J. exp. Biol. **165**, 195–212 (1992)
Printed in Great Britain © *The Company of Biologists Limited 1992*

INTERACTIVE EFFECTS OF SEASONAL TEMPERATURE AND LOW pH ON RESTING OXYGEN UPTAKE AND SWIMMING PERFORMANCE OF ADULT BROWN TROUT *SALMO TRUTTA*

BY P. J. BUTLER[1], N. DAY[1] AND K. NAMBA[2]

[1]*School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK* and [2]*Faculty of Applied Biological Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, 724 Japan*

Accepted 6 January 1992

Summary

Adult brown trout were acclimated for 2–4 weeks to artificial, soft water (Ca^{2+} 25 μmol l^{-1}) at neutral pH and at summer (15°C) or winter (5°C) temperatures. During this period they swam against a current of approximately 0.25 m s^{-1}. They were then exposed to neutral or sublethal pH for 4 days in still water. For fish with their dorsal aorta catheterized, sublethal pH was 4 at 5°C and 4.5 at 15°C.

After 4 days of exposure to sublethal pH, resting oxygen uptake ($\dot{M}_{O_2}$) was 40 % higher than that at neutral pH for fish held at 15°C and 38 % higher for fish held at 5°C. Critical swimming speeds (U_{crit}), in contrast, were 35 % and 31 % lower, respectively. These two phenomena may be related in as much as the 'metabolic cost' of exposure to low pH may increase as swimming speed increases, thus reducing the scope for activity. Another important factor could be an impairment of oxygen delivery to the red muscle fibres. Although arterial O_2 concentrations and heart rate are both similar for fish at U_{crit} in neutral and acid water, there are signs of haemoconcentration in fish exposed to low pH, and the consequent increase in blood viscosity could disrupt the local circulation in the red fibres. Whatever its causes, an impairment of swimming activity resulting from exposure to acid water may have severe consequences for active fish such as salmonids.

Exposure to sublethal pH caused significant reductions in plasma Na^+ and Cl^- concentrations at both temperatures, although these were more substantial at 5°C than at 15°C. Swimming at U_{crit} had no significant effect on plasma concentrations of Na^+, K^+ and Cl^- except at sublethal pH at 5°C, when there were significant reductions in all three.

Seasonal temperature had significant but small effects on resting $\dot{M}_{O_2}$ and U_{crit}, and these are discussed in terms of the possible effects of low temperature and continued swimming activity (training) on hypertrophy of skeletal and cardiac muscles and on the aerobic capacity of the 'red' muscle fibres.

Introduction

The effects of naturally occurring environmental factors, such as temperature

Key words: temperature, pH, swimming, brown trout, *Salmo trutta*.

and oxygen availability, on the swimming performance of fish have been reasonably well studied (Beamish, 1978). However, as Beamish (1978) points out, studies on the influence of factors introduced into the environment directly or indirectly by man have largely been restricted to the determination of concentrations that are lethal to inactive animals, while only a few of the many factors that may contribute to disturbances in the environment have been examined for their influence on swimming performance. This is rather strange, because scope for activity has been recommended for use as a criterion in the determination of the sublethal effects of pollutants on fish (Sprague, 1971).

Sustained swimming activity, e.g. during migration, is aerobic, with little accumulation of lactic acid in the blood (Driedzic and Kiceniuk, 1976; Butler *et al.* 1986), although there may be temporary accumulation of lactate in the muscles at the onset of relatively high sustainable swimming speeds in rainbow trout (Wokoma and Johnston, 1981). These authors suggested that this lactate may actually be oxidized by the red fibres, although this seems not to be the case (Weber, 1991). In addition to problems relating to oxygen supply and the degree of net anaerobic metabolism during exercise, there are also increased passive fluxes of ions and water across the gills of freshwater fish when swimming (Wood and Randall, 1973*a*,*b*).

In rainbow trout *Oncorhynchus mykiss* (Walbaum), acid water (pH 4.0–6.0) causes decreases in plasma concentrations of Na^+ and Cl^-, to a greater or lesser extent, depending on water hardness (McDonald *et al.* 1980; Playle *et al.* 1989), together with increases in haematocrit and plasma protein concentration (McDonald *et al.* 1980). The latter two effects are the result of at least three factors: (a) movement of water from the extracellular to the intracellular space (itself a consequence of branchial ion loss); (b) swelling of the red blood corpuscles; and (c) an increase in the number of red blood cells. Such haemoconcentration causes a large increase in blood viscosity, which may eventually lead to circulatory failure (Milligan and Wood, 1982). Exposure to soft, acid water also causes an increase in blood lactate concentration (McDonald *et al.* 1980), which may be indicative of impaired oxygen transport. In very soft water there is little or no blood acidosis (Wood, 1989).

The synergistic effect of acid water and strenuous exercise on rainbow trout has been demonstrated by Graham *et al.* (1982). Severe (i.e. burst) exercise caused approximately 40 % mortality during the 12 h recovery period when the fish were at near neutral pH (approximately 7.5). Severe exercise in soft water at a pH of approximately 4.5 (which in resting fish caused no mortality over a 12 h period) increased mortality during the 12 h period following exercise to 80 %. These authors conclude that their data indicate 'that physiological tests on resting fish may underestimate or misinterpret the toxic effects of acid in the wild where fish are intermittently very active'.

It is certainly true that fish may be very active for short periods when escaping from predators or capturing prey, but sustained, aerobic swimming is also an important aspect of the lives of some fishes, particularly during migration. There

are three reports on the effect of acid water on the swimming ability of rainbow trout. Graham and Wood (1981) found that critical swimming speed (U_{crit}; Brett, 1964) of fingerlings was significantly depressed at pH values below 4.6 in soft water. The fish were not acclimated to the test pH and no physiological measurements were made. The authors suggested that impairment of gas exchange and/or of oxygen transport were the major factors affecting the fishes' swimming performance. More recently, Ye and Randall (1991) found that 24 h of exposure to high pH (>pH 9) or low pH (<pH 6) reduced swimming performance in adult trout. In an accompanying paper, Ye *et al.* (1991) suggested (but did not demonstrate) that exposure to low pH reduced the oxygen content of arterial blood and, therefore, that oxygen transport capacity was impaired. Waiwood and Beamish (1978) found that exposure to water at pH 6 had no effect on the swimming performance of fingerling trout.

Environmental acidification is essentially unique to soft water (Wood and McDonald, 1982). Acute exposure to low pH (4.0–4.5) is not uncommon in natural soft waters in Northern Europe as a result of snow melt in the spring or highly acidic run-off in the summer and autumn (Wood, 1989). Because temperature affects the survival of fingerling rainbow trout exposed to lethal, low pH (Kwain, 1975), the sublethal values of pH for adult trout may be different in winter and summer. There is no doubt that, in many field situations, metals such as aluminium and copper are mobilized by acidified soft water. However, it is likely that acidity itself is the dominant toxic agent under acute conditions (Wood, 1989).

