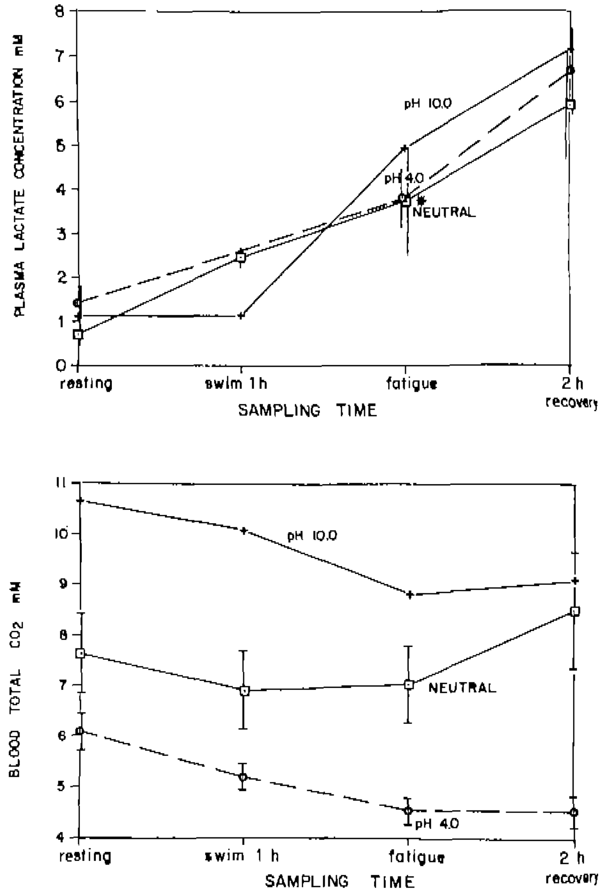

Fig. 3. The effect of water pH on the plasma lactate and blood total CO_2 concentrations in rainbow trout before, during and after being swum to fatigue. Data expressed as mean ± SEM for n see Fig. 2; * = significantly different from resting level and ** = significantly different from neutral control ($p < 0.05$).

a further increase in pH_e from 8.29 to 8.36, but exhaustive swimming resulted in a marked drop in pH_e to 7.98; pH_e was back up to 8.11 after 2h recovery. Alkaline water induced a marked increase in pH_i in resting fish. During swimming, pH_i increased slightly from 7.52 to 7.56 and increased to 7.65 after 2h recovery in alkaline water.

The change of La^-, and $CaCO_2$ during swimming in acid, alkaline and neutral water is shown in Fig. 3 and Table 1. La^- levels at the time of fatigue were increased significantly above resting levels, and continued to rise during 2h recovery in neutral, acid and alkaline water. Similar increases in La^- were observed despite the fact that fish swimming in acid and alkaline water showed a decrease in U_{crit} and fatigued more quickly than fish in neutral water.

Compared with fish in neutral water, $CaCO_2$ in fish exposed to acid water decreased significantly during and after burst swimming. Conversely, $CaCO_2$ increased markly from 7.65 mM in neutral water to 10.66 mM in alkaline water, but then decreased to 8.89 mM after exercise.

Discussion

The results of these experiments demonstrate that exposure of fish to either acid or alkaline water reduces the critical velocity of trout. Waiwood and Beamish (1978) found that critical speed was not appreciably influenced by hardness, pH, or time of exposure. The water pH they used, however, was from 6 to 8. In our experiments, we also found that U_{crit} was not appreciably decreased in water between pH 6 and 9, but in water pH below 5 and above 9 the U_{crit} decreased significantly. Graham and Wood (1980) measured the effect of low pH on fingerling rainbow trout, weight: 3.50 ± 0.09 g, length: 7.56 ± 0.30 cm, using sulfuric acid acidification. They found that, below pH 4.6 (soft water) or 4.4 (hard water), critical velocity declined linearly by about 4% per 0.1 pH unit. We observed a larger decrease in the maximum critical velocity in acid waters. In Graham and Wood's (1980) study, the chemical composition of the water was different, the fish were smaller and were exercised as soon as they were exposed to the low pH water. When we exercised fish as soon as they were exposed to the low pH water (pH 4) the reduction of critical velocity was less than 20%, whereas after 24h exposure, the reduction was 46%. Thus exposure time, as well as the pH of water, influences the U_{crit} of fish. The longer the fish is exposed to low pH water, the greater the reduction in swimming speed.

