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THE PHYSIOLOGY OF ACID/ALUMINIUM STRESS IN TROUT

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ABSTRACT

Recent work in the authors' laboratories is reviewed on the responses of brook (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) to acid/Al exposure under environmentally realistic conditions in softwater. The studies involve measurements of net and unidirectional ion exchange, blood gases, acid-base status, hematology, and electrolytes by chronic cannulation, whole body ions, and gill Al. The principal conclusions are: (i) the toxic effects of low pH alone reflect direct actions of H^+ on branchial ionoregulatory mechanisms; (ii) Al is additionally toxic to ionoregulation, largely through effects on the passive efflux component; (iii) Al is also toxic to O_2 and CO_2 exchange across the gills, with resultant hypoxemia and acidosis; (iv) these effects are associated with the accumulation of Al on the gills; (v) the rainbow trout is more sensitive than the brook trout; and (vi) the relative importance of these two mechanisms varies with water pH, Ca, and species. The actions of H^+ and Al are discussed in the context of the branchial micro-environment.

INTRODUCTION

While reproductive failure and early life stage mortality are probably the critical factors in fisheries decline under environmental acidification, sublethal and lethal toxicity to juveniles and adults may also make important contributions. The physiological effects of acidity on fish have been studied intensively over the past decade (see McDonald, 1983; Howells, 1984; Wood, 1987, for reviews). It is now clear that over the pH range of environmental relevance in softwater (4.0 - 6.0), the key toxic mechanism of pure acid stress is disturbance of electrolyte balance at the gills and ultimate ionoregulatory failure. This is thought to kill the fish through secondary fluid volume shifts, hemoconcentration, and circulatory collapse. Water Ca is protective against ionoregulatory disturbance. Acid stress may cause other problems

(acidosis, O_2 and CO_2 exchange disturbances), but these are of sufficient magnitude to cause mortality only under abnormal circumstances (eg. pH's below 4.0 and/or very high Ca water).

However fisheries losses and direct fish kills have often been noted at higher pH's than anticipated from the known effects of acidity alone. A growing body of evidence implicates Al (Grahm, 1980; Muniz and Leivestad, 1980a; Schofield and Trojnar, 1980; Henriksen *et al.*, 1984), for Al is abundant in rocks and soil, highly soluble at low pH, relatively innocuous at neutral pH, but extremely toxic to fish around pH = 5.0. The aqueous chemistry of Al is complex, with speciation critically dependent on pH and the presence of Al-binding ligands. Far less is known about the physiology of acid/Al stress than about pure acid stress. The limited information available suggests a much more complicated situation (Muniz and Leivestad, 1980a,b; Rosseland, 1980; Stuarne *et al.*, 1984; Neville, 1985; Witters, 1986). Ionoregulatory, respiratory, and acid-base effects have all been observed in softwater over the pH range of environmental relevance. The goal of our recent research, reviewed here, has been to identify sublethal and lethal mechanisms of toxicity of acid/Al stress in salmonids, and to understand how their importance may vary with water pH and Ca levels. The majority of the work has been carried out on the brook trout (*Salvelinus fontinalis*) with some comparative studies on the rainbow trout (*Salmo gairdneri*).

MATERIALS AND METHODS

Adult (100 - 300 g) and juvenile (10 - 30 g) brook and rainbow trout were obtained from hardwater hatcheries and acclimated to artificial softwater (ASW; pH = 5, 10 - 12°C) for at least two weeks. The ASW was produced by reverse osmosis followed by re-addition of Ca^{2+} (25 or 400 uequiv/L) and Na^+ (~50 uequiv/L) to levels typical of acid-threatened softwaters in eastern North America and northern Europe. In some experiments, adult trout were surgically fitted with indwelling serial catheters to allow repetitive blood sampling without disturbance; fish were allowed to recover for 48 - 72 h prior to test.