The objective of the present study was to determine the effects of exposure to sublethal (acid) pH on the resting oxygen uptake and sustainable swimming capabilities of adult brown trout in soft water, and at summer and winter temperatures. Physiological data were obtained in order to determine whether impaired gas exchange and/or oxygen transport, or circulatory failure resulting from excessive ion loss, could be the cause of any observed differences in swimming performance.

Materials and methods

The animals and their holding conditions

Brown trout, *Salmo trutta* (L.) (total length 30–46 cm, mass 320–520 g) were obtained from the Leadmill trout farm, Hathersage, Derbyshire (water composition, $\mu mol\,l^{-1}$: Ca^{2+}, 245–290; Na^{+}, 330–370; K^{+}, 40–90), and kept for 2–4 weeks in large (1400 l) circular glass fibre tanks through which dechlorinated Birmingham tapwater (composition, $\mu mol\,l^{-1}$: Ca^{2+}, 130–200; Na^{+}, 260–400; K^{+}, 6–26) was flowing at a rate of $120\,l\,h^{-1}$. The water was aerated vigorously and circulated around the tank using a pump and spray bar which produced jets of water that were nearly horizontal when they hit the water surface. The fish were provided with plastic tubes (10 cm in diameter 55 cm in length) suspended in mid water, in which they could position themselves. Because the water was circulating

around the tank (at approximately 0.25 m s^{-1}) and through the tubes, the fish had to swim to maintain position.

Following the initial acclimation period, the fish were transferred into a similar tank containing artificial lakewater (Dalziel *et al.* 1985), also circulating at 0.25 m s^{-1} and of the following composition (μmol l^{-1}): Ca^{2+}, 25; Na^{+}, 50; K^{+}, 5; Mg^{2+}, 40; Cl^{-}, 100; SO_4^{2-}, 65; NO_3^{-}, 5. This concentration of Ca^{2+} is similar to that found in some areas of the UK, e.g. Galloway (Harriman *et al.* 1987), Mid Wales and North Wales (Turnpenny *et al.* 1987). Dechlorinated water was passed through a deioniser (Permutit TS100) at a rate of 120 l h^{-1} and into a mixing tank. A concentrated solution of the above ions was then added to the deionized water at a rate known to give the required composition. Titanium cooling coils and small aquarium heaters maintained the temperature of the water in the two large holding tanks at the required level, which was 5°C in winter (November to March inclusive, 4–7°C at the trout farm) and 15°C in summer (June to mid-September, 12–16°C at the trout farm). The pH of this water was maintained at 7±0.1 (range) using an Analytical Instruments controller, a Russell CT757 low-conductivity pH electrode and a CP Instruments variable-speed peristaltic pump which delivered 0.25 mol l^{-1} NaOH when required. The fish were kept under these conditions for a further 2–4 weeks after the initial period in Birmingham tapwater. Throughout the acclimation period the fish were exposed to the natural photoperiod and were fed daily on floating pellets [Mainstream trout diet, B.P. Nutrition (UK) Ltd]. All uneaten pellets were removed from the tank an hour after feeding. No food was given the day before transfer of the fish to an experimental tank or during the experimental period.

Determination of sublethal pH

The first task was to determine the sublethal pH for the fish. Following acclimation to artificial lakewater, the dorsal aorta was catheterized in five fish, which were anaesthetized in buffered MS222 as described by Butler *et al.* (1986). The five fish were placed in a smaller circular plastic tank (280 l) through which artificial lakewater (pH 7) at the experimental temperature was flowing at 120 l h^{-1}. (Note: the water was not circulated around this tank.) The fish were allowed to recover for 2 days. They were then kept at a low pH for a further 4 days. Initially the pH was set at a level which was thought to be close to, but above, the lethal level (Grande *et al.* 1978; Wood *et al.* 1988), so that all the fish survived. Successive batches of five fish were placed in water of progressively lower pH (pH was reduced by 0.25 units for each batch) until one or more fish died during the 4-day exposure period. The pH immediately above that value was designated the sublethal level. This proved to be a very repeatable test, and at 5°C the sublethal pH was found to be 4, while at 15°C it was 4.5. Similar tests were performed on fish that had been anaesthetized, but not cannulated. The sublethal pH values in this case were 3.75 at 5°C and 4.25 at 15°C. pH was controlled to an accuracy of ±0.05 units (range) by a Radiometer TTT2 titrator, a Russell CT757 low-conductivity electrode and a CP Instruments variable-speed peristaltic pump

which delivered NaOH ($0.25\,mol\,l^{-1}$) or H_2SO_4 ($1\,mol\,l^{-1}$), as required. The accuracy of the pH measurement was tested against a system selected for use with solutions of low ionic strength: a Radiometer PHM 71 Mk2 acid–base analyser with a Corning 003 11 101J triple-purpose glass electrode and Beckman Futura Plus reference electrode (Davison and Woof, 1985).

Experimental procedure

Experiments on uncannulated fish

Resting oxygen uptake ($\dot{M}_{O_2}$) and critical swimming speed (U_{crit}) were determined in the anaesthetised but uncannulated fish following 2 days of recovery and 4 days of exposure to artificial lakewater at neutral pH or at the sublethal pH relevant to the particular temperature. For determination of $\dot{M}_{O_2}$, fish were placed individually in a darkened respirometer tube either 40 cm or 50 cm in length and 9 cm in diameter. Water flowed through the tube at 300–400 ml min^{-1} at 5 °C and at 400–600 ml min^{-1} at 15 °C, so that the difference between the P_{O_2} of incurrent water (P_{inc,O_2}) and that of excurrent water (P_{exc,O_2}) was approximately 2 kPa. The fish were placed in the respirometer at approximately 11:00 h on day 1 and $\dot{M}_{O_2}$ was determined 3–4 times during a 60- to 90-min period at approximately 18:00 h, and then in the same way and at the same times (11:00 h and 18:00 h) on each of the successive 6 days. The mean of the 3–4 determinations was calculated for each sampling time. Because the respirometer tubes were darkened to avoid disturbing the fish, it was not always possible to determine the level of activity of the animals. However, when it was obvious that a fish was active, data obtained during that period were not incorporated into the subsequent analysis. For fish at neutral pH, $\dot{M}_{O_2}$ declined over the first 2–3 days, so the mean resting values were calculated from the lowest four data points obtained after the settled rate had been reached. For fish initially at neutral pH and subsequently transferred to sublethal pH, the mean of the last two data points (see Fig. 1A,B) was taken to represent $\dot{M}_{O_2}$ at sublethal pH.

U_{crit} (Brett, 1964) was determined in a variable-speed, Blazka-type water channel (see Axelsson and Nilsson, 1986, for details) of total volume 200 l with a test section 65 cm long and 15 cm in diameter. Water flow through the complete system was at $40\,l\,h^{-1}$ and water speed through the test section could be varied between 0 and $1\,m\,s^{-1}$ and was determined by a revolution counter calibrated against a Braystoke BFM 002 current flowmeter. Each fish was swum for 15 min at 0.2 or $0.3\,m\,s^{-1}$ and then for the same time at increasing $0.1\,m\,s^{-1}$ increments until it ceased to swim and rested on the back grid. The only attempts made to encourage the fish to swim were to cover the anterior portion of the swim chamber while brightly illuminating the rear and to reduce the water speed slightly after the fish had stopped swimming. Invariably it began to swim again and the water speed was returned to its previous value. This usually gave sufficient time for a blood sample (2–3 ml) to be taken from cannulated fish (see next section) before they stopped swimming again. This was taken as the U_{crit} (Butler *et al.* 1986) and was

not corrected for the presence of the fish in the water channel (see Jones *et al.* 1974, for a discussion).

