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Swimming performance, whole body ions, and gill Al accumulation during acclimation to sublethal aluminium in juvenile rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Juvenile rainbow trout (2–5 g) were chronically exposed (for 22 days) to acidified softwater (Ca^{2+} = 25 μEq/l, pH 5.2) in the presence or absence sublethal Al (30 μg/l). Al-exposed fish (5.2/Al group) suffered 20% whole body Na^+ and Cl^- losses and a 30% reduction in the maximum sustainable swimming speed (U_{crit}) over the initial 7 days. These disturbances were approximately 2 fold greater than those observed in the fish exposed to low pH alone (5.2/0 group). However, whole body ion levels were completely restored in the 5.2/Al fish by day 22, whereas they merely stabilized at a new reduced level in the 5.2/0 group. Increased resistance to acutely lethal Al (200 μg/l at pH 5.2) was observed from day 17 onwards in the 5.2/Al fish. Despite this acclimation and recovery of whole body ions, U_{crit} remained significantly lower than in the 5.2/0 group throughout. Growth on a restricted diet of 1% body wt./day was normal in the 5.2/0 group compared with controls maintained in pH 6.5 softwater, whereas 5.2/Al fish suffered a 50% reduction in growth rate on the same diet. The 5.2/Al fish accumulated large amounts of Al on the gills, reaching an initial peak after 4 days, followed by a decline at 7 days, and a secondary rise thereafter. Therefore acclimation and recovery of whole body ionic status was not associated with a reduction in the gill Al burden. Some of the metabolic costs of acclimation to Al, namely a continued impairment of swimming speed and growth, are discussed in light of the physiological and structural changes reported to occur at the gills.

Introduction

Dissolved aluminium concentrations are frequently elevated in low pH softwater due to the acid-induced leaching of Al from soils and sediments (Wright and Gjessing 1976; Cronan and Schofield 1979; Dickson 1980). It is now clear that at pH values between 4.7 and 5.5 fish kills may be primarily due to the presence of Al rather than the H^+ concentration *per se* (Schofield and Trojnar 1980; Baker and Schofield 1982). Physiological studies have demonstrated that Al causes *acute* ionoregulatory and respiratory disturbances in this pH range (*e.g.*, Neville 1985; Playle *et al.* 1989; Witters *et al.* 1990). However, the continued presence of fish populations in softwater lakes and rivers containing levels of aluminium in excess of 100 μg/l at these pH's (Wright and Snekvik 1978; Schofield and Trojnar 1980; Kelso *et al.* 1986) has encouraged research into the effects of *chronic* exposure to sublethal Al concentrations in acidified low Ca^{2+} water (Wood and McDonald 1987; Mount *et al.* 1988; McDonald and Milligan 1988; Wood *et al.* 1988a,b; Walker *et al.* 1991).

Acclimation to Al, *i.e.*, increased resistance to acutely toxic levels established through *continued* sublethal exposure, has now been well documented (Guthrie 1982; Orr *et al.* 1986; Siddens *et al.* 1986; Reid *et al.* 1991; McDonald *et al.* 1991). Previous studies from this laboratory and others have demonstrated that the gills are the primary site of toxic impact during Al exposure (Neville 1985; Booth *et al.* 1988; Wood *et al.* 1988a; Playle *et al.* 1989; Playle and Wood 1991) and that the acclimation process results from a 'damage/repair' phenomenon involving physiological, biochemical, and structural changes at the gills (McDonald *et al.* 1991; Meuller *et al.* 1991; McDonald and Wood 1992). Using juvenile brook trout (*Salvelinus fontinalis*), the latter studies demonstrated that the initial damage phase (the first 4 days) was characterized by substantial accumulation of gill Al, a corresponding reduction in gill sialic acid content (an indicator of gill mucous content), inhibition of branchial Na^+ transport, and severe histopathologies (necrosis and fusion of secondary lamellae, hyperplasia, hypertrophy, and desquamation of pavement and mucous cells). These changes were associated with a reduction in whole body electrolytes, haemoconcentration, and impaired tissue oxygen delivery (whole body lactate levels were elevated). Acclimation (increased tolerance to lethal Al) was observed from day 10 onwards, and was accompanied by at least partial recovery of whole body ion and lactate levels, and a progressive reduction of total gill Al. However, many gill histopathologies remained even after acclimation had been established. Although resting aerobic metabolism was restored with time (resting whole body lactate had returned to control levels by day 24) the continuation of structural abnormalities at the gills suggests that the capacity of the respiratory gas exchange system may still be impaired. This would only be apparent during increased aerobic activity such as sustained swimming.

