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PLASMA AMMONIA CONCENTRATION IN BROWN TROUT IN SOFT ACIDIC WATER AND ITS RELATIONSHIP TO DECREASED SWIMMING PERFORMANCE

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Summary

Adult brown trout (300–600 g) were acclimated for 2 weeks to an artificial soft water (Ca^{2+}, 50 μmol l^{-1}) and maintained at either 5 °C (October to March) or 15 °C (May to August). Following insertion of a cannula into the dorsal aorta under MS-222 anaesthesia and a recovery period of 2 days, the fish were exposed to a 4 day episode of sub-lethal copper levels at pH 5 or kept at control conditions of pH 7 without copper. The copper concentrations had been predetermined by toxicity testing and were approximately 0.47 μmol l^{-1} at 5 °C and 0.08 μmol l^{-1} at 15 °C. At 5 °C, a group of fish was also exposed to approximately 0.08 μmol l^{-1} copper at pH 5.

Plasma total ammonia (T_{amm}) concentration was significantly elevated by exposure to copper and pH 5. In resting trout exposed to the appropriate sub-lethal copper concentration at pH 5, T_{amm} was six and 7.5 times greater at 5 and 15 °C, respectively, than those of control trout at the respective temperatures. Although unconfirmed, an elevation of ammonia production alone seems unlikely to account for such substantial increases. From previous studies, there is little evidence of impairment of respiratory gas exchange in trout exposed to these copper concentrations and yet, in the acidic test waters, the gradient of NH_3 partial pressure between fish and water was 5.5–6 times greater than that under control conditions.

Swimming performance determined by the critical swimming speed (U_{crit}) was reduced by copper and acid exposure, and a significant relationship existed between U_{crit} and the plasma ammonia concentration of exercised trout. Ammonium ions influence several key enzymes involved in energy metabolism, and elevated ammonia levels might, therefore, reduce the capacity of muscle to exercise. Alternatively, ammonia may have affected the nervous coordination of exercise either centrally or by disrupting peripheral motor innervation.

Key words: copper, low pH, ammonia, swimming, brown trout, *Salmo trutta*.

Introduction

Although it is an essential trace element in the diet of fish, copper is one of the more toxic heavy metals. It appears in the aquatic environment from both natural and anthropogenic sources, such as mine washings and direct application as an algicide and molluscicide. Low pH in natural waters is often accompanied by the mobilisation of metal ions such as aluminium, copper and zinc (Leivestad and Muniz, 1976; Cronan and Schofield, 1979; Spry *et al.* 1981; Turnpenny, 1989; Bulger *et al.* 1993), and Turnpenny *et al.* (1987) concluded that, in the soft streams of north and mid-Wales, copper and zinc concentrations were more important determinants of the status of fish populations than acidity alone.

In a previous paper (Beaumont *et al.* 1995), it was reported that the swimming performance of brown trout, as determined by the critical swimming speed U_{crit} (Brett, 1964), was impaired by 4 days of exposure to sub-lethal copper levels at pH 5. Despite apparent ultrastructural damage to the gills, there was no resultant systemic hypoxia, indicating that oxygen uptake by diffusion was not limiting performance. Instead, it was suggested that haematological disturbances may have disrupted the transport of oxygen to the working muscle and resulted in local hypoxia. Observations of tissue metabolite status provide some evidence to support this hypothesis (J. L. Mair, E. W. Taylor and P. J. Butler, unpublished observations). Another possible factor, an elevation of plasma ammonia concentration, that may contribute to the reduced swimming performance of trout exposed to sub-lethal copper levels and acid will be discussed in the present paper.

Tashiro (1922) measured the formation of ammonia during stimulation of the frog sciatic nerve and speculated over its role in fatigue. Since then, ammonia accumulation has often been linked to exercise fatigue, particularly in humans and other mammals (e.g. Mutch and Banister, 1993). Ammonium ions are produced in exercising muscle by the deamination of adenosine 5′-monophosphate in the purine nucleotide cycle (Lowenstein, 1972; Aragon and Lowenstein, 1980; Weicker *et al.* 1990). In mammals, there is a wide variation in deaminase

activity between muscle fibre types (Raggi *et al.* 1969). Slow twitch fibres with a high mitochondrial density tend to have a relatively low deaminase activity (Meyer and Terjung, 1979). Ammonia production is greater in fast twitch fibres. Increased ammonia levels are, therefore, primarily related to intense exercise during which production increases exponentially with work rate as recruitment of fast twitch fibres occurs (Meyer *et al.* 1980). For this reason, ammonia production has been largely discarded as an agent of fatigue by those searching for a general mechanism applicable to all fibre types. For example, in his recent review of the cellular factors resulting in muscle fatigue, Fitts (1994) does not even discuss ammonia production.