Experiments on cannulated fish

After acclimation, fish were anaesthetised in buffered MS222 and the dorsal aorta was cannulated. The animals were placed into a Blazka-type water channel and left to recover for 2 days. They were then left for a further 4 days at neutral pH or at the sublethal pH relevant to the particular temperature. The pH of the experimental water was controlled to an accuracy of ±0.05 unit by a Radiometer PHM82 standard pH meter, with a TTT80 titrator, a Russell CT757 combination electrode and a CP Instruments variable-speed peristaltic pump, as described above. Experiments were performed alternately at neutral and sublethal pH to avoid seasonal bias. At the end of the exposure period, data were collected from resting fish or from fish that had been swum up to their U_{crit}. Dorsal aortic blood pressure was recorded on a two-channel pen recorder (Lectromed Ltd), from which heart rate could also be determined. 2–3 ml of arterial blood was removed from the dorsal aorta (see previous section) for the determination of partial pressure of oxygen (Pa_{O_2}), using a Radiometer BMS3 blood micro system and a PHM 73 pH/blood gas monitor, oxygen content (Ca_{O_2}), using a Lexington Instruments Lex-O_2-Con, haematocrit (Hct), using a Hawksley microhaematocrit centrifuge, concentrations of plasma Na^+ and K^+, using a Pye Unicam SP9 atomic absorption spectrophotometer, and of plasma Cl^-, using an Aminco chloride titrator. Haemoglobin concentration [Hb] and plasma protein concentration were determined with Sigma kits no. 525 and no. P5656, respectively. Water in the holding tanks and water channel was monitored weekly for P_{O_2}, CO_2 content and total ammonia content (Verdouw *et al.* 1978). The first was always greater than 20 kPa, the second less than 0.1 mmol l^{-1} and the third less than 20 μmol l^{-1} in the holding tanks and less than 10 μmol l^{-1} in the water channel.

All values are given as the mean±S.E. and the significance of the differences between mean values was assessed using Student's *t*-test, with $P<0.05$ being taken as the probability level for significance.

Results

Resting $\dot{M}_{O_2}$ and U_{crit} in uncannulated fish (Table 1)

Seasonal temperature had small but significant effects on resting $\dot{M}_{O_2}$ and U_{crit}. Resting $\dot{M}_{O_2}$ at neutral pH was 22 % lower at 5 °C than at 15 °C, and U_{crit} (BL s^{-1}) was 15 % lower at 5 °C. Exposure to sublethal pH had much larger effects, which are highly significant at both temperatures. At 15 °C, resting $\dot{M}_{O_2}$ at sublethal pH was 40 % higher than that at neutral pH, whereas U_{crit} (BL s^{-1}) was 35 % lower. At 5 °C, resting $\dot{M}_{O_2}$ at sublethal pH was 38 % higher than that at neutral pH, and U_{crit} (BL s^{-1}) was 31 % lower. After exposure to sublethal pH, there was an immediate increase in $\dot{M}_{O_2}$ and these levels were maintained for approximately 3 days before $\dot{M}_{O_2}$ declined somewhat (Fig. 1A,B).

Table 1. *Resting oxygen uptake and critical swimming speed of uncannulated brown trout after exposure for 4 days to artificial lake water at 15 or 5°C and at neutral or sublethal pH*

N	Temperature (°C)	pH	Total length (cm)	Mass (kg)	Resting $\dot{M}_{O_2}$ (mmol kg^{-1} h^{-1})	U_{crit} ($BL\,s^{-1}$)	U_{crit} ($m\,s^{-1}$)
10	15	7	35.0±2.4	0.43±0.018	2.17±0.12*,††	2.18±0.13*,†††	0.78±0.04*,†††
14	15	4.25	35.2±3.6	0.43±0.013	3.03±0.21††	1.42±0.05†††	0.50±0.02†††
18	5	7	33.7±1.6	0.40±0.006	1.70±0.12*,†	1.85±0.08*,†††	0.62±0.02*,†††
14	5	3.75	32.7±2.1	0.37±0.011	2.35±0.22†	1.27±0.08†††	0.42±0.03†††

Values are given as mean±S.E., N=number of fish.
* Indicates significant effect of temperature at a given pH.
† Indicates significant effect of pH at a given temperature.
One, two or three symbols signify $P<0.05$, 0.01 or 0.001, respectively.
U_{crit}, critical swimming speed; $\dot{M}_{O_2}$, oxygen uptake; BL, body length.

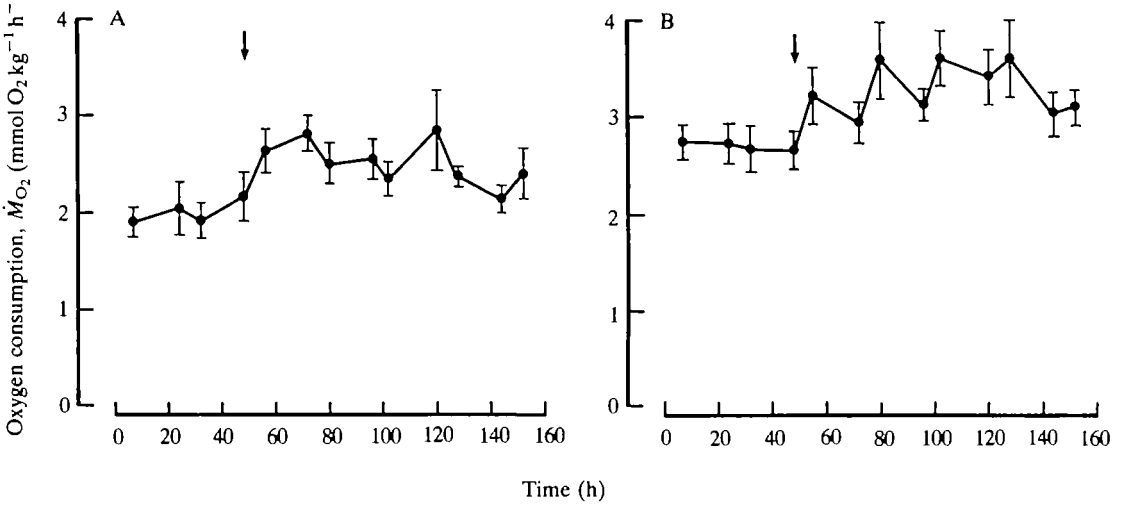

Fig. 1. The effect of exposure to soft water at sublethal pH on oxygen uptake in adult brown trout at 5°C (A) and at 15°C (B). Fish in an open-circuit respirometer were exposed to soft water at neutral pH for the first 2 days, after which (indicated by the arrows) the water flowing through the respirometer was changed to pH 3.75 (A) or 4.25 (B). The values are mean±S.E. For further details see the text and Table 1.