The main objective of the present study was to examine some of the costs of acclimation to Al. To determine how acclimation influences the capacity for aerobic activity, we measured the critical swimming velocity (U_{crit}) during chronic exposure (22 days) to sublethal Al concentrations in low pH (5.2) softwater. To control for the effects of low pH alone, we performed comparable tests on fish chronically exposed to pH 5.2 in the absence of Al. Growth was also measured under a restricted feeding regime as an indicator of cumulative metabolic cost. For comparison with previous acclimation studies on brook trout and rainbow trout (McDonald *et al.* 1991; Reid *et al.* 1991), we additionally monitored whole body electrolytes and gill Al accumulation as an index of the disruption of branchial ionoregulation and changes associated with acclimation at the gills.

Materials and methods

Animals

Juvenile rainbow trout, *Oncorhynchus mykiss*, (2–5 g) were obtained from Rainbow Springs Trout Farm, Thamesford, Ontario. Whilst being held in dechlorinated Hamilton city tapwater ($[Ca^{2+}] \approx 2.0$, $[Na^+] \approx 0.6$ mEq/l, pH $\approx$ 8.0), fish were freeze-branded (Mighell 1969) for later separation into three groups (see below). After 2 days recovery in dechlorinated tapwater, all fish were transferred to a holding tank supplied with synthetic very soft water ($[Ca^{2+}] \approx 25$, $[Na^+] \approx 50$ μEq/l, pH 6.5–7.0) designed to duplicate the composition of acid-threatened lakes and streams in Eastern North America and Scandinavia.

Softwater was generated from dechlorinated tapwater passed through a reverse osmosis unit (Culligan MP1000) or deionizing resin cannisters (J.W. Anderson Co. Ltd., Dundas, Ontario, Canada). The required amounts of reagent grade $CaCl_2$, NaCl, and KOH were added by peristaltic pump to give the desired ionic composition and pH (see Table 1).

Experimental protocol

After 4 weeks acclimation to normal softwater conditions, fish were divided into three groups (according to brand marks) and transferred to separate 70 l holding tanks. Each tank contained approximate-

Table 1. Measured water chemistry variables and temperatures during 22 day chronic exposures

Exposure group	$[Na^+]$ (μEq/l)	$[Ca^{2+}]$ (μEq/l)	pH	Temperature (°C)	Total [Al] (μg/l)
6.5/0	44.6 ± 0.5	24.5 ± 0.6	6.58 ± 0.04	15.5 ± 0.1	< 2.8[a]
5.2/0	46.7 ± 0.4	26.3 ± 0.8	5.17 ± 0.02	15.1 ± 0.1	< 2.8[a]
5.2/Al	46.7 ± 0.4	26.3 ± 0.8	5.20 ± 0.02	15.1 ± 0.1	31.4 ± 1.5

Data shown as mean ± SEM (n = 20); [a] below the sensitivity limit of the pyrocatecholviolet method.

ly 200 fish and was served with a constant flow (~0.7 l/min) of softwater without recirculation. After a further 3 days these groups were exposed to one of three conditions (all in softwater) for a period of 22–24 days: 1) normal softwater at pH 6.5 (6.5/0 group); 2) low pH (5.2) softwater (5.2/0 group); and 3) low pH (5.2) softwater with 30 μg/l Al added (5.2/Al group). Softwater acclimation and exposures were performed at 15°C (range ± 0.5°C). Fish were fed a 1% body weight/day diet of sinking pellets (Purina Trout Chow) during the last 3 weeks of softwater acclimation and throughout the experimental exposure period.

The softwater supply to the low pH exposure tanks was acidified with 1.0N H_2SO_4 using an automatic titration assembly (Radiometer TTT80 Titrator, PHM84 pH meter, and GK2401C combination electrode). Al was supplied to a 250 ml mixing vessel situated immediately above the 5.2/Al tank from an $AlCl_3.6H_2O$ stock solution using a peristaltic pump. Water pH was measured daily in all tanks using an independent electrode (Radiometer PHM82 meter and GK2401C electrode). Daily water samples were also collected for the measurement of Ca^{2+} and Na^+ concentrations by atomic absorption spectrophotometry (Varian 1275) and total Al by the pyro-catechol violet method (Dougan and Wilson 1974). Exposure tanks were cleaned daily by temporarily lowering the external standpipe which rapidly cleared the bottom surface of uneaten food and waste products *via* a mesh-covered drainhole situated at the lowermost point of each tank.