There has also been little discussion of this topic with regard to exercise in fish, no doubt as a result of the lack of agreement regarding the role of ammonia accumulation in other organisms and the relative ease with which ammonia is usually excreted in the aquatic environment. Fish have a remarkable capacity to use dietary proteins as an energy source (van Waarde, 1983), and ammonia is the major end-product of nitrogen metabolism. Under resting conditions, its main site of production is probably the liver (Pequin and Serfaty, 1963). Over 90 % of ammonia is excreted across the gills (Cameron and Wood, 1978) and this probably mainly occurs *via* the passive diffusion of free ammonia, although the contribution of an active excretory mechanism is still the subject of considerable discussion (see Cameron and Heisler, 1983; Randall and Wright, 1987; Wood, 1993; Wilson and Taylor, 1992; Wilson *et al.* 1994).

Despite the importance of ammonia as an end-product of metabolism in fish and a report that muscle ammonia levels can rise by up to 14 times following exhaustive exercise (T. P. Mommsen and P. W. Hochachka, unpublished data reported by Randall and Wright, 1987), the role of ammonia in fatigue has not been investigated in fish. Randall and Brauner (1991) and Ye and Randall (1991) suggest that ammonia accumulation may have been the cause of the reduced swimming performance of rainbow trout after exposure to high pH. However, none of these authors actually measured ammonia concentrations in the fish.

Materials and methods

The experimental protocol was similar to that employed by Butler *et al.* (1992), although a slightly harder water was used. Briefly, adult brown trout *Salmo trutta* (L.) (mass 300–600 g) were obtained from Leadmill trout farm, Hathersage, Derbyshire, and were kept for 2–4 weeks in a 1400 l circular fibreglass tank through which dechlorinated Birmingham tap water ([Ca^{2+}] 130–200 μmol l^{-1}) flowed at 120 l h^{-1}. The water was aerated vigorously and circulated around the tank at approximately 0.25 m s^{-1} by means of a spray bar. The fish swam to maintain their position within a number of plastic tubes (10 cm diameter, 55 cm length) suspended in mid-water. After this initial period, the fish were transferred to identical holding tanks with circulating water and acclimated to an artificial soft water (composition, μmol l^{-1}; Ca^{2+}, 50; Na^{+}, 75; K^{+}, 5; Mg^{2+}, 40; Cl^{-}, 100; SO_4^{2-}, 65; NO_3^{-}, 5) for a minimum of 2 weeks.

All tanks were maintained at neutral pH and at the seasonal mean temperatures 5±0.2 °C (winter, October to March) or 15±0.2 °C (summer, May to August). The fish were fed *ad libitum* on a diet of trout pellets (BP Mainstream 'floaters') but were not fed for 24 h before or during any experiment. The trout were anaesthetised in a solution of 100 mg l^{-1} MS-222 (Sigma) and a catheter was inserted into the dorsal aorta following the methods of Soivio *et al.* (1972). The fish were allowed to recover for 48 h in the swimming flumes in the artificial soft water prior to starting the experimental exposure regime (see Butler *et al.* 1992, for details). As there were three flume systems linked in parallel to the same water supply, it was possible to have up to three experimental fish swimming simultaneously. Twelve animals at each temperature were then exposed to the appropriate maximum sub-lethal copper concentration (SLCC) at pH 5 for 96 h. These levels were predetermined by toxicity testing and were approximately 0.47 μmol l^{-1} copper at 5 °C and 0.08 μmol l^{-1} copper at 15 °C. In addition, 12 winter trout were exposed to the summer SLCC (pH 5, 0.08 μmol l^{-1}) and 12 trout at each temperature were left in the acclimation water only (pH 7, no added copper) as controls. At pH 5, 99 % of the copper should be in the form of Cu^{2+} (see Beaumont *et al.* 1995).