U_{crit} *and cardiovascular variables (Table 2)*

Although the sublethal pH was 0.25 units higher at both temperatures in the cannulated fish compared with that for the uncannulated animals, there were no significant differences between any of the U_{crit} values between the two groups (compare values in Tables 1 and 2). At neutral pH, temperature and exercise both had significant effects on heart rate. Resting heart rate at 5°C was 42% lower than that at 15°C, and heart rate at U_{crit} was 2.1 times that at rest at 15°C and 2.4 times that at rest at 5°C. At sublethal pH, resting heart rate was 41%, but not significantly, higher than that at neutral pH at 15°C whereas it was a significant 25% higher at 5°C. At sublethal pH and U_{crit}, heart rate was 29%, but not significantly, higher than that at rest at 15°C but a significant 57% higher at 5°C. There were no significant effects of low pH or of exercise on mean dorsal aortic blood pressure at 15°C. At 5°C and neutral pH, mean pressures were significantly lower than those at 15°C in resting, but not in swimming, fish. Mean dorsal aortic pressure was, however, significantly higher in resting fish at sublethal pH at 5°C than in those at neutral pH. There were no significant differences between dorsal aortic blood pressures in fish at sublethal pH at either temperature.

Blood gases and [Hb]

Despite a significant reduction in Pa_{O_2} at 15°C when swimming at U_{crit} and at neutral pH (Fig. 2A), there was a significant increase in Ca_{O_2} (Fig. 2B). This was the result of a significant increase in [Hb] (Fig. 2D), which also occurred in response to exposure to sublethal pH at both temperatures.

Table 2. *Critical swimming speed, heart rate and mean dorsal aortic blood pressure of cannulated brown trout after exposure for 4 days to artificial lakewater at 15 or 5°C and at neutral or sublethal pH*

N	Temperature (°C)	pH	Total length (cm)	Mass (kg)	U_{crit} ($BL\,s^{-1}$)	U_{crit} ($m\,s^{-1}$)	Heart rate (beats min^{-1})	Mean dorsal aortic blood pressure (kPa)
7	15	7	31.9±8.8	0.43±0.017	Rest	Rest	41±4.2**·‡‡‡	4.2±0.11**
7	15	7	33.3±6.9	0.40±0.023	2.21±0.08*·†††	0.73±0.02*·†††	85±4.5‡‡‡	4.0±0.39
8	15	4.5	34.0±5.5	0.42±0.024	Rest	Rest	58±6.8*	4.1±0.12
8	15	4.5	34.9±6.3	0.45±0.019	1.37±0.08†††	0.48±0.03†††	75±6.5	4.5±0.32
6	5	7	33.8±5.4	0.41±0.025	Rest	Rest	24±2.0**·‡‡·†	3.4±0.14**·†
6	5	7	34.0±6.0	0.40±0.023	1.95±0.06*·††	0.66±0.02*·††	57±6.7‡	3.1±0.27
6	5	4	33.0±5.6	0.35±0.014	Rest	Rest	31±3.2‡·†	4.0±0.16†
7	5	4	33.1±3.7	0.36±0.018	1.25±0.14††	0.41±0.05††	47±7.1‡	4.0±0.37

Values are mean±S.E., N=number of fish.
* Indicates significant effect of temperature at a given pH or level of activity.
† Indicates significant effect of pH at a given temperature or level of activity.
‡ Indicates significant effect of swimming at U_{crit} (compared with rest) at a given temperature or pH.
One, two or three symbols signify $P<0.05$, 0.01 or 0.001, respectively.
U_{crit}, critical swimming speed; BL, body length.

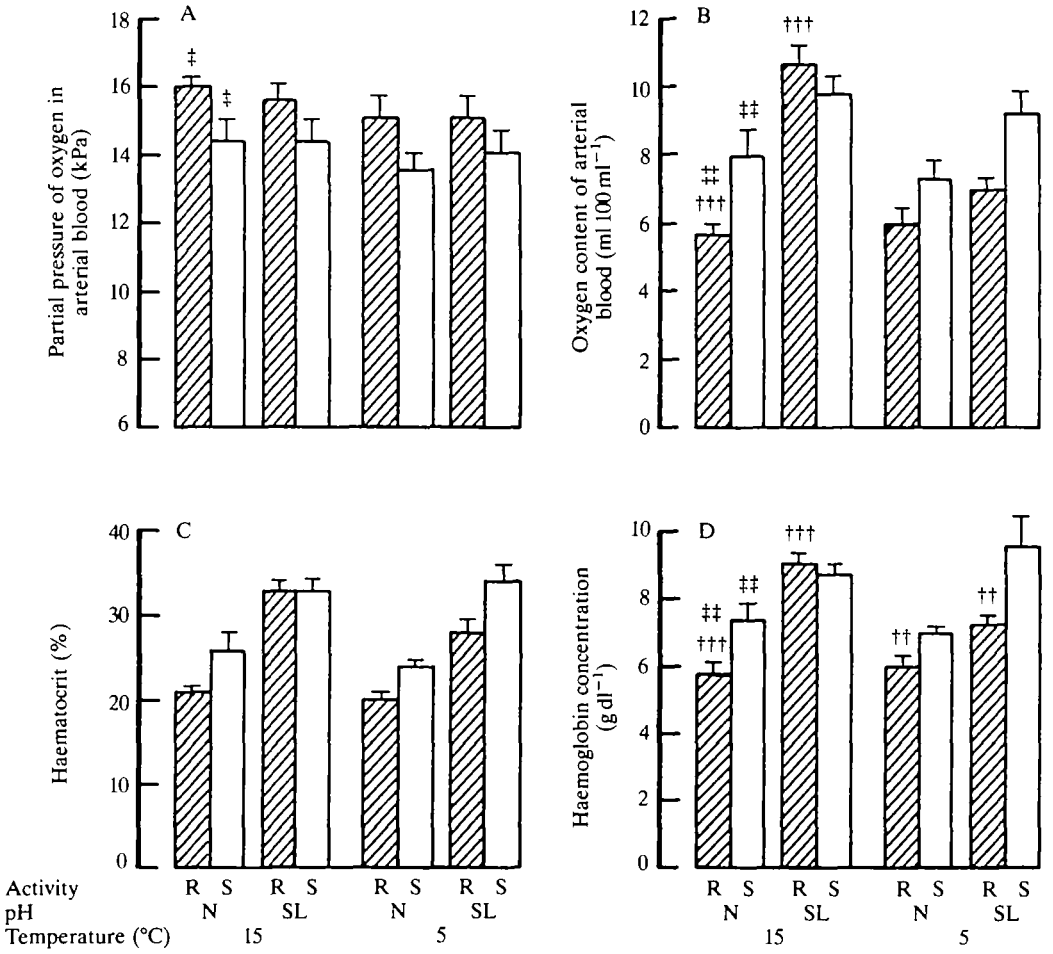

Fig. 2. Histograms showing mean values of partial pressure of oxygen (A) and oxygen content in arterial blood (B), haematocrit (C) and haemoglobin concentration (D) in adult brown trout at rest (R) and while swimming at U_{crit} (S) in soft water at neutral pH (N) or after 4 days at sublethal pH (SL) at both 5°C and 15°C. For further details, see Table 2.

