

**REDUCTION IN SWIMMING PERFORMANCE OF BROWN TROUT FOLLOWING
COPPER EXPOSURE IN SOFT ACIDIC WATER.**

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Introduction and Methods

Toxicity tests have provided investigators with a simple and relatively rapid procedure by which to assess the effects of a pollutant upon an organism. However, the limitations of measures of lethality, such as the LC₅₀, in predicting the environmental consequence of a pollutant to a population of organisms have been recognised for some time. Swimming performance is an indicator of the ability of the fish to feed, escape predation and maintain position in a current (Beamish, 1978) and is particularly important to fish such as salmonids that migrate upstream to spawn. Petersen *et al.* (1987) observed near normal behaviour of rainbow trout exposed to acrylamide in an aquarium but a complete lack of swimming ability of the same fish in a swimming tunnel and conclude that a toxicant's sub-lethal effects may be as important as its lethal effects in determining the long term viability of fish. It is, therefore, perhaps surprising to find that the number of studies of swimming performance of fish exposed to pollutants is relatively meagre. As well as providing a more realistic appraisal of the toxicity of a pollutant like copper, such studies can provide the opportunity to uncover the morphological and physiological changes leading to reduced swimming performance which may in turn provide some insights into the little understood mechanisms of fatigue.

Toxicity tests were performed to determine the 96 h sub-lethal copper concentration at pH 5 (SLCC) for adult brown trout acclimated to 5°C (Oct.-Mar.) or 15°C (May-Aug.) in an artificial softwater (Ca²⁺ 50 µmol l⁻¹). These were found to be 0.47 and 0.08 µmol l⁻¹ Cu²⁺ at 5 and 15°C respectively. Critical swimming speed (Ucrit) of groups of trout exposed to the SLCC for each temperature were measured and compared to trout maintained in the absence of copper and at pH 7. At 5°C, an additional group were

exposed to the summer copper level of $0.08 \mu\text{mol l}^{-1} \text{Cu}^{2+}$ at pH 5. At the end of each experiment, samples of arterial blood were taken from the dorsal aorta via chronically indwelling cannulae, gill samples were excised and fixed for light and electron microscopic analysis and samples of red, white and cardiac muscle, liver, and gill tissues were freeze clamped for later analysis of metabolite concentrations and enzyme activities.

Swimming Performance

Exposure to sub-lethal copper at low pH significantly reduced swimming performance. Trout exposed to $0.08 \mu\text{mol l}^{-1} \text{Cu}^{2+}$ at pH 5 achieved a critical swimming speed some 25-50% slower than control trout with no significant temperature effect. Of the six winter trout exposed to $0.47 \mu\text{mol l}^{-1} \text{Cu}^{2+}$, only one swam steadily at the lowest test speed of 0.3ms^{-1} , the remainder achieving at most a brief burst of activity. Prior to the present study and the recent experiments of Butler *et al.* (1992), a reduction in swimming performance observed following exposure to a pollutant was generally attributed to a disruption of oxygen transfer at the gill or a decrease in the oxygen carrying capacity of the blood. Specifically, in the case of acid and of copper exposures, the studies of Graham and Wood (1981), Ye and Randall (1991) and of Waiwood and Beamish (1978) each cited reductions in oxygen transfer and carrying capacity as probable contributory factors to a measured reduction in Ucrit. However no direct measurements of blood oxygen levels were made in any of these investigations although Ye and Randall (1991) did measure a minor blood and red cell acidosis from which they inferred that a reduced oxygen carrying capacity may follow the reduction in oxygen affinity and capacity resulting from combined Bohr and Root shifts.

Unlike Ye and Randall (1991), but in common with Wood (1989), Butler and Day (1993) observed no significant changes in pH of either the plasma or red blood cells following acid exposure. More importantly, there was not a decrease in arterial oxygen content (Butler *et al.* 1992). In fact, at 15°C , oxygen content was significantly increased by exposure to acidity.