There was a continuous flow of water through the flume systems at a rate of 3 l min^{-1}, representing a 90 % replacement time of 12 h (Sprague, 1969). Cu^{2+} was added constantly from a stock solution of $CuCl_2$ to maintain the desired concentration. Cu^{2+} concentration was regularly monitored by aniodic stripping voltammetry (EG+G model 264 polarograph with a hanging-drop mercury electrode) which had an experimental detection limit of approximately 0.01 μmol l^{-1}. The concentration never deviated from the nominal value by more than 15 %. In both the dechlorinated tap water and the control, artificial soft water, Cu^{2+} concentration was always below the detection limit (0.01 μmol l^{-1}). A control group of fish, exposed to pH 5 without addition of Cu^{2+}, was considered unnecessary, as a recent study in our laboratory has described the effects of low pH alone on the swimming performance of brown trout (Butler *et al.* 1992). The present data enable the specific effects of added Cu^{2+} to be identified.

After 96 h of exposure, the swimming performance at their acclimation temperature of six fish from each group was determined. Swimming performance was measured as critical swimming speed (U_{crit}; Brett, 1964); the fish swam against water flowing at 0.3 m s^{-1} for 15 min, after which the water flow was increased by 0.1 m s^{-1}. This speed increment was repeated every 15 min until the trout failed to maintain position and fell back onto the mesh at the rear of the flume. No electrical stimuli were applied to 'encourage' swimming. Instead, the rear of the flume was illuminated and the front covered. The trout swam to maintain position in the darkened area. When the fish first fell back to the mesh, the water speed was briefly reduced. This was often sufficient for the fish to begin to swim once more.

The time and speed at which the fish could or would not swim any further was recorded and an arterial blood sample was taken *via* the dorsal aortic cannula. This forms part of our standard protocol based on observations of the development of fatigue in the trout and of the repeatability of measurements of U_{crit} (e.g. Butler *et al.* 1992; Butler and Day, 1993). Blood samples were also taken from the six remaining trout in each group at rest, which were also held in flumes.

Each blood sample was centrifuged at approximately 9500***g*** for 5 min and the plasma was analysed for total ammonia (T_{amm}) concentration using a method based upon the reductive amination of 2-oxaloglutarate in the presence of glutamate dehydrogenase (Sigma kit no. 171). When this determination could not be performed immediately, plasma samples were frozen at −70 °C. The assay was always completed within 24 h of sampling. Plasma pH was measured immediately using a Radiometer BMS (Mk3) analyser and G229A capillary electrode thermostatically controlled to the experimental temperature. Water samples were analysed for ammonia concentration using the salicylate method (Verdouw *et al.* 1978).

Free (NH_3) and ionised ammonia (NH_4^+) concentrations in water and plasma were calculated from the Henderson–Hasselbalch equation:

$$[NH_4^+] = \frac{T_{amm}}{1 + 10^{pH-pK'}},$$

$$[NH_3] = T_{amm} - [NH_4^+].$$

Values of pK′ were estimated from the nomogram of Cameron and Heisler (1983). The NH_3 concentration and appropriate solubility coefficient (αNH_3) determined from Cameron and Heisler (1983) were used to calculate the partial pressure of ammonia (P_{NH_3}):

$$P_{NH_3} = \frac{[NH_3]}{\alpha NH_3}.$$

Transbranchial gradients (ΔP_{NH_3} and $\Delta[NH_4^+]$) were calculated by simple subtraction of the water values from those of the arterial plasma. This calculation will underestimate the true gradients, which ideally should have been determined from the mean concentration in the gills, which would have required the simultaneous measurement of venous T_{amm} and pH (Wilson *et al.* 1994).

Plasma samples for ion analysis were stored frozen at −70 °C. Plasma K^+ concentration was measured within 3 months using a Pye Unicam SP9 atomic absorption spectrophotometer.