Plasma ions and protein concentrations

At neutral pH, temperature had little effect on plasma ion concentrations. Exposure to sublethal pH caused significant reductions in plasma Na^+ (Fig. 3A) and Cl^- (Fig. 3B) concentrations at both temperatures, although these were more substantial at 5°C than at 15°C. There were no effects on plasma K^+ concentration (Fig. 3C). Swimming at U_{crit} had no significant effect on plasma concentrations of Na^+, K^+ and Cl^- under any conditions, except at sublethal pH at 5°C when there were significant reductions in all three (Fig. 3A,B,C).

Plasma protein concentration was not influenced by temperature or swimming

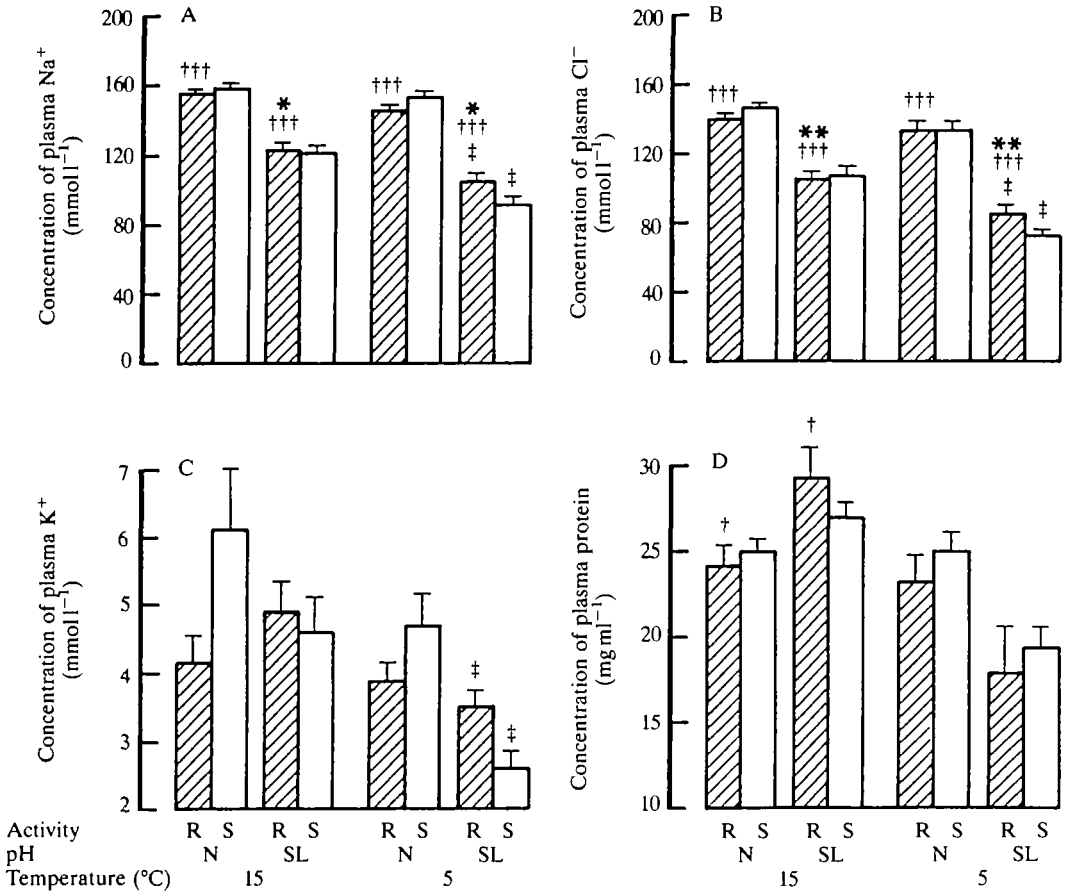

Fig. 3. Histograms showing mean values of plasma concentrations of Na^+ (A), Cl^- (B), K^+(C) and protein (D) in adult brown trout at rest (R) and while swimming at U_{crit} (S) in soft water at neutral pH (N) or after 4 days at sublethal pH (SL) at either 5°C or 15°C. For further details, see Table 2.

at U_{crit} (Fig. 3D). It was significantly higher than that at neutral pH after exposure to sublethal pH at 15°C, but there was no such effect at 5°C.

Discussion

Effect of sublethal pH on resting $\dot{M}_{O_2}$ *and* U_{crit}

The major finding of this study is that, when exposed to their sublethal pH for 4 days, the swimming ability of adult brown trout, as indicated by the U_{crit}, is impaired at both 15°C and 5°C. Although cannulation of the dorsal aorta does have an effect on sublethal pH, the effect of sublethal pH on swimming performance is the same in cannulated and uncannulated fish. This effect of low

pH on the swimming performance of trout contrasts with the finding of Waiwood and Beamish (1978), but is in agreement with the findings of Graham and Wood (1981) and Ye and Randall (1991). The first two groups studied fingerling rainbow trout, and the first group exposed their fish to pH 6, whereas the last two groups exposed their animals to pH <4.6.

There are no signs of a reduction in arterial oxygen content (cf. Ye *et al.* 1991) or of a substantial rise in metabolic rate in response to exposure to sublethal pH for 4 days, indicating that neither of these factors was the likely cause of the reduced U_{crit}. Although previous data on the effect of low pH on metabolic rate are contradictory (Wood and McDonald, 1982), Rosseland (1980) did report an increase in standard oxygen consumption in brown trout exposed to pH 4.5 in brookwater (Tovdal), although he concluded that this effect was not due to low pH alone. In addition, both Hargis (1976) and Waiwood and Beamish (1978) described an increase in oxygen consumption at low swimming speed in rainbow trout exposed to low pH. In contrast, Ye *et al.* (1991) reported no change in oxygen uptake of rainbow trout exposed to pH 4 for 24 h. The causes of the increased resting $\dot{M}_{O_2}$ and heart rate at sublethal pH in the present experiments are unknown. Milligan and Wood (1982) conclude that the latter effect is the result of increased adrenergic stimulation, which would indicate an increased level of stress. The increase in $\dot{M}_{O_2}$ may be another manifestation of this stress, which may be associated with increased low-level activity of the fish although, as stated earlier, this was not determined in the present experiments. It is interesting to note that there was no significant increase in levels of plasma catecholamines in rainbow trout following 24 h of exposure to pH 4 (Ye *et al.* 1991).

