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INFLUENCE OF MILD HYPERCAPNIA ON THE EFFECTS OF ENVIRONMENTAL ACIDIFICATION ON RAINBOW TROUT (*SALMO GAIRDNERI*)

BY C. M. NEVILLE

Department of Zoology, University of Toronto, Toronto, Ontario, Canada

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The physiological consequences of lethal ambient acid levels in adult fish have been previously investigated (Lloyd & Jordan, 1964; Packer & Dunson, 1972; Lievestad & Muniz, 1976). Acidaemia associated with a loss of total carbonate and/or electrolyte loss have been implicated as the cause of death but the sublethal physiological effects leading to one or both of these conditions have not been fully clarified. Neville (1979) has shown that the acidaemia of trout exposed to pH 4 under normocapnic conditions for 5 days is not due to build-up of lactic acid.

It is possible that the reduction in ambient total carbonate concentration after a pH change from 7 to 4 may be the underlying cause of total carbonate loss from the fish. Douderoff & Katz (1950) concluded that most freshwater fish can tolerate pH 5 indefinitely in the absence of other adverse conditions. By imposing a slight hypercapnia it is possible to elevate the bicarbonate concentration at pH 4 to the level which would be found at pH 5 with normocapnia. This report deals with the effects of mild hypercapnia upon fish exposed to water of pH 4.

Rainbow trout (*Salmo gairdneri*; 295–405 g) were exposed at 10 °C in well-aerated water to pH 7·0 (controls), pH 4·0 (acid group), or pH $4{\cdot}0 + P_{CO_2} = 4{\cdot}5$ mmHg (acid + mild hypercapnia group). The pH was controlled ($\pm 0{\cdot}1$ unit) by automatic titrators, and water P_{CO_2} was monitored using a P_{CO_2} electrode system (Radiometer E5036 and PHM 71).

After 12 days arterial and venous blood samples were taken anaerobically from the caudal vessels for measurement of pH, total CO_2, Hb, Na^+, Cl^-, and Ca^{2+}. Tissue samples approximately 1 g in weight were taken from the epaxial musculature for measurement of water content and electrolytes.

Arterial blood pH was measured anaerobically at 10 °C (Radiometer microelectrode unit, E 5021 and acid–base analyser PHM 71). Total CO_2 was measured on 100 μl freshly centrifuged arterial blood plasma (Harleco micro CO_2 apparatus, no. 64987). Values were estimated to be within ± 1 mM l^{-1} by this method. Mean HCO_3^- and P_{CO_2} for each group were calculated from the mean pH and total CO_2 using the Henderson-Hasselbalch equation, with values for pK^1 and αCO_2 from Albers (1970). Haemoglobin was measured colorimetrically, serum Cl^- on a Buchler Cotlove chloridometer, and serum Na^+ and Ca^{2+} with an atomic absorption spectrophotometer (Varian Techtron Model AA-6).

Tissue samples were dried overnight at 110 °C for estimation of percentage water

Table 1. *Blood and tissue respiratory and electrolyte characteristics (mean ± S.D.) after 12 days exposure to pH 7·0 (control), pH 4·0 (acid group), or pH 4·0 + 4·5 mmHg CO_2 (acid + mild hypercapnia group).*