Values are given as mean ±1 S.E.M. Three-factor (temperature, $[Cu^{2+}]$ and exercise) analysis of variance (ANOVA) was performed using the GLM procedure of the SAS computer program (SAS Institute, 1982). When appropriate, pair-wise comparisons were performed *post hoc* using a modified Tukey test (Cicchetti, 1972).

Results

Neither acclimation temperature nor exercise had an effect upon plasma T_{amm} of control trout (Fig. 1). At rest and at both temperatures, trout exposed to Cu^{2+} and pH 5 had a greater T_{amm} than the corresponding controls. At 15 °C, exposure to 0.08 μmol l^{-1} Cu^{2+} and pH 5 elevated the plasma T_{amm} of resting fish by over 7.5 times to 697.5±105.4 μmol l^{-1}. The same levels of Cu^{2+} and pH at 5 °C caused a smaller, but still significant, rise of almost 4.5 times the control value. Winter trout exposed to the sub-lethal Cu^{2+} concentration for this temperature (0.47 μmol l^{-1}, pH 5) had a plasma T_{amm} of 607.7±58.3 μmol l^{-1}, almost six times greater than levels in the control trout. Exercise had no effect upon the plasma T_{amm} of control trout at either temperature, and nor were there any significant differences between resting and exercised groups of winter fish exposed to either Cu^{2+} concentration. At 15 °C, however, the Cu^{2+}-exposed trout at pH 5 that had been exercised had a plasma T_{amm} of 259.9±30.4 μmol l^{-1}, only one-third of that of the Cu^{2+}-exposed fish at pH 5 at rest (Fig. 1). ΔP_{NH_3} and $\Delta[NH_4^+]$ values for trout at rest are given in Table 1.

Exercise under control conditions caused a significant increase in plasma K^+ concentration at both temperatures (Fig. 2). This increase was 2.6-fold at 15 °C and 2.8-fold at 5 °C. Cu^{2+}-exposure at pH 5 had no significant effect upon plasma $[K^+]$ at rest and there was no significant effect of exercise in the

Table 1. *Calculated NH_4^+ concentration, NH_3 partial pressure (P_{NH_3}) and plasma–water gradients (Δ) for groups of six resting brown trout* Salmo trutta *after 4 days of exposure to either control conditions (no added Cu^{2+}, pH 7) or to the stated Cu^{2+} concentration at pH 5*

Temperature (°C)	Water $[Cu^{2+}]$ (μmol l^{-1})	Water pH	$[NH_4^+]$ (μmol l^{-1})			P_{NH_3} (Pa)		
			Plasma	Water	$\Delta[NH_4^+]$	Plasma	Water	ΔP_{NH_3}
5 (Control)	–	7	103.1±13.0	7.9	95.1	3.5±0.5	0.063	3.4
5	0.08	5	456.5±35.0	12.3	444.2	18.8±1.6	0.001	18.8
5	0.47	5	598.4±57.3	16.0	582.4	20.1±2.6	0.001	20.1
15 (Control)	–	7	90.0±19.4	7.2	82.8	4.4±0.9	0.070	4.4
15	0.08	5	688.7±103.5	10.2	678.5	4.4±5.3	0.001	24.0

For plasma samples, values are mean ± S.E.M. but, because there were up to three experimental fish used simultaneously, the number of individual water samples varied between two and four. Thus, no errors are given for the water values.