In experimental exposures, adult fish were isolated in individual 2.5 L flux chambers (McDonald and Rogano, 1986) supplied with flowing ASW at 0.5 L/kg-min. Juvenile fish were exposed in batches under similar conditions. Al solutions in ASW

were made up fresh daily (as $AlCl_3$ at 0, 111, 333, and 1000 ug/L), pH was controlled automatically (with H_2SO_4 at 4.4, 4.8, 5.2 or 6.5), and the toxicants were run once through the chambers and then to waste. This approach minimized complications associated with aging of Al solutions, organic complexation or precipitation of Al, and pH changes in the bulk water, and ensured that the majority of the Al remained in the inorganic monomeric form.

Net ion fluxes were determined on a flow-through basis by monitoring concentrations in the inflowing and outflowing water of the flux chambers and applying the Fick principle. Unidirectional Na^+ fluxes were determined by briefly closing the chambers (30 min) and monitoring the disappearance of added ^{22}Na (McDonald and Rogano, 1986). Blood samples were obtained by terminal caudal puncture, or repetitively by catheter, and analyzed for blood gases, acid-base status, electrolytes, metabolites, and cortisol by standard clinical techniques. Whole body ions were determined by acid digestion, and gill Al levels by a pyrocathechol violet procedure.

RESULTS AND DISCUSSION

Ion Fluxes

Exposure of adult brook trout to acid stress alone for 10 days was not lethal, but resulted in net losses of Na^+ and Cl^- (eg. Fig. 1). The magnitude of the fluxes was dependent on the severity of the pH, and reduced by elevated water Ca. Losses were greatest over the first 6h, and zero balance was generally re-established within 24 - 48 h. The addition of Al to the exposures exacerbated the losses, and in many acid/Al combinations, resulted in severe mortality, most deaths occurring within 1 - 4 days (eg. Fig. 1). Terminal blood samples reflected these electrolyte losses, with severe hemoconcentration in lethal exposures. Net fluxes were most negative during the first 12 h, and thereafter attenuated; some survivors were able to attain zero or positive balance after several days of continuous exposure. The initial ion losses were a good predictor of eventual mortality. At low Ca = 25 uequiv/L, the magnitude of the net Na^+ loss and toxicity increased with the Al concentration at any one pH, and there was a tendency for Al to be less toxic at the lowest pH. At high Ca = 400 uequiv/L, these effects were less clear-cut, and toxicity and losses generally ameliorated.

Unidirectional flux measurements with ^{22}Na revealed that the initial effect of acid or acid/Al exposure was invariably a combination of influx inhibition and efflux stimulation (Fig. 2). The stimulation of efflux was larger, and therefore the more important factor in the initial losses, but this returned to control levels, or even below, within 24 - 48 h in all but acutely lethal exposures. In contrast, the inhibition of influx persisted indefinitely, and was responsible for ion losses over the long term. The specific effects of Al in the presence of acid were a concentration-dependent stimulation of efflux above that caused by low pH alone, and a further inhibition of influx (Fig. 2).

These results suggest that the ionoregulatory effects of acid/Al challenge are qualitatively similar to those previously demonstrated for low pH alone, and can be considered as two relatively distinct phases (cf. McDonald, 1983; Wood, 1987). An initial 'shock' phase (0 - 12 h) of heavy losses appears critical to acute mortality, may kill by setting up intolerable fluid volume disturbances. The large efflux stimulation at this time is likely due to an opening of paracellular channels in the gill epithelium (McDonald, 1983), while the influx inhibition may be explained by a blockade of active uptake mechanisms and transport enzymes (Stuarnes et al., 1984). Probably protects by binding to gill surface ligands, thereby stabilizing apical membranes and the integrity of the tight junctions on the paracellular channels. A 'recovery' phase (12 h +) reflects a return of efflux to control levels or below, appears to be an important adaptive response to acid/Al exposure. This may result from mucification, lamellar cell swelling, hormonal effects, or other mechanisms which restore paracellular permeability.