In the present study, considerable gill damage was observed following exposure to sub-lethal copper, the extent of which was related to the copper concentration. In the extreme, there was hyperplasia of epithelial cells, proliferation of mucocytes in the secondary lamellae, necrosis of chloride cells and fusion of neighbouring secondary lamellae. Morphometric analysis showed the harmonic mean diffusion distance across the lamellae to have increased by over threefold from $3.62 \pm 0.42 \mu\text{m}$ in control fish to $11.54 \pm 1.63 \mu\text{m}$ in fish exposed to $0.47 \mu\text{mol l}^{-1} \text{Cu}^{2+}$ at pH 5. In these fish, although there was no change in the arterial partial pressure of oxygen (P_{aO_2}) at rest, a significant decline in the exercised fish indicates an underlying diffusional limitation.

Trout exposed to $0.08 \mu\text{mol l}^{-1} \text{Cu}^{2+}$ at pH 5 also possessed gill ultrastructural abnormalities. However, there was little indication that oxygen uptake ever became limiting, P_{aO_2} was not significantly decreased even following exercise. Indeed, as for exposure to acid alone (Butler *et al.*, 1992), the oxygen content of arterial blood was unchanged or even elevated after exposure to either level of copper.

While neither oxygen uptake nor the apparent oxygen carrying capacity were limiting, oxygen transport to the tissues may have still been disrupted. Both acid and copper exposure have a number of haemoconcentrating effects such as the osmotic loss of plasma water and an increase in number and size of red cells. Indeed, at least during acid exposure, haemoconcentration and the concomitant increase in blood viscosity are

believed to be the primary causes of death (Wood, 1989). Randall and Brauner (1991) suggested that sub-lethal changes in blood viscosity during acid exposure may affect exercise through changes to cardiac output, principally reduced stroke volume, that reduce oxygen transport to the tissues. Changes in blood viscosity may also affect the local circulation of blood through the peripheral capillaries (Wells and Weber, 1991). Following copper/low pH exposure, increases in mean arterial blood pressure, haemoglobin concentration, haematocrit and protein concentration were measured. While these alterations were not consistent between treatments, there were also some evidence of changes to tissue metabolites, consistent with hypoxia. These were found particularly in the trout exposed to the higher copper concentration where, for example, resting glycogen levels in the white muscle was over 40% lower and red muscle lactate concentration almost double that of control animals. Lactate dehydrogenase activity also displayed a trend to increase following low pH and copper exposure.

Ammonia accumulation and swimming performance.

Following sub-lethal copper/low pH exposure, there was a very significant elevation of plasma ammonia. When the critical swimming speed of the trout exposed to copper and their controls was plotted against plasma [Tamm] or $[\text{NH}_4^+]$, a significant negative correlation, with an r^2 value of almost 0.7, was established (Figure 1). A limited pilot experiment, in which ammonium bicarbonate was infused into the dorsal aorta of brown trout and the Ucrit of these animals determined, indicate this to be a viable method of raising plasma [Tamm] without the other physiological changes associated with copper exposure. Raising [Tamm] above the highest levels observed during sub-lethal copper exposure caused Ucrit to be reduced by over 50%. These infusions took place over 24 h, shorter infusions had less effect and infusions up to 96 h in duration may therefore produce changes in swimming performance of more similar magnitude to those that occurred following copper exposure.

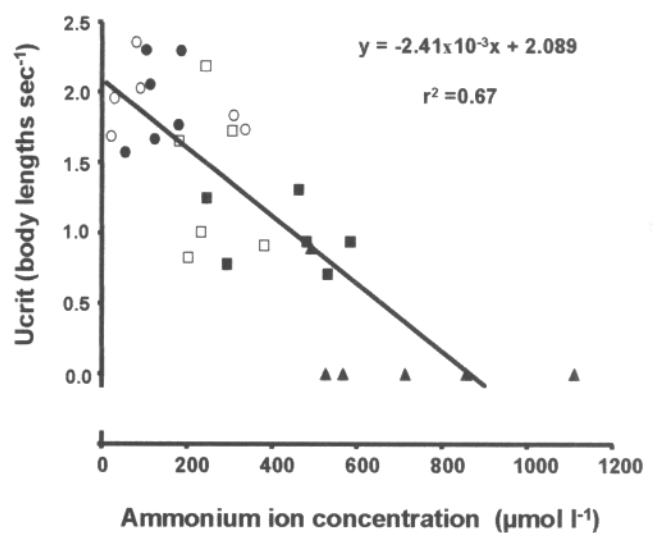


Figure 1. The relationship between swimming performance and plasma NH_4^+ concentration.