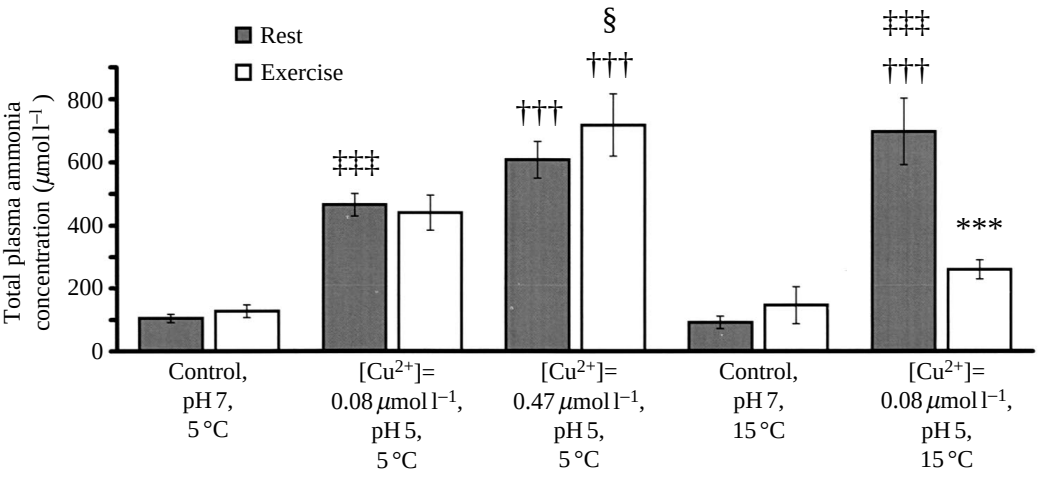

Fig. 1 Mean (±S.E.M.) total plasma ammonia concentration (μmol l^{-1}) for brown trout acclimated to either 5 or 15 °C, exposed for 96 h to either control conditions (pH 7, no added Cu^{2+}) or to the stated Cu^{2+} concentrations at pH 5 and sampled either at rest or after exercise (N=6). Significant differences are indicated by the following symbols: * a significant difference due to exercise; ‡ a significant difference due to the addition of 0.08 μmol l^{-1} Cu^{2+}, at pH 5; † a significant difference due to the addition of 0.47 μmol l^{-1} Cu^{2+}, pH 5; § a significant difference between 0.08 μmol l^{-1} and 0.47 μmol l^{-1} Cu^{2+}. One symbol indicates P<0.05, two represent P<0.01 and three represent P<0.001.

Cu^{2+}-exposed trout at pH 5. However, at both temperatures, the plasma [K^+] of the Cu^{2+}-exposed trout that were exercised was greater than that of control animals at rest and was not significantly different from that of exercised controls.

The swimming performance of these trout has been documented elsewhere (Beaumont *et al.* 1995) and is summarised in Table 2. U_{crit} was greatest in the control groups, where plasma T_{amm} was lowest, and reduced in the Cu^{2+}-exposed trout at pH 5 in which plasma T_{amm} was elevated. Regression analysis of U_{crit} and plasma T_{amm} shows there to be a significant linear relationship between the two variables with an r^2 value of 0.64 (results not shown). Using the estimated values of plasma [NH_4^+] as the independent variable gives a linear relationship with a similar slope and slightly greater r^2 of 0.67 (Fig. 3).

Discussion

The accumulation of ammonia

A consistent effect of exposure to Cu^{2+}, low pH or both is an elevation of plasma ammonia concentration (Lauren and McDonald, 1985; Wilson and Taylor, 1993*a*,*b*; Beaumont *et al.* 1995; N. Day and P. J. Butler, unpublished observations). Increased ammonia production can arise from a general corticosteroid-mediated stress response that includes increased protein catabolism and gluconeogenesis (Freeman and Idler, 1973). However, exposure of carp to sub-lethal Cu^{2+} levels does not cause a marked increase in ammonia excretion in carp (De Boeck *et al.* 1994); the scale of the increase in T_{amm} in the present study on brown trout suggests that there is likely to be some effect on ammonia excretion. There has been considerable research effort on this topic. The passive diffusion of NH_4^+ across the gills has been dismissed, at least in freshwater fish, because of the low permeability of most biological membranes to ionic compounds and the diffusion barrier presented by tight junctions (Wright and Wood, 1985). Thus, both passive diffusion of free NH_3 and an active pathway, either involving the direct exchange of NH_4^+ or a proton pump coupled to NH_3 excretion, are considered to be probable mechanisms of ammonia excretion (Cameron and Heisler, 1983; Cameron, 1986; Avella and Bornancin, 1989; Wilson and Taylor, 1992; Wilson *et al.* 1994).