Parameters by Cannulation

Cannulation experiments with brook trout allowed us to follow the development of gas, acid-base, ionic, and hematological disturbances, and to assess the internal condition immediately prior to death. Cannulated fish were more sensitive than uncannulated, but trends in mortality with pH and Al were qualitatively similar. Ten days exposure to pH = 4.8 in the absence of Al (Ca = 25 uequiv/L) caused a significant internal disturbance (Fig. 3), but the same conditions at pH = 4.4 resulted in no mortality. Death was preceded by 35% decreases in plasma Na^+ and

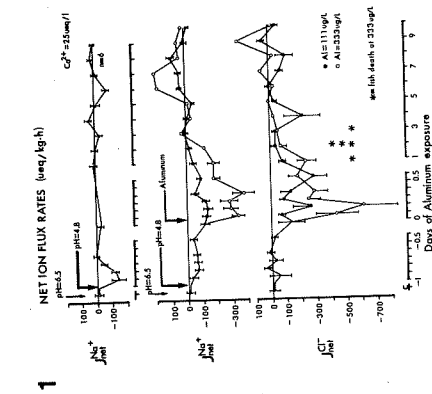


FIG. 1. Net Na^+ and Cl^- flux rates of adult brook trout exposed to acid or acid/Al for 10 days. Mean $\pm$ 1 SEM (N = 6). (Booth et al., 1987).

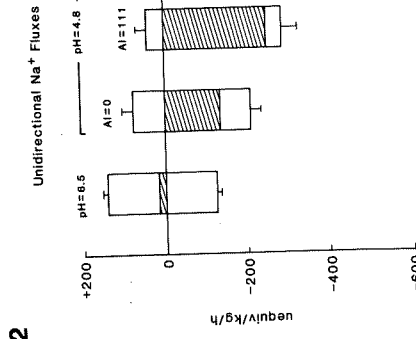


FIG. 2. Unidirectional Na^+ fluxes (crosshatching = net) in adult brook trout exposed to acid or acid/Al for 6 h. Mean $\pm$ 1 SEM (N = 6). (Booth et al., 1987).

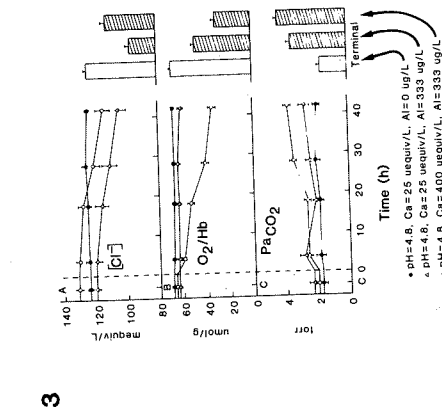


FIG. 3. Changes in plasma Cl^- , hemoglobin O_2 , and CO_2 tension of arterial blood in cannulated adult brook trout exposed to acid (N = 10) or acid/Al at low (N = 19) and high Ca (N = 28). Graphs show responses of 42+ h survivors (ie. most resistant fish), and crosshatched bars the last measurements prior to death in all fish. Under acid alone, no fish died so open bars show values at 10 days. Mean $\pm$ 1 SEM. (Wood et al., 1987a).

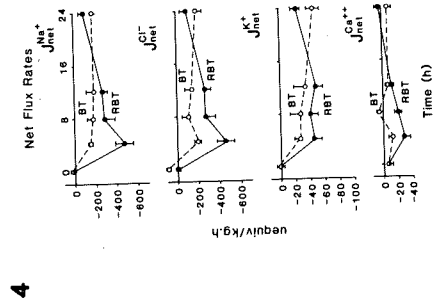


FIG. 4. A comparison of net ion flux rates between adult brook (BT) and rainbow trout (RT) exposed to an identical acid/Al challenge (pH = 4.8, Al = 333 ug/L, Ca = 25 uequiv/L) for 24 h. Mean $\pm$ 1 SEM (N = 6). (D.G. McDonald and C.M. Wood, unpubl.).

vels and marked red cell swelling, but no change in blood gases, confirming the lethal mechanism in pure acid stress is ionoregulatory failure. Interestingly, in this and the acid/Al exposures (eg. Fig. 3A), plasma ions fell in an almost fashion over time, despite the fact that branchial losses were greatest during initial 'shock' phase, and thereafter attenuated. This suggests an accompanying fall in ions from the intra- to extracellular compartments, a conclusion later confirmed by body ion measurements.