An increase in H^+ ions causes a greater reduction in swimming performance than an equivalent increase in OH^- ion concentration. The reason for this may be that when fish exercise, they generate many acidic products and, in acid water, may have problems dealing with the resulting acidotic state. Alkaline water also impairs swimming performance, but presumably due to different causes than those in acid waters.

Fish exercised in acid water, compared to neutral water, had a much lower initial pH_e and pH_i. In addition, blood pH_e was not restored to the resting levels after 2h recovery in acid water. Fish in neutral water excrete some of the acid produced during exercise across the gills (Heisler 1984). In acid water, the large H^+ gradient from water to the body of the fish probably reduced acid excretion across the gills and impaired the ability of the fish to recover from the exercise induced acidotic state.

It has been reported that oxygen transport (Primmett *et al.* 1986) and U_{crit} (Randall *et al.* 1987) are not inhibited by exercise-induced extracellular acidosis in salmonids. The maintenance of blood oxygen content is associated with a maintenance of red cell pH due to an increase in plasma catecholamine levels (Nikinmaa 1983). Adrenergic regulation of red cell pH allows normal Hb-O_2 saturation in the face of an exercise-induced extracellular acidosis (Primmett *et al.* 1986). In acid exposed resting fish, red cell pH decreased significantly from 7.46 in neutral water to 7.33 in acid water (Fig. 2). Thus, during acid exposure of fish, the Bohr and Root effects were not ameliorated by the action of catecholamines and, as a result, blood oxygen content was probably reduced. Blood oxygen content was adequate for the requirements of the resting fish as oxygen consumption was not affected (Ye *et al.* 1990). During exercise, however, the capacity to deliver oxygen to the muscles determines critical swimming velocity (Jones 1971) and the reduced blood oxygen content due to acid exposure caused a decrease in U_{crit}. pH_i in fish exposed to acid water, however, was higher at fatigue than before swimming, probably as a result of the release of catecholamines into the blood. Nevertheless, pH_i was still below that seen in fish recovering in neutral water (Fig. 2).

In alkaline water, the H^+ ions produced by exercise only offset the already high pH_e so that the pH_e decreased from 8.29 to 7.98. Clearly, the development of an acidotic state cannot account for the reduction in swimming ability under alkaline conditions and there is no other clear reason as to why swimming is impaired. One possibility is the accumulation of ammonia in the body. Water pH has a marked effect on ammonia excretion (Wright and Wood 1985) and ammonia is retained in the body under alkaline conditions (Randall and Wright 1989).

The tilapia, *Oreochromis alcalicus grahami*, survives in alkaline Lake Magadi (pH 9–10.5) in the Kenyan Rift Valley, in part, because it can detoxify ammonia by producing urea *via* the ornithine-urea cycle (Randall *et al.* 1989). Tilapia sp. have extremely low surface permeabilities to ions and this also may confer upon them the capacity to survive in these extreme conditions which also have high ionic content (Maloiy *et al.* 1978). Maetz and De Renzis (1978) studied aspects of the adaptation of fish to high external alkalinity and found pH_e

increased (see also Johansen *et al.* 1975), gill potential decreased and there was a 9-fold stimulation of Na^+ and Cl^- influx in *T. mossambica*. Rainbow trout cannot produce large amounts of urea (Olson and Fromm 1971) and alkaline conditions reduce Na^+ influx and ammonium excretion (Wright and Wood 1985). Thus, unlike the Lake Magadi tilapia, trout seem to lack those characteristics that allow fish to survive in alkaline environments.

In conclusion, acid or alkaline conditions clearly reduce the critical swimming velocity of rainbow trout. The farther the water pH is from neutrality and the longer the fish is exposed, the more the swimming capacity of the fish is impaired.

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