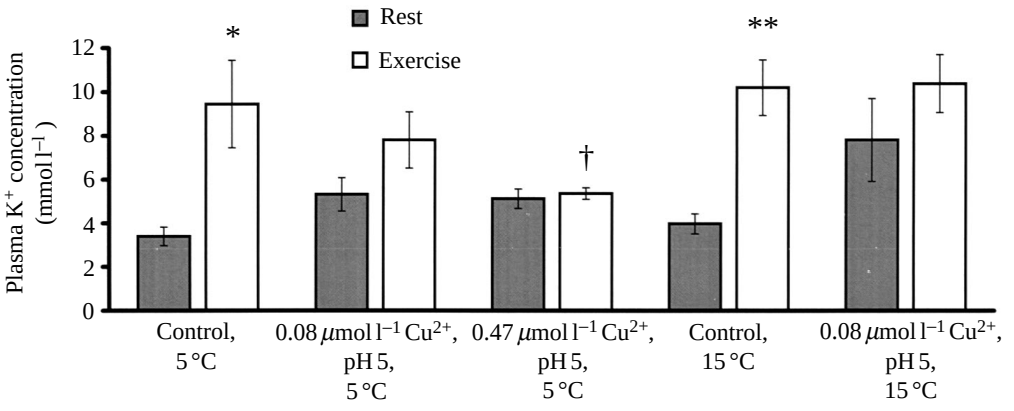

Fig. 2. Mean values (±S.E.M.) of plasma K^+ concentration (mmol l^{-1}) for brown trout acclimated to either 5 or 15 °C, exposed for 96 h to either control conditions (pH 7, no added Cu^{2+}) or to the stated Cu^{2+} concentrations at pH 5 and sampled either at rest or after exercise (N=6). Significant differences are indicated by the same symbols as in Fig. 1.

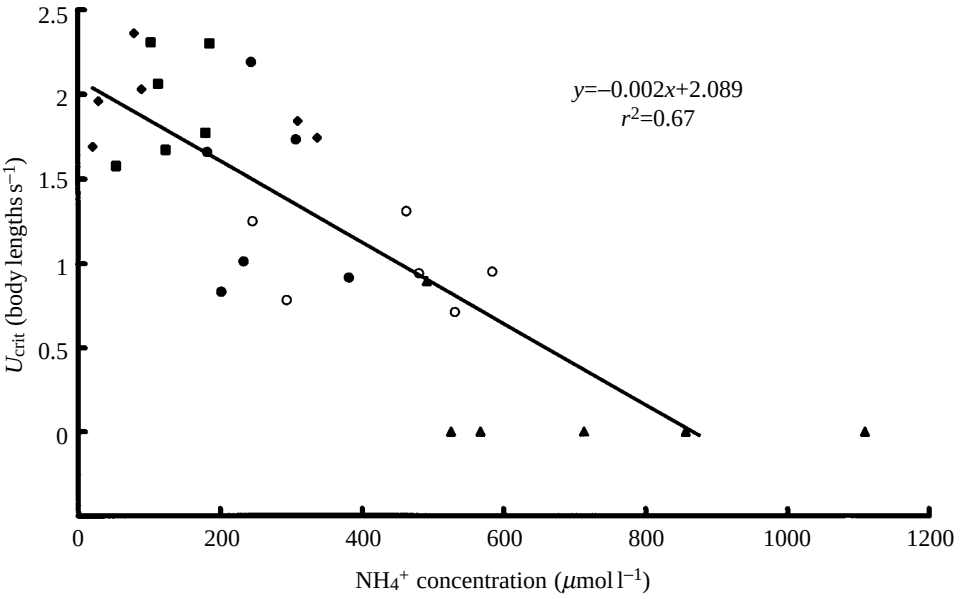

Fig. 3. The relationship between post-exercise NH_4^+ concentration in arterial plasma and critical swimming speed (U_{crit}) of brown trout acclimated to either 5 or 15 °C and exposed for 96 h to either control conditions (pH 7, no added Cu^{2+}) or to Cu^{2+} concentrations of either 0.08 or 0.47 μmol l^{-1} at pH 5 (N=6). Fish exposed to a Cu^{2+} concentration of 0.47 μmol l^{-1} at pH 5 which did not swim at the lowest water speed (0.3 m s^{-1}) (N=5) have been designated as having a U_{crit} of 0 m s^{-1}. ■, 5 °C, no Cu^{2+}, pH 7; ▲, 5 °C, Cu^{2+} at 0.47 μmol l^{-1} and pH 5; ○, 5 °C, Cu^{2+} at 0.08 μmol l^{-1} and pH 5; ◆, 15 °C, no Cu^{2+}, pH 7; ●, 15 °C, Cu^{2+} at 0.08 μmol l^{-1} and pH 5.