Exposure to pH = 4.8 plus Al = 333 ug/L at low Ca = 25 uequiv/L proved extremely with an LT50 of only 39 h. While a depression in plasma Na^+ (Fig. 5B) and (Fig. 3A) was again the most prominent physiological disturbance, fish died at higher plasma electrolyte levels than under acid stress alone. There was also evidence of respiratory problems (Figs. 3B,C, 5B,C: decreased Pa_{O_2} , hemoglobin saturation, and pH; increased Pa_{CO_2} and blood lactate) prior to death. At pH = 4.8 plus Al = 25 uequiv/L, and the same Al = 333 ug/L, average survival time was about twice as long, confirming the observation of the flux experiments that Al is less toxic at low pH. However the internal syndrome prior to eventual death (ionoregulatory failure compounded by respiratory inhibition) was very similar to that under the conditions at pH = 4.8. Exposure to acid/Al stress (pH = 4.8, Al = 333 ug/L) at Ca = 400 uequiv/L killed the fish almost as quickly as at lower Ca, but caused a different internal toxic syndrome. While ionic depressions were ameliorated by Ca (Fig. 3A), respiratory disturbance was clearly exacerbated to the point where it became the important cause of lethality (Fig. 3B,C). Arterial blood gases reached typical values, pH fell, and there were large elevations in lactate and obvious ventilation prior to death.

Thus acid/Al stress may involve respiratory blockade as a second mechanism of ionoregulatory failure, in agreement with the results of Muniz and Leivestad (1980b), and Neville (1985). The present data indicate that this mechanism is favoured by higher Ca (though still within the softwater range) and Neville's results indicate that it is favoured by higher pH's, especially above 5.0. Mucous production, the build-up of Al complexes on the gill surface, oedema, swelling, or even separation of the epithelial cell layers

(Schofield and Trojnar, 1980; Tandjung, 1982; Karlsson-Norrgren et al., 1986) may all serve to increase the water to blood diffusion distance for O_2 and CO_2 .

Brook Trout versus Rainbow Trout

Adult rainbow trout were far more sensitive to acid, or acid/Al, than were adult brook trout. For example, in flux studies, rainbows consistently lost greater amounts of electrolytes and showed earlier and larger mortalities than brooks under identical acid/Al challenge (Fig. 4). Blood glucose and plasma cortisol levels are two well documented indices of stress in fish (Wedemeyer and McLeay, 1981). Glucose and cortisol elevations were as great at Al = 111 ug/L in rainbow trout as at Al = 333 ug/L in brook trout (at pH = 4.8, low Ca; Table 1). Furthermore there was an initial rise in cortisol during the first 24 h of exposure to acid alone (pH = 4.8) in rainbows but not in brooks, though this had disappeared after 3 days in both (Table 1). These physiological conclusions are in accord with toxicological and field observations, indicating that *Salvelinus fontinalis* is the most resistant, and *Salmo gairdneri* the most sensitive of a range of salmonids (Grande et al., 1978). At least in part, this difference may stem from the long evolutionary history of brook trout in softwater, to which it is widely endemic.

Cannulation experiments suggest that the respiratory problems caused by Al may be of greater significance in the rainbow trout. Fig. 5 compares selected blood parameters in rainbows exposed to Al = 111 ug/L with those in brooks exposed to Al = 333 ug/L (both at low Ca, pH = 4.8), treatments with very similar LT50's. Prior to death, rainbows suffered lesser plasma ion depressions (Fig. 5A), but more severe reductions in Pa_{O_2} (Fig. 5B), acidosis (Fig. 5C), and elevations in Pa_{CO_2} . Indeed, the response of the rainbow trout at lower Ca and Al was comparable to that of the brook trout at higher Ca and Al (cf. Fig. 3). The mechanism(s) responsible for these differences remain unknown, but the observations emphasize that caution is needed in extrapolating between species in terms of both sensitivity and causes of death.