At each sampling time (days 1, 2, 4, 7, 12, and 22) 24 fish were removed from the 5.2/0 and 5.2/Al tanks to evaluate: 1) whole body electrolytes (Na^+ and Cl^-), wet weight, and gill Al content (n = 8); 2) LT50 during challenge with an acutely lethal concentration of Al (pH 5.2, nominal Al = 200 μg/l; n = 8); and 3) swimming performance (critical swimming velocity) and post-exercise whole body electrolytes (n = 8). On day 0 fish (n = 22 per test) were randomly selected from all three tanks and combined for the same tests as above. Fish from the 6.5/0 control group (n = 8 per test) were also tested on day 24.

For analysis of whole body electrolytes and gill Al content, 8 fish were netted individually from exposure tanks, killed by a blow to the head, decapitated with a sharp knife, and the body portion immediately frozen with pre-cooled aluminium tongs in liquid nitrogen (−196°C). Freeze-clamping was employed to facilitate some preliminary biochemical tests (data not reported here). The frozen body plus the head (unfrozen) were then weighed together to the nearest 0.01 g. Gill baskets were dissected out and stored at −20°C for later analysis of Al content, and the frozen carcasses were stored in a −80°C freezer until preparation for measurement of whole body ions.

Al challenge tests involved transferring 8 fish from each relevant exposure tank to a single 10 l flow-through (flow rate = 320 ml/min) softwater tank at pH 5.2 with a nominal Al concentration of 200 μg/l. Cumulative mortalities were then recorded over the following 72 h, or until all fish were dead. LT50 values (the time to 50% mortality) were estimated by log/probit analysis for each group exposed to the challenge. Previous branding allowed us to place fish from all groups tested into the same challenge tank. All groups were therefore exposed to exactly the same challenge conditions, thus avoiding miscellaneous 'tank effects'. On day 17 an additional Al challenge test was made using 8 fish

from the 5.2/Al group only.

Swimming performance was assessed using a 150 l plexiglas respirometer described by Graham and Wood (1981). On each sampling day 8 fish from each of the 5.2/0 and 5.2/Al groups were transferred to the swimming respirometer and maintained at the minimum water velocity (18 cm/s) for 3 h. Water speed was then increased in 6 cm/s steps, each increment lasting 60 min. Critical swimming speed was recorded for each fish according to Brett (1964). Fish were considered exhausted once they became impinged on the rear screen and were non-responsive to gentle prodding with a blunt rod. Once exhausted, fish were removed from the swimming chamber *via* a siphon tube (30 mm inside diameter), and quickly killed, their fork length measured to the nearest 0.1 mm, freeze-clamped in liquid nitrogen, weighed, and stored at −80°C for later analysis for whole body ions. Water pH in the respirometer was maintained at 5.2 by regular pH measurements and manual additions of H_2SO_4. Sufficient $AlCl_3.6H_2O$ was added to the respirometer water at the beginning of the swimming test to raise the Al concentration to 30 μg/l. Water samples were taken at the beginning and end of the swimming test for measurement of total aluminium concentration. The same conditions (*i.e.*, Al present) were employed during the swim testing of both experimental groups (5.2/0, 5.2/Al) as we wished to compare differences due to the *prior* exposure regimes, rather than differences due to water chemistry during the swim test itself. The control group tested on days 0 and 24 were put through the same swimming test except that the water pH was maintained at 6.5 with no aluminium added. This served as an absolute control.

Analytical techniques

Frozen carcasses (minus head) were ground to a fine powder under liquid nitrogen with a mortar and pestle. The powder was weighed in scintillation vials (once all residual liquid nitrogen had gassed off) to which was added 15 ml of ice-cold 8% perchloric acid. The homogenate was shaken thoroughly, allowed to stand for 24 h at 4°C, and centrifuged at 10,000 g for 2 min. Duplicate aliquots of the supernatant were analysed for Na^+ by atomic absorption spectrophotometry (Perkin Elmer, Model 2380) following 1:100 dilution with deionized water. Supernatant Cl^- was measured directly on duplicate 200 μl aliquots by coulometric titration (Radiometer CMT-10).

Gill baskets were thawed, weighed, and digested with 5 times their weight of 1.0N H_2SO_4 in 2 ml air-tight screw top microcentrifuge tubes at 80°C overnight. Gill digests were vortexed, centrifuged (10,000 g for 2 min), and a sample of the supernatant diluted 1:10 in deionized water. Five μl aliquots were analysed for Al content using a Varian AA-1275 AAS with a GTA-95 graphite tube atomiser. Atomiser parameters were 20s ramp to 120°C, 20s at 120°C, 2s ramp to 1200°C, 10s at 1200°C, 0.1s ramp to 2700°C, 5.0s at 2700°C, (read during the last 5.0s) λ = 309.3 nm, N_2 gas.