The branchial permeability coefficient of NH_3 is greater than that for CO_2 and considerably greater than that for O_2 (Cameron and Heisler, 1983). Thus, as diffusional oxygen uptake was apparently not affected by exposure of brown trout to sub-lethal copper and low pH (Beaumont *et al.* 1995), and yet there was an accumulation of ammonia, there may be an active component in ammonia excretion that is damaged by Cu^{2+}-exposure and/or exposure to pH 5 (Wilson and Taylor, 1992, 1993*a*).

Ammonia and swimming performance

We found a significant linear relationship between U_{crit} and post-exercise plasma [NH_4^+]. The value for the coefficient of determination (r^2) implies that almost 67 % of the variation in swimming performance can be explained by the plasma [NH_4^+]. There is no evidence from this study that ammonia accumulation is a normal cause of fatigue in trout, for there is no increase in ammonia levels in control animals at U_{crit}. Instead, these data suggest that elevation in ammonia levels due to Cu^{2+}-exposure at pH 5 may be the basis of reduced performance, i.e. ammonia accumulation may cause fatigue to occur earlier in these fish. A pilot study (N=4) has been conducted in which ammonium bicarbonate was infused into the dorsal aorta of brown trout and the U_{crit} of these animals was then determined. On average, T_{amm} was increased to approximately six times the resting value (1.7 mmol l^{-1}), which is well above the highest levels observed during the present study. These high levels caused an average 30 % reduction in U_{crit}. The effect was less than that caused by Cu^{2+}-exposure at pH 5 (see Fig. 3) which may reflect, at least in part, an additional contribution of haematological disturbances, resulting in local hypoxia (Beaumont *et al.* 1995). However, the infusions took place over 24 h, shorter infusions had less effect and it is possible that infusions of up to 96 h in duration could produce changes in swimming performance of a magnitude more similar to those that occurred following 96 h of Cu^{2+}-exposure at pH 5. Ammonia and ammonium ions are known to have a number of metabolic and physiological effects that may influence swimming performance. They may interfere with the metabolic status of the muscle or affect central or peripheral nervous activity, transmission at the neuromuscular junction, excitation/contraction coupling or muscle electrophysiology.

Metabolism is influenced by ammonia at a number of stages. Ammonium ions are an allosteric activator of phosphofructokinase (Su and Storey, 1994) and inhibit pyruvate carboxylase (Zaleski and Bryla, 1977). Elevated ammonia levels may, therefore increase the rate of flux through the glycolytic pathway, depleting stored glycogen levels and possibly also disrupting its regeneration. Although evidence indicates that swimming at up to 70–80 % of U_{crit} is essentially aerobic in

Table 2. *Critical swimming speed of cannulated brown trout* Salmo trutta *acclimated to either 5 or 15 °C and exposed for 96 h to either control conditions (pH 7, no added Cu^{2+}) or to the stated Cu^{2+} concentration at pH 5*

Temperature (°C)	Test water [Cu^{2+}] (μmol l^{-1})	pH	U_{crit} (body lengths s^{-1})
5 (control)	–	7	1.95±0.13
5	0.08	5	0.99±0.10***
5	0.47	5	<0.3 m s^{-1}
15 (control)	–	7	2.01±0.14
15	0.08	5	1.48±0.25*

Data are from Beaumont *et al.* (1995).
*P<0.05; ***P<0.001, compared with relevant control values.
Values for U_{crit} are given as means ± S.E.M. (N=6).