TABLE 1. Indices of stress in brook and rainbow trout under acid and acid/Al exposure. Means $\pm$ 1 SEM (N = 6-13). (Goss and Wood, 1987; Wood et al., 1987b).

	Brook Trout		Rainbow Trout	
	Control (pH = 6.5)	Acid (pH = 4.8)	Acid/Al (Al = 333 ug/L)	Acid/Al (pH = 4.8) (Al = 111 ug/L)
Cortisol (ng/ml)	131.5 $\pm$ 27.8	175.0 $\pm$ 34.4	322.2* $\pm$ 51.3	75.4 $\pm$ 28.7
Glucose (mmol/L)	3.24 $\pm$ 0.34	3.98 $\pm$ 0.46	10.93* $\pm$ 0.92	4.39 $\pm$ 0.70
				3.49 $\pm$ 0.53
				8.06* $\pm$ 0.66

Fish were cannulated. Control and acid data were taken after 3 days exposure; acid/Al data were last measurements prior to death within this period.
*p $\leq$ 0.05 relative to control.

Juvenile Fish

Juvenile fish offer several advantages for acid/Al studies. Many more juveniles than adults can be exposed in a given volume of ASW, the generation of which is a major limitation and expense in this type of work. Furthermore, they can be terminally blood sampled, and then easily digested for measurement of whole body ions and gill Al content. Our research to date with juvenile rainbow and brook trout indicates that their responses are both qualitatively and quantitatively similar to those of adults.

For example, pH = 4.8, Al = 111 ug/L at Ca = 25 uequiv/L had only small effects on juvenile brook sacrificed from 4 to 48 h during the challenge. In contrast, juvenile rainbows subjected to the same challenge showed progressive decreases in plasma Na^+ and Cl^- , increases in hematocrit and plasma protein indicative of hemoconcentration, and elevations in lactate indicative of respiratory disturbance.

In all exposures, changes in whole body Na^+ (eg. Fig 6B) paralleled those in plasma Na^+ , indicating that this easily measured parameter may be employed when blood samples cannot be obtained. Gill Al accumulation was a very sensitive indicator of toxicity, and well correlated with decreases in plasma and whole body electrolytes (Fig 6). Gill Al levels increased in a time- and concentration- dependent fashion, were greatest in dying fish, and were clearly related to differences in species sensitivity. Thus in any given exposure, branchial Al accumulation was least in the smallmouth bass (*Micropterus dolomieu*), which is even more resistant than the brook trout, and greatest in the rainbow trout (Fig. 6B).