Statistics

LT50 values were compared between groups using the nomographic methods of Litchfield (1949) and Litchfield and Wilcoxon (1949) to compute 95% confidence limits. LT50's were considered different when the 95% confidence limits did not overlap. For all other measured variables the 5.2/0 and 5.2/Al groups were compared with each other on each sampling day, and with the combined control group values (6.5/0 fish from day 0 and 24), using a Student's unpaired t-test at a 5% level of significance.

Results

Approximately 4% mortality (39 out of 1000 fish) was observed during the first two weeks of acclimation to the control softwater conditions. However, no mortalities occurred in any of the three groups during the 22–24 day experimental exposures, confirming that all exposure regimes were truly sublethal.

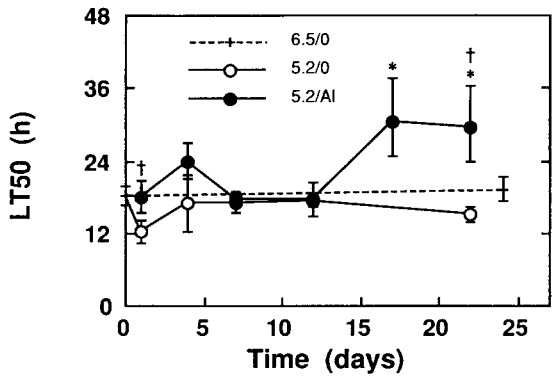

Fig. 1. Estimated times to 50% mortality (LT50's) during challenge with acutely lethal aluminium (200 μg/l at pH 5.2), in rainbow trout previously exposed to pH 5.2 alone (open circles), pH 5.2 plus sublethal Al present (30 μg/l; filled circles), or control softwater conditions (pH 6.5, zero Al; crosses joined by a dashed line) for upto 22 days. Vertical bars represent 95% confidence limits. Asterisks indicate significantly different from the 6.5/0 control group, and daggers indicate significantly different from the 5.2/0 group.

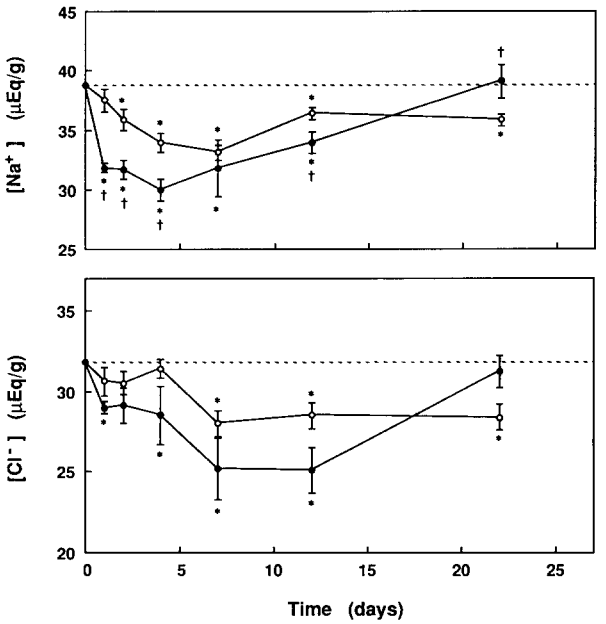

Fig. 2. Whole body Na^+ and whole body Cl^- ions during 22 days exposure to pH 5.2 (open circles), or pH 5.2 with sublethal aluminium present (30 μg Al/l; filled circles). The dashed line represents the combined mean value for control fish (maintained at pH 6.5, zero Al) on days 0 and 24 ([Na^+] = 38.8 ± 0.6, [Cl^-] = 31.8 ± 0.6 μEq/g wet weight, n = 29). Asterisks indicate means significantly different ($p < 0.05$) from the 6.5/0 control group, and daggers indicate means significantly different from the 5.2/0 group.

Al challenge tests

The LT50 values for 6.5/0 control and 5.2/0 fish exposed to 210.1 ± 1.9 μg Al/l (range 203.4 to 224.8) at pH 5.2 varied between 12.5 and 17.4 h throughout the study (Fig. 1). Significantly increased resistance to Al challenge was only observed from day 17 onwards for the 5.2/Al group. Their LT50 values were 30.5 and 29.5 h on days 17 and 22 respectively, at least 1.7 fold greater than the corresponding LT50 for the other two groups. The Al-exposed fish were therefore only considered 'acclimated' from day 17 onwards.