salmonids (Jones, 1982), some recruitment of white muscle occurs even at sustainable speeds and particularly as the animal nears fatigue (Johnston and Moon, 1980; Butler and Day, 1994). The high ammonia concentration prior to exercise might, therefore, have reduced swimming performance by impairing anaerobic capacity. Butler and Day (1993, 1994) have presented evidence that there is a lack of activity in white muscles at U_{crit} in brown trout exposed to sub-lethal pH (pH 4 at 5 °C, pH 4.5 at 15 °C) for 96 h. Under these conditions, there is a significant elevation of plasma ammonia concentration associated with a 50 % reduction in U_{crit} (N. Day and P. J. Butler, unpublished observations). However, this does not explain the results for the Cu^{2+}-exposed fish at pH 5 at 15 °C in the present study (Fig. 1). Plasma T_{amm} was lower in the exercised group than in the resting trout and it is the *post-exercise* $[NH_4^+]$ value that lies on the regression line between $[NH_4^+]$ and swimming performance (Table 2; Fig. 3). If glycogen depletion is the cause of reduced swimming capacity, then it is possible that a prior history of elevated ammonia concentration is important rather than the level during exercise. However, a lowering of ammonia levels at the onset of exercise may allow sufficient gluconeogenesis to occur for some recovery of glycogen stores prior to the recruitment of the white muscle.

Ammonium ions also attenuate the oxidative decarboxylation of pyruvate to lactate (McKhann and Tower, 1961) and, in the cat cerebral cortex, inhibit the activity of pyruvate dehydrogenase (Katunuma *et al.* 1966). This latter enzyme is important for the conversion of pyruvate to acetyl coenzyme A, the link between glycolysis and the tricarboxylic acid (TCA) cycle, which occurs in the mitochondria. Finally, owing to an accelerated decrease in pyridine nucleotide levels, ammonium ions have an inhibitory effect upon isocitrate dehydrogenase, a rate-limiting enzyme within the TCA cycle (Katunuma *et al.* 1966). Indeed Avillo *et al.* (1981) refer to unpublished data showing that high ammonia levels cause an impairment of the TCA cycle in rainbow trout. Elevated plasma ammonia levels could, therefore, have reduced swimming performance by slowing and even uncoupling oxidative phosphorylation, lowering the efficiency of aerobic metabolism.

Another area in which increased ammonia levels could be of influence is the neuromuscular coordination of exercise. Ammonium ions are able to substitute for K^+ in exchange mechanisms, resulting in depolarisation of neurones (Binstock and Lecar, 1969). Sjøgaard (1991) has proposed that K^+ loss and subsequent depolarisation are the causes of fatigue associated with low contraction forces in human skeletal muscle. The plasma $[K^+]$ of exercised trout that had been exposed to 0.08 μmol l^{-1} Cu^{2+} at pH 5 was not significantly different from that of control trout, despite differences in swimming performance. However, plasma $[K^+]$ was also not elevated above that of control resting trout in the trout exposed to 0.47 μmol l^{-1} Cu^{2+} at pH 5 and which would not swim. Unless K^+ loss from muscle to the extracellular fluid is masked by fluxes from plasma to the environment, this seems an unlikely cause of the failure to swim in these trout.

Ammonium ions may affect the neuromuscular system in other ways. The inhibition of glutaminase (O'Neill and O'Donovan, 1979) decreases glutamate, aspartate and GABA concentrations, all essential synaptic neurotransmitters. In the cat spinal cord, Raabe and Lin (1984) have shown that ammonia can decrease the hyperpolarising action of postsynaptic inhibition. This effect was due to the inactivation of Cl^- extrusion from the neurones and occurred at ammonium concentrations much lower than those required to produce any other nervous effect and without any metabolic changes. The resulting disruption of synaptic transmission has been proposed as the cause of ammonia-induced convulsions and coma (Hillaby and Randall, 1979) but, at lower doses of ammonia, subtle disruption to the coordination of exercise could lead to the loss of performance observed in this study. The effect of ammonia upon nervous function would also be easily reversible and thus fits the observations at 15 °C in this study.

Elevation of plasma ammonia concentration is not a phenomenon related to Cu^{2+}-exposure alone. For example, low pH (pH 4 at 5 °C, pH 4.5 at 15 °C; N. Day and P. J. Butler, unpublished observations.), high pH (pH 9.9; Lin and Randall, 1990) and increased aluminium levels (Booth *et al.* 1988) elevate plasma ammonia levels, and these pollutants also decrease swimming performance (Ye and Randall, 1991; Butler *et al.* 1992; Wilson and Wood, 1992). Thus, this single factor may at least partly explain reduced swimming performance arising from a variety of environmental pollutants.

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