Ammonia and ammonium ions have a number of possible physiological effects that may influence swimming performance by interfering with central or peripheral nervous activity, with transmission at the neuromuscular junction, with excitation/contraction coupling and muscle electrophysiology or by affecting the metabolic status of the muscle. NH_4^+ ions are an allosteric activator of phosphofructokinase (PFK), one of the rate limiting enzymes of glycolysis (Su and Storey, 1994) and an inhibitor of pyruvate carboxylation, a first step in gluconeogenesis (Zaleski and Bryla, 1977). Elevated ammonia may therefore increase the rate of flux through the glycolytic pathway depleting stored glycogen and, furthermore, disrupt its regeneration. The metabolite data from his study does not support this conclusion, however, since glycogen appears to have been significantly depleted only in the muscles of the fish exposed to the highest level of copper.

Ammonium ions also attenuate oxidative decarboxylation of pyruvate to lactate (McKhann and Tower, 1961) and, in the cat cerebral cortex, inhibit 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 cycle which occurs in the mitochondria. Finally, due to an accelerated decrease in pyridine nucleotides, ammonium ions have an inhibitory role upon isocitrate dehydrogenase, a rate limiting enzyme within the TCA cycle itself (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 could therefore have reduced swimming performance by slowing and even uncoupling oxidative phosphorylation, thus lowering the efficiency of aerobic metabolism.

Ammonium ions are able to substitute for K^+ in exchange mechanisms, resulting in a depolarisation of neurons (Binstock and Lecar, 1969) and it is possible that the neuromuscular coordination of exercise is another area in which ammonia could have an influence. Disturbances of sarcolemma or t-tubule membrane excitability due to an inability to maintain K^+ gradients has been postulated as a possible mechanism of fatigue (Sjøgaard, 1991). Ammonium ions might exacerbate this effect. Heald (1975) found ammonium ions to depress the twitch tension of stimulated frog sartorius muscle through the progressive loss of fibres as they became electrically inexcitable. Ammonium inhibition of glutaminase (O'Neill and O'Donovan, 1979) decreases glutamate, aspartate and GABA concentrations, all essential synaptic neurotransmitters. In a recent study of the sub-lethal effects of copper exposure to carp, De Boeck *et al.* (1994) found the concentration of some neurotransmitters in the telencephalon to decline by almost 50%. In the cat spinal cord, Raabe and Lin (1984) have shown that ammonia can decrease the hyperpolarising action of postsynaptic inhibition. This was due to the inactivation of Cl^- extrusion from the neurons 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 coordination of exercise may lead to the loss of performance that has been observed in our previous studies.

Concluding remarks.

The limitations of lethal toxicity testing are now well recognised. Critical swimming performance, which involves the use of both aerobic and anaerobic metabolic pathways and has obvious ecological application, can be widely applied as a more satisfactory indicator of the sub-lethal effect of pollutants on fish. Elevation of plasma ammonia concentration is not a phenomenon related solely to copper exposure. For example, low pH (Day and Butler, in press), high pH (Lin and Randall, 1990) and aluminium (Booth *et al.* 1988) elevate plasma ammonia and these pollutants also decrease swimming performance (Butler *et al.* 1992, Ye and Randall, 1991, Wilson and Wood, 1992). If a relationship between U_{crit} and ammonia can be confirmed, then perhaps this single effect may at least partly explain the reduced swimming performance arising from a variety of environmental pollutants.

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