Bearing in mind that rainbow trout swimming at U_{crit} increase their $\dot{M}_{O_2}$ by 7.5–9 times above resting (Webb, 1971; Kiceniuk and Jones, 1977), it could be argued that the relatively small increase in resting $\dot{M}_{O_2}$ of brown trout exposed to sublethal pH would not in itself have a major effect on U_{crit}. This conclusion is certainly consistent with the data from Waiwood and Beamish (1978), who showed that exposure of rainbow trout to low pH caused a 25 % increase in $\dot{M}_{O_2}$ at very low swimming speeds, but had no 'appreciable' effect on U_{crit}. However, the metabolic cost of exposure to low pH may increase as swimming speed increases (cf. Febry and Lutz, 1987), thus reducing the fishes' scope for activity.

It is known that exposure to low environmental pH causes an imbalance in the fluxes of Na^+ and Cl^-, leading to net losses of both of these ions (Wood, 1989), although the precise mechanisms may be different in fish exposed to lethal levels of pH compared to those in fish chronically exposed to sublethal pH (McDonald and Wood, 1981; McDonald, 1983; McDonald *et al.* 1983; Audet *et al.* 1988). Ion regulatory failure is thought to lead to the eventual death of rainbow trout exposed to water of lethal low pH by causing haemoconcentration and ensuing circulatory failure (McDonald *et al.* 1980; Milligan and Wood, 1982). In the present experiments, reductions in plasma concentrations of both Na^+ and Cl^- occurred in fish exposed to sublethal pH at both temperatures, reaching lower levels at 5 °C than at 15 °C. Associated with these reductions in plasma ion concentrations were

signs of haemoconcentration, although these were different at the two temperatures.

There were substantial increases in [Hb] and plasma [protein] but not in dorsal aortic blood pressure in resting fish at sublethal pH at 15°C. At 5°C, however, there was a smaller increase in [Hb] and no change in plasma [protein], whereas dorsal aortic pressure was elevated in resting fish at sublethal pH. Thus, although neither Ca_{O_2} nor heart rate was significantly lower at U_{crit} in fish exposed to sublethal pH compared with those at neutral pH, the possible impairment of oxygen transport to the red muscles of swimming trout when exposed to low pH cannot be ruled out. An increase in blood viscosity, by whatever cause, may have had subtle, adverse effects on the local circulation in the red muscles. This may have contributed to the impaired swimming performance of fish at sublethal pH. It is interesting to note that swimming at U_{crit} had no effect on the plasma concentrations of Na^+ and Cl^- under most conditions (cf. Wood and Randall, 1973*a*), the exception being fish at 5°C and sublethal pH.

Whatever the underlying cause(s), the effect of sublethal pH on swimming performance of brown trout is clear (see also Nelson, 1989, for yellow perch, *Perca flavescens*). Exposure to sublethal levels of other pollutants, such as copper (Waiwood and Beamish, 1978) and the water-soluble fraction of crude oil (Thomas and Rice, 1987), also reduces the swimming performance of salmonid fish, although this is not the case with all pollutants (Webb and Brett, 1973). So, although resting fish are able to survive certain levels of environmental pollutants, their ability to swim under such conditions may be diminished. This could have severe consequences on the ability of active fish, such as salmonids, to maintain station in fast-running rivers, especially at the 'swim up' stage (Heggenes and Traaen, 1988), or to migrate.

The influence of seasonal temperature

It was not the intention of the present study to determine the separate effects of temperature and season on the responses being studied. It was, however, intended to investigate whether the responses to sublethal pH are different in fish kept at a low (seasonal) temperature in winter compared with those kept at a higher (seasonal) temperature in summer.

In terms of the aims of the present study, the important finding is that brown trout can survive, for 4 days at least, at a lower pH (by 0.5 units) at 5°C in winter than they can at 15°C in summer. Also, some of the physiological responses to exposure to sublethal pH and to swimming in water at sublethal pH are different at the lower temperature. These observations indicate that seasonal temperature should be taken into account in studies of the influence of sublethal pH on fish. In addition, it is of some interest and worthy of discussion that seasonal temperature had only minor influences on resting $\dot{M}_{O_2}$ and U_{crit} of fish kept at neutral pH.

Most earlier studies on trout, which were not specifically investigating the effect of temperature, have been performed on rainbow trout acclimated to 10–15°C at variable (unreported) times of the year and photoperiod. The measurements

obtained in the present study from the brown trout at 15 °C and in water at neutral pH are very similar to those obtained during previous studies on rainbow trout. The values of resting (standard) $\dot{M}_{O_2}$ and of U_{crit} are similar to those reported by Webb (1971) and Bushnell *et al.* (1984) for slightly smaller rainbow trout at 15 °C. The decline in Pa_{O_2} during swimming has previously been reported by Kiceniuk and Jones (1977) and Thomas *et al.* (1987), the latter authors also noting that, as in the present study, Ca_{O_2} is maintained or even increased as a result of an associated increase in [Hb]. Priede (1974) noted a doubling of heart rate (from 45 beats min^{-1}) in rainbow trout exercising normally at 15 °C. Mean dorsal aortic pressure and haematocrit in the resting brown trout are similar to those recorded in rainbow trout at approximately 10 °C (Kiceniuk and Jones, 1977) and 14 °C (Milligan and Wood, 1982) and, as in the former of these two studies, mean dorsal aortic pressure at U_{crit} in the brown trout was no different from that at rest.

There have been many studies specifically investigating the effect of temperature on metabolism and swimming performance of fish. In a study of young sockeye salmon *Oncorhynchus nerka*, Brett (1964) showed that, although standard metabolic rate was 43 % lower at 5 °C than at 15 °C, U_{crit} was only 20 % lower. Griffiths and Alderdice (1972) working on coho salmon, *O. kisutch,* also indicated that there is a seasonal factor involved, so that swimming performance is improved at low temperature during winter. Data from 'wild' fish are somewhat contradictory. Jones *et al.* (1974) studied 17 species of freshwater fish and found no significant influence of acclimation temperature (between 7 and 20 °C) on their swimming performance. In contrast, the water speed against which juvenile rainbow trout could hold position in winter (5 °C, 10.5 h:13.5 h L:D) was significantly (40 %) lower than that in summer (15 °C, 14 h:10 h L:D) (Facey and Grossman, 1990).

There is no doubt that, in many species of fish, acclimation to low temperature causes a number of anatomical and biochemical adaptations that would enhance swimming performance. In cold-acclimated goldfish *Carassius auratus*, crucian carp *C. carassius* and striped bass *Morone saxatilis*, there are increases in the proportion of aerobic 'red' and 'pink' muscle fibres, in mitochondrial density (and hence in the activity of aerobic enzymes) and in capillary supply, and a decrease in diffusion path between the sarcoplasmic and mitochondrial compartments (Johnston and Lucking, 1978; Johnston and Maitland, 1980; Sidell, 1980; Johnston, 1982; Jones and Sidell, 1982; Egginton and Sidell, 1989). There appear to have been no such studies on salmonid fish. In addition to the above adaptations to low temperature, there are increases in myofibrillar ATPase activity in both 'red' and 'white' muscles in cyrinids (Johnston *et al.* 1975; Sidell, 1980), but not in 'white' muscle of trout (Penney and Goldspink, 1981*a*,*b*). These authors point out that this discovery is somewhat surprising in view of the active life style of salmonids. Like the striped bass (Sisson and Sidell, 1987), salmonids are presumably able to enhance their swimming ability at low temperature by increasing the proportion of 'red' fibres, but this remains to be demonstrated. There is certainly hypertrophy of cardiac muscle in rainbow trout at winter temperature, resulting in stroke volume

being the same as, or even greater than, that in trout at summer temperature (Graham and Farrell, 1989). These authors point out that such compensation may be important in enabling the fish to maintain a high level of swimming performance during the winter.