	Control (n = 10)	Acid (n = 8)	Acid + CO_2 (n = 9)
	Blood		
pH	7·66	7·35	7·55
H^+ (nM.l^{-1})	21·8 ± 2·6	45·5 ± 8·6**	28·8 ± 7·2*††
Hb (g%)	5·6 ± 1·1	7·4 ± 0·7*	5·3 ± 1·2†
	Plasma		
ΣCO_2 (mM.l^{-1})	9·9 ± 1·0	3·2 ± 1·5**	10·1 ± 1·8††
HCO_3^- (mM.l^{-1})	9·6	3·0	9·6
P_{CO_2} (mmHg)	4·9	3·3	6·9
Na^+ (mM.l^{-1})	149 ± 5·3	114 ± 11·7**	119 ± 10·7**
Cl^- (mM.l^{-1})	130 ± 6·5	102 ± 10·1**	105 ± 11·3**
Ca^{2+} (mM.l^{-1})	2·3 ± 0·3	2·1 ± 0·3	2·2 ± 0·2
	Epaxial muscle		
% water content	76·4 ± 1·7	77·1 ± 1·7	77·7 ± 1·0
Na^+ (mM.kg^{-1})	11·8 ± 2·6	7·2 ± 0·8**	8·1 ± 1·4*
Cl^- (mM.kg^{-1})	11·5 ± 2·5	6·7 ± 0·5**	7·1 ± 1·2**
Ca^{2+} (mM.kg^{-1})	3·0 ± 1·4	2·2 ± 0·3	3·3 ± 1·9

Significant difference from the control group – ** < 0·001, * < 0·01. Significant difference between the acid and acid + CO_2 groups – †† < 0·001, † < 0·01. (F test for analysis of variance.)

content, then digested in 0·1 N HNO_3 for extraction of electrolytes. The time course of electrolyte loss was determined in further experiments in control and acid groups only for exposure periods of 3–12 days.

The results of the blood and muscle analyses of fish after 12 days of the experimental treatments are presented in Table 1. Blood acid–base characteristics in the acid + CO_2 group were closer to the controls than to the acid group. However, the Na^+ and Cl^- concentrations in both blood and tissue resembled the acid group rather than the controls. H^+ ion concentration was greatly increased in the acid group despite an augmented buffering capacity provided by an increase in haemoglobin concentration (Table 1). The arterial P_{CO_2} was lowest in the acid group and highest in the acid + CO_2 group. Total CO_2 and HCO_3^- were also considerably reduced in the acid group and slightly raised above control levels in the acid + CO_2 group.

The decrease in Na^+ and Cl^- concentrations in the blood and tissue of both experimental groups as compared to the controls is unlikely to be due to water influx as there were no significant differences in tissue water content or blood and tissue Ca^{2+} concentrations amongst all three groups (Table 1). However, tissue Ca^{2+} levels in the acid group were consistently low, suggesting a depletion of intracellular Ca^{2+}. There were no significant differences (5% level) between acid groups of consecutive 3-day intervals except the 0- and 3-day Na^+ concentrations (Fig. 1). However, the loss of Na^+ and Cl^- was significant over the 12-day period and presumably would have continued if the experiment had been prolonged.

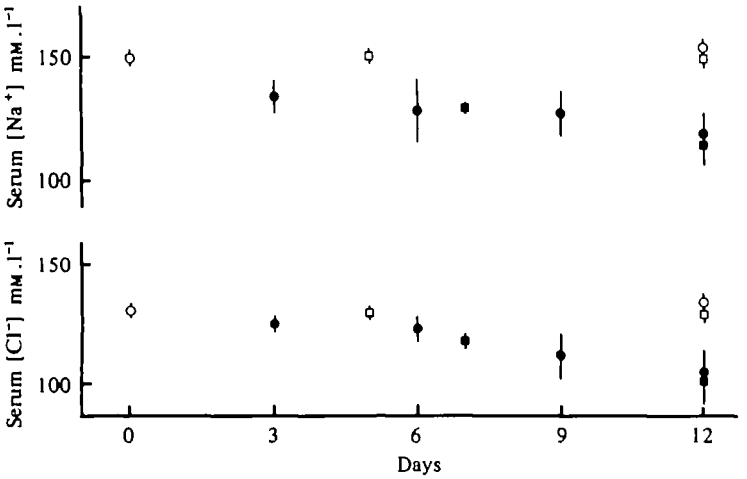

Fig. 1. Effects of exposure to pH 4·0 on serum Na^+ and Cl^- concentrations in confined fish (●), and free swimming fish (■). Controls – open symbols. Mean ± S.E. of at least 6 fish except day 3, Na^+ (n = 3) and day 7 Na^+ and Cl^- (n = 2). No significant difference between tank and individual chamber experiments, nor between acid groups of consecutive 3-day intervals except 0–3-day Na^+ concentrations. (Wilcoxon non-parametric ranked sum test, 5% level.)