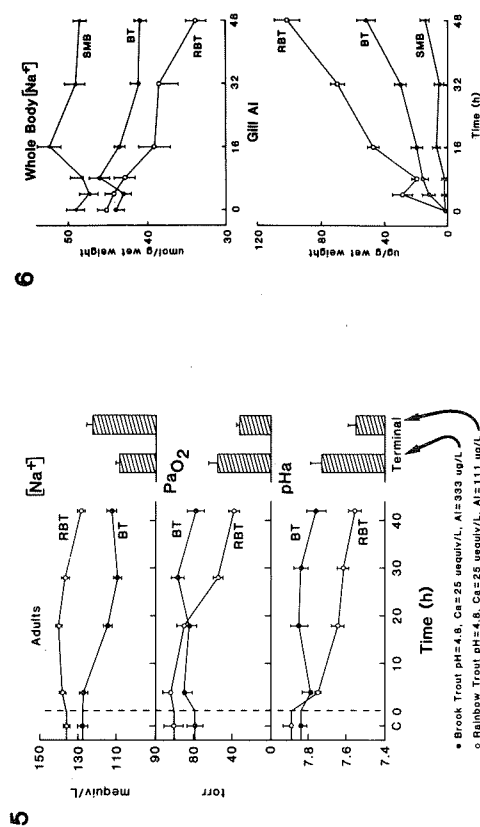


FIG. 5. A comparison of changes in plasma Na^+ , O_2 tension, and pH of arterial blood between cannulated adult brook (BT; N = 19) and rainbow trout (RBT; N = 12) exposed to acid/Al challenge at low Ca. BT were exposed to higher Al than RBT. Other details as in Fig. 3. (Wood et al., 1987a; R.C. Playle, G.C. Goss, and C.M. Wood, unpubl.).

FIG. 6. A comparison of gill Al and whole body Na^+ between juvenile smallmouth bass (SMB), brook trout (BT), and rainbow trout (RBT) exposed to an identical acid/Al challenge (pH = 4.8, Al = 111 ug/L, Ca = 25 uequiv/L) for 48 h. Means $\pm$ 1 SEM (N = 8-11 at each point). (D.C. McDonald and C.M. Wood, unpubl.).

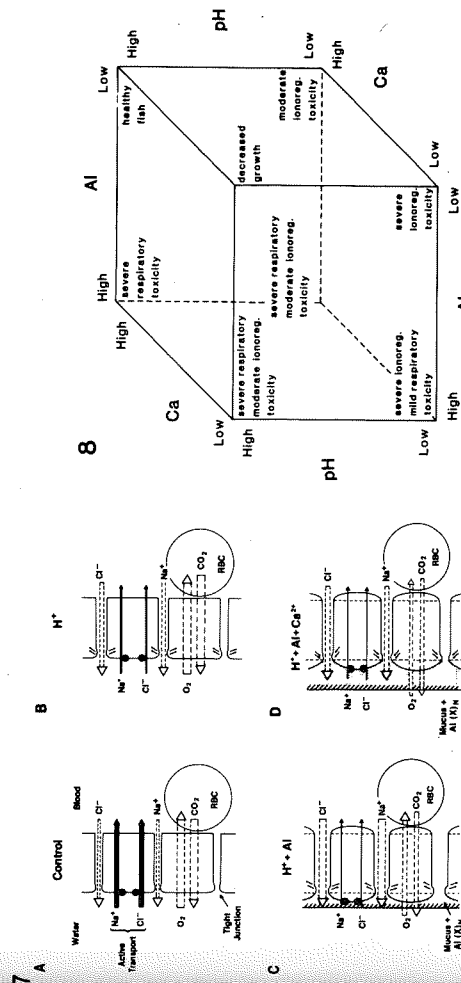


FIG. 7. Some simple models of hypothesized action on the gill epithelium of acid alone, acid/Al, and acid/Al in the presence of higher Ca, and resultant effects on ion and gas exchange. Width of arrow indicates size of flux. See text for details.
FIG. 8. Predictions, based on laboratory data, of qualitative responses of fish to natural waters of varying pH, Al, and Ca levels. See text for details.

These results agree with previous observations of branchial Al accumulation (Grahn, 1980; Neville, 1985; Karlsson-Norrgrén et al., 1986) and suggest that this phenomenon is intrinsic to toxicity. We view this as a surficial uptake, onto or into the gill cells. Neither we nor Neville (1985) have been able to find any evidence of Al entry into the bloodstream or accumulation in the liver and other tissues in exposures lasting up to 10 days. Interestingly, we also have no evidence that water Ca affects net gill Al accumulation despite its documented influence on the nature and degree of toxicity. More detailed studies are currently in progress on this point.