It is also of some interest that continued swimming activity (training) of trout improves their U_{crit}, causes hypertrophy of 'red' and 'white' skeletal muscle and of cardiac muscle and causes an increase in standard $\dot{M}_{O_2}$ (Davison and Goldspink, 1977; Greer Walker and Emerson, 1978; Nahhas *et al.* 1982; Farrell *et al.* 1990). The brown trout used in the present study were kept active for the entire holding period in Birmingham and, if the effects of activity were relatively more pronounced at the lower temperature, this could explain the relatively small differences in resting $\dot{M}_{O_2}$ and U_{crit} at neutral pH during summer and winter. Interestingly, seasonal temperature had no effect on U_{crit} at sublethal pH.

This work was supported by the Natural Environmental Research Council.

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.

AXELSSON, M. AND NILSSON, S. (1986). Blood pressure control during exercise in the Atlantic cod, *Gadus morhua*. *J. exp. Biol.* **126**, 225–236.

BEAMISH, F. W. H. (1978). Swimming capacity. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar and D. J. Randall), pp. 101–187. New York: Academic Press.

BRETT, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish Res. Bd Can.* **21**, 1183–1226.

BUSHNELL, P. G., STEFFENSEN, J. F. AND JOHANSEN, K. (1984). Oxygen consumption and swimming performance in hypoxia-acclimated rainbow trout *Salmo gairdneri*. *J. exp. Biol.* **113**, 225–235.

BUTLER, P. J., METCALFE, J. D. AND GINLEY, S. A. (1986). Plasma catecholamines in the lesser spotted dogfish and rainbow trout at rest and during different levels of exercise. *J. exp. Biol.* **123**, 409–421.

DALZIEL, T. R. K., MORRIS, R. AND BROWN, D. J. A. (1985). The effects of low pH, low calcium concentrations and elevated aluminium concentrations on sodium fluxes in brown trout, *Salmo trutta*. CEGB report no. TPRD/L/2861/N85.

DAVISON, W. AND GOLDSPINK, G. (1977). The effect of prolonged exercise on the lateral musculature of the brown trout (*Salmo trutta*). *J. exp. Biol.* **70**, 1–12.

DAVISON, W. AND WOOF, C. (1985). Performance tests for the measurement of pH with glass electrodes in low ionic strength solutions including natural waters. *Analyt. Chem.* **57**, 2567–2570.

DRIEDZIC, W. R. AND KICENIUK, J. W. (1976). Blood lactate levels in free-swimming rainbow trout (*Salmo gairdneri*) before and after strenuous exercise resulting in fatigue. *J. Fish. Res. Bd Can.* **33**, 173–176.

EGGINTON, S. AND SIDELL, B. D. (1989). Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am. J. Physiol.* **256**, R1–R9.

FACEY, D. E. AND GROSSMAN, G. D. (1990). The metabolic cost of maintaining position for four North Americal stream fishes: Effects of season and velocity. *Physiol. Zool.* **63**, 757–776.

FARRELL, A. P., JOHANSEN, J. A., STEFFENSEN, J. F., MOYES, C. D., WEST, T. G. AND SUAREZ,

R. K. (1990). Effects of exercise-training and coronary ablation on swimming performance, heart size and cardiac enzymes in rainbow trout, *Oncorhynchus mykiss*. *Can. J. Zool.* **68**, 1174–1179.

FEBRY, R. AND LUTZ, P. (1987). Energy partitioning in fish: the activity-related cost of osmoregulation in a euryhaline cichlid. *J. exp. Biol.* **128**, 63–85.

GRAHAM, M. S. AND FARRELL, A. P. (1989). The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. *Physiol. Zool.* **62**, 38–61.

GRAHAM, M. S. AND WOOD, C. M. (1981). Toxicity of environmental acid to the rainbow trout: interactions of water hardness, acid type and exercise. *Can. J. Zool.* **59**, 1518–1526.

GRAHAM, M. S., WOOD, C. M. AND TURNER, J. D. (1982). The physiological responses of the rainbow trout to strenuous exercise: interactions of water hardness and environmental acidity. *Can. J. Zool.* **60**, 3153–3164.

GRANDE, M., MUNIZ, I. P. AND ANDERSEN, S. (1978). Relative tolerance of some salmonids to acid waters. *Verh. int. Verein. Limnol.* **20**, 2076–2084.

GREER WALKER, M. AND EMERSON, L. (1978). Sustained swimming speeds and myotomal muscle function in the trout, *Salmo gairdneri*. *J. Fish Biol.* **13**, 475–481.

GRIFFITHS, J. S. AND ALDERDICE, D. F. (1972). Effects of acclimation and acute temperature experience on the swimming speed of juvenile coho salmon. *J. Fish. Res. Bd Can.* **29**, 251–264.

HARGIS, J. R. (1976). Ventilation and metabolic rate of young rainbow trout (*Salmo gairdneri*) exposed to sublethal environmental pH. *J. exp. Zool.* **196**, 39–44.

HARRIMAN, R., MORRISON, B. R. S., CAINES, L. A., COLLEN, P. AND WATT, A. W. (1987). Long-term changes in fish populations of acid streams and lochs in Galloway, south west Scotland. *Water Air Soil Poll.* **32**, 89–112.

HEGGENES, J. AND TRAAEN, T. (1988). Downstream migration and critical water velocities in stream channels for fry of four salmonid species. *J. Fish Biol.* **32**, 717–727.

JOHNSTON, I. A. (1982). Capillarisation, oxygen diffusion distances and mitochondrial content of carp muscles following acclimation to summer and winter temperatures. *Cell Tissue Res.* **222**, 325–337.

JOHNSTON, I. A., DAVISON, W. AND GOLDSPINK, G. (1975). Adaptations in Mg^{2+}-activated myofibrillar ATPase activity induced by temperature acclimation. *FEBS Lett.* **50**, 293–295.

JOHNSTON, I. A. AND LUCKING, M. (1978). Temperature induced variation in the distribution of different types of muscle fibre in the goldfish (*Carassius auratus*). *J. comp. Physiol.* **124**, 111–116.

JOHNSTON, I. A. AND MAITLAND, B. (1980). Temperature acclimation in crucian carp, *Carassius carassius* L., morphometric analyses of muscle fibre ultrastructure. *J. Fish Biol.* **17**, 113–125.

JONES, D. R., KICENIUK, J. W. AND BAMFORD, O. S. (1974). Evaluation of the swimming performance of several fish species from the Mackenzie River. *J. Fish. Res. Bd Can.* **31**, 1641–1647.

JONES, D. R. AND SIDELL, B. (1982). Metabolic responses of striped bass (*Morone saxatilis*) to temperature acclimation. II. Alterations in metabolic carbon sources and distributions of fiber types in locomotory muscle. *J. exp. Zool.* **219**, 163–171.