Sampling technique

The values for arterial pH in the control fish are approximately 0·25 pH units lower than those observed in cannulated fish (Neville, 1979). Undoubtedly this is a consequence of the method of blood sampling where the fish have undergone about 1 min of struggling followed by a few seconds of apnoea. However, the overall pattern of blood acid–base change between control and pH-4-exposed fish is essentially the same as observed in chronically cannulated trout under the same experimental circumstances (Neville, 1979). The sampling method had little effect on the control fish blood Na^+ and Cl^- levels since these are the same as those reported for salmonids in fresh water by Holmes & Donaldson (1969).

Experimental results

The following conclusions can be drawn as to the cause of acidaemia and electrolyte loss in rainbow trout.

(*a*) The acidaemia is not due to hypercapnia since in the acid group both ambient and arterial P_{CO_2} were low (Table 1).

(*b*) The acidaemia cannot be explained exclusively by inhibition of possible Na^+/H^+ or Na^+/NH_4^+ ion exchange mechanisms in the gills since the acid + hypercapnia fish were only slightly acidaemic but continued to lose electrolytes at the same rate as the acid group (Table 1).

(*c*) The acidaemia is not due to lactic acid accumulation (Neville, 1979).

(*d*) The electrolyte loss is due to ambient low pH and not to acidaemia (Table 1).

Although the experiments do not reveal the exact cause of acidaemia and electrolyte

loss the results show clearly that the presence of mild hypercapnia in an acidic environment does prevent loss of total carbonate and acidaemia, but not the loss of electrolytes. Without measurements of flux rates it is not possible to define a mechanism by which this occurs. However, the increased ambient HCO_3^- concentration may reach critical levels required for a HCO_3^- uptake system, as proposed by de Renzis (1975) for goldfish, or after hydration of the obligatory increased CO_2 tension within the gill epithelium, HCO_3^- may be transported into the blood where it would increase the buffering capacity, whilst the resultant H^+ ions would be actively transported to the environment either as H^+ or NH_4^+ in exchange for Na^+ as proposed by Maetz (1973). Both of these hypotheses would require intact active transport systems for ion exchange, and increased gill epithelial permeability to account for increased diffusive loss of ions. McWilliams & Potts (1978) calculated from measurements of gill potentials and Na^+ flux rates in brown trout (*S. trutta*) that H^+ ion influx at pH 4·0 alters the trans-epithelial potential and is the major cause of increased efflux and reduced influx of Na^+ ions. However, their calculated 40% decrease in Cl^- efflux does not comply with the loss of Cl^- found in my experiments, nor by Lievestad & Muniz (1976) in tank experiments on brown trout where Na^+ and Cl^- ions were lost in equimolar concentrations. These results indicate that electrolyte loss is more likely due to increased gill permeability to both ions rather than to changes in trans-epithelial potential.

My results support those of Lloyd & Jordan (1964). They found that an acidic environment (pH 3·5–4·5) with 10 ppm CO_2 (i.e. $P_{CO_2} \sim 3{\cdot}5$ mmHg) caused less stress to rainbow trout than with 1·5 or 50 ppm CO_2. They tested whether the increased survival time was caused by the free CO_2 content of the water by exposing fish to ambient pH values of 3·3 and 3·6 with five levels of free CO_2 ranging from 1·5 to 48 ppm. At these extreme pH values the free CO_2 levels had no significant effect. However, if the tests had been repeated at pH 4·0–4·5 they might have found that there was indeed a significance.

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