Some Simple Models

Based on our own work and that of many others cited herein, we offer some speculation on the mechanisms of Al and H^+ action. Both are probably surface-active toxicants which alter the structure, chemistry, and physiology of the gill epithelium. The branchial micro-environment is rich in organic anions on mucus and cell surfaces, and at low pH is undoubtedly more alkaline than the bulk water due to NH_3 efflux and acid uptake/base excretion processes. Higher pH exponentially reduces Al solubility and favours the predominance of cationic Al hydroxides over Al^{3+} . These factors will favour the binding of Al to organic ligands, the nucleation of Al polymers, and/or the precipitation of aluminum hydroxide complexes on the gill epithelium.

H^+ alone (Fig. 7B) acts to inhibit transcellular active Na^+ and Cl^- uptake processes, and to open up the paracellular channels, perhaps by displacing Ca, thereby increasing diffusive losses of these and other electrolytes; gas exchange is not affected. In the additional presence of Al (Fig. 7C), the accumulation of Al complexes on the gill surface acts as an irritant which stimulates the secretion of mucus and causes an inflammatory response. This weakens the tight junctions of the paracellular channels even more, while the surface coating further inhibits active ion uptake, either by physical blockade of access to the carriers, or interference with the carriers themselves. At the same time, the cell swelling, separation, and oedema of inflammation, together with the mucus/Al layer, all increase the diffusion distance from water to blood, thereby reducing O_2 and CO_2 exchange. The action of higher water Ca under these circumstances (Fig. 7D) is to help close the paracellular channels,

thereby decreasing ion loss. However, Ca also intensifies the gas exchange problem, perhaps by promoting the formation of Al polymers or thickening the mucous coat. A great deal more work on the nature of the gill surface will be needed to confirm or disprove these ideas.

Fig. 8 relates laboratory data to the field situation for the three variables Ca, pH, and Al. We have purposely not quantified the axes in view of documented differences in species sensitivity. The combination of high Ca, high pH, and low Al (i.e. hardwater) is optimal for fish health, and the major effect of lower Ca under these conditions (i.e. softwater) is reduced growth. Low pH alone cause severe ionoregulatory toxicity which is ameliorated by higher Ca. Elevated Al exacerbates acid toxicity by both ionoregulatory and respiratory mechanisms. The ionoregulatory actions are probably greatest at intermediate pH, and increase with reductions in Ca. The respiratory effects generally increase with both pH and Ca. Based on this model, an episodic acid/Al 'surge' to very low pH would kill mainly by ionoregulatory actions, but one at higher pH could be equally toxic through combined respiratory and ionoregulatory effects. Similarly, a treatment such as liming, which raises both Ca and pH substantially without altering total Al in the short term, may be toxic to fish by exchanging one toxic mechanism for another.

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IONOREGULATORY AND HAEMATOLOGICAL RESPONSES OF RAINBOW TROUT *SALMO GAIRDNERI* RICHARDSON TO CHRONIC ACID AND ALUMINIUM STRESS

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ABSTRACT

Chronic exposure experiments were performed to compare physiological responses in adult rainbow trout, *Salmo gairdneri* Richardson during acid exposure (pH 5.0) and during a subsequent period of combined acid (pH 5.0) and aluminium (200 µg Al/l) stress in soft water (1 mg Ca/l).

These experiments gave evidence that even after a pre-exposure to acid water for 14 days, Al was highly toxic to the fish. About 50% of the fish were death within 2 days due to the presence of Al.

Measurements of the whole body ion fluxes and the plasma ion concentrations revealed that, compared to the foregoing acid exposure period where fish recovered from acid stress within some days, the exposure to 200 µg Al/l (at pH 5.0) caused a significant disturbance of the ion balance. A net whole body loss of Na and Cl (400-500 µequiv/kg.hr) was observed during the first 4 hours, which resulted in decreased plasma Na and Cl levels. These acute ionoregulatory disturbances are supposed to give rise to severe haematological changes, observed on the 3rd day of Al stress. The lowered plasma osmolality could induce a changed body fluid distribution resulting in the observed swelling of blood cells. This process and the increase of the number of erythrocytes could be responsible for the doubled haematocrit value in Al-exposed fish.

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