KICENIUK, J. W. AND JONES, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247–260.

KWAIN, W.-H. (1975). Effects of temperature on development and survival of rainbow trout, *Salmo gairdneri*, in acid waters. *J. Fish. Res. Bd Can.* **32**, 493–497.

MCDONALD, D. G. (1983). The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. I. Branchial and renal net ion and H^+ fluxes. *J. exp. Biol.* **102**, 123–140.

MCDONALD, D. G., HÖBE, 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.

MCDONALD, D. G., WALKER, R. L. AND WILKES, P. R. H. (1983). The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. II. Branchial ionoregulatory mechanisms. *J. exp. Biol.* **102**, 141–155.

MCDONALD, D. G. AND WOOD, C. M. (1981). Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH. *J. exp. Biol.* **93**, 101–118.

MILLIGAN, C. L. AND WOOD, C. M. (1982). Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **99**, 397–415.

NAHHAS, R., JONES, N. V. AND GOLDSPINK, G. (1982). Some aspects of sustained training of rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* **20**, 351–358.

NELSON, J. A. (1989). Critical swimming speeds of yellow perch *Perca flavescens*: comparison of populations from a naturally acidic lake and a circumneutral lake in acid and neutral water. *J. exp. Biol.* **145**, 239–254.

PENNEY, R. K. AND GOLDSPINK, G. (1981*a*). Short term temperature acclimation in myofibrillar ATPase of a stenotherm *Salmo gairdneri* Richardson and an eurytherm *Carassius auratus*. *J. Fish Biol.* **18**, 715–722.

PENNEY, R. K. AND GOLDSPINK, G. (1981*b*). Temperature adaptation by the myotomal muscle of fish. *J. therm. Biol.* **6**, 297–306.

PLAYLE, R. C., GOSS, G. G. AND WOOD, C. M. (1989). Physiological disturbances in rainbow trout (*Salmo gairdneri*) during acid and aluminium exposures in soft water of two calcium concentrations. *Can. J. Zool.* **67**, 314–324.

PRIEDE, I. G. (1974). The effect of swimming activity and section of the vagus nerves on heart rate in rainbow trout. *J. exp. Biol.* **60**, 305–319.

ROSSELAND, B. O. (1980). Physiological responses to acid water in fish. II. Effects of acid water on metabolism and gill ventilation in brown trout, *Salmo trutta* L. and brook trout, *Salvelinus fontinalis* Mitchill. In *Ecological Impact of Acid Precipitation* (ed. D. Drabløs and A. Tollan), pp. 348–349. SNSF Project.

SIDELL, B. D. (1980). Responses of goldfish (*Carassius auratus*, L.) muscle to acclimation temperature: alterations in biochemistry and proportions of different fiber types. *Physiol. Zool.* **53**, 98–107.

SISSON, J. E. AND SIDELL, B. D. (1987). Effect of thermal acclimation on muscle fiber recruitment of swimming striped bass (*Morone saxatilis*). *Physiol. Zool.* **60**, 310–320.

SPRAGUE, J. B. (1971). Measurement of pollutant toxicity to fish. III. Sublethal effects and 'safe' concentrations. *Water Res.* **5**, 245–266.

THOMAS, R. AND RICE, S. D. (1987). Effect of water-soluble fraction of Cook Inlet crude oil on swimming performance and plasma cortisol in juvenile coho salmon (*Oncorhynchus kisutch*). *Comp. Biochem. Physiol.* **87C**, 177–180.

THOMAS, S., POUPIN, J., LYKKEBOE, G. AND JOHANSEN, K. (1987). Effects of graded exercise on blood gas tensions and acid–base characteristics of rainbow trout. *Respir. Physiol.* **68**, 85–97.

TURNPENNY, A. W. H., SADLER, K., ASTON, R. J., MILNER, A. G. P. AND LYNAM, S. (1987). The fish populations of some streams in Wales and northern England in relation to acidity and associated factors. *J. Fish Biol.* **31**, 415–434.

VERDOUW, H., VAN ECHTELD, C. J. A. AND DEKKERS, E. M. J. (1978). Ammonia determination based on indophenol formation with sodium salicylate. *Water Res.* **12**, 399–402.

WAIWOOD, K. G. AND BEAMISH, F. W. H. (1978). Effects of copper, pH and hardness on the critical swimming performance of rainbow trout (*Salmo gairdneri* Richardson). *Water Res.* **12**, 611–619.

WEBB, P. W. (1971). The swimming energetics of trout. II. Oxygen consumption and swimming efficiency. *J. exp. Biol.* **55**, 521–540.

WEBB, P. W. AND BRETT, J. R. (1973). Effects of sublethal concentrations of sodium pentachlorophenate on growth rate, food conversion efficiency, and swimming performance in underyearling sockeye salmon (*Oncorhynchus nerka*). *J. Fish Res. Bd Can.* **30**, 499–507.

WEBER, J.-M. (1991). Effect of endurance swimming on the lactate kinetics of rainbow trout. *J. exp. Biol.* , 463–476.

WOKOMA, A. AND JOHNSTON, I. A. (1981). Lactate production at high sustainable cruising speeds in rainbow trout (*Salmo gairdneri* Richardson). *J. exp. Biol.* **90**, 361–364.

WOOD, C. M. (1989). The physiological problems of fish in acid waters. In *Acid Toxicity and Aquatic Animals* (ed. R. Morris, E. W. Taylor, D. J. A. Brown and J. A. Brown), pp. 125–151. Cambridge: Cambridge University Press.

WOOD, C. M. AND MCDONALD, D. G. (1982). Physiological mechanisms of acid toxicity to fish. In *Acid Rain/Fisheries* (ed. R. E. Johnson), pp. 197–226, Bethesda. MD: American Fisheries Society.

WOOD, C. M., PLAYLE, R. C., GOSS, G. G. AND MCDONALD, D. G. (1988). Blood gases, acid–base status, ions and hematology in adult brook trout (*Salvelinus fontinalis)* under acid/aluminium exposure. *Can. J. Fish aquat. Sci.* **45**, 1575–1586.

WOOD, C. M. AND RANDALL, D. J. (1973*a*). Sodium balance in the rainbow trout (*Salmo gairdneri*) during extended exercise. *J. comp. Physiol.* **82**, 235–256.

WOOD, C. M. AND RANDALL, D. J. (1973*b*). The influence of swimming activity on water balance in the rainbow trout (*Salmo gairdneri*). *J. comp. Physiol.* **82**, 257–276.

YE, X. AND RANDALL, D. J. (1991). The effect of water pH on swimming performance in rainbow trout (*Salmo gairdneri,* Richardson). *Fish Physiol. Biochem.* **9**, 15–21.

YE, X., RANDALL, D. J. AND HE, X. (1991). The effect of acid water on oxygen consumption, circulating catecholamines and blood ionic and acid–base status in rainbow trout (*Salmo gairdneri*, Richardson). *Fish Physiol. Biochem.* **9**, 23–30.