Whole body electrolytes

The mean values for whole body Na^+ and Cl^- of the 6.5/0 control group were very similar on days 0 and 24. Data have therefore been pooled from these two sample times to give a single mean control value for whole body Na^+ and Cl^-.

Both the 5.2/0 and 5.2/Al fish underwent major whole body ion losses during the 22 day exposure when compared with the 6.5/0 control group (Fig. 2). These losses of Na^+ and Cl^- occurred entirely during the first 7 days. Over this period the 5.2/Al group suffered approximately double the losses experienced by fish exposed to acid alone (5.2/0). Thereafter, ion levels more or less stabilized in the 5.2/0 group (preceded by a slight recovery in Na^+); both Na^+ and Cl^- remained significantly depressed throughout the remainder of the exposure (by −3.0 and −3.4 μEq/g respectively on day 22). In contrast, the 5.2/Al fish had completely recovered their whole body ion status by day 22.

In the groups used to determine U_{crit}, the mean whole body Na^+ and Cl^- values (measured post-exercise; data not shown) were always lower than the mean value for the corresponding group sampled at rest on the same day. On average whole body Na^+ and Cl^- values were 2.2 and 2.4 μEq/g lower in the groups exercised to exhaustion, corresponding to a loss of approximately 6 and 8% of their whole body Na^+ and Cl^- respectively. No

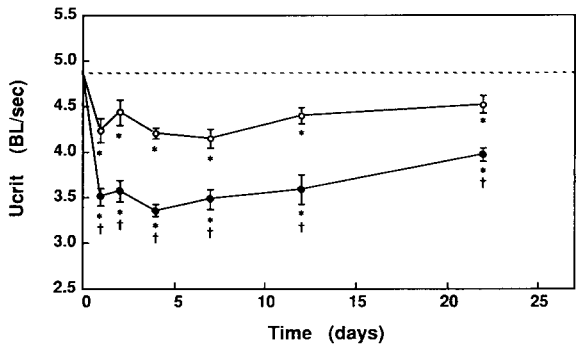

Fig. 3. Critical swimming velocities (U_{crit}) expressed as body lengths per second, for fish exposed for 22 days to pH 5.2 (open circles), or pH 5.2 plus 30 μg Al/l (filled circles). These swim tests were performed at pH 5.2 with 30 μg Al/l present. The dashed line represents the combined mean value for control fish (maintained *and swam* at pH 6.5, zero Al) on days 0 and 24 (U_{crit} = 4.82 ± 0.11 BL/sec, n = 30). Asterisks indicate means significantly different ($p < 0.05$) from the 6.5/0 control group, and daggers indicate means significantly different from the 5.2/0 group.

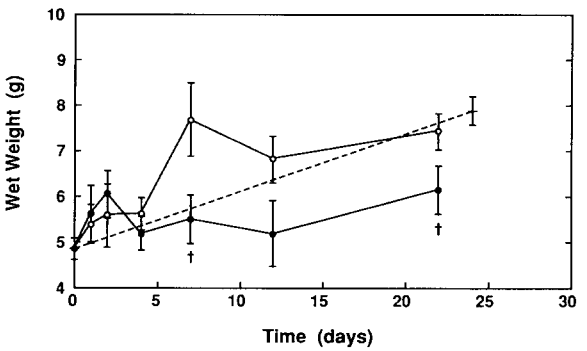

Fig. 4. Body mass of fish exposed for 22 days to pH 5.2 (open circles), or pH 5.2 plus 30 μg Al/l (filled circles). Mean values for control fish maintained in pH 6.5 (zero Al) are shown on days 0 and 24 (crosses joined by a dashed line). Daggers indicate means significantly different ($p < 0.05$) from the 5.2/0 group.

differences were noted between the 5.2/0 and 5.2/Al groups in this respect, except on day 22 when the post exercise values were almost identical in the two groups even though the 5.2/Al group had completely recovered their resting whole body ion status.

Swimming performance

Critical swimming velocity (U_{crit}) for control fish swam in pH 6.5 water (zero Al) were 4.88 ± 0.11 BL/s (38.77 ± 1.28 cm/s; n = 22) on day 0 and 4.65 ± 0.28 BL/s (40.4 ± 2.77 cm/s; n = 8) on day 24. The mean fork length of control fish used for the swimming tests increased by 10% over this 24 day period, from 79.3 ± 1.5 mm (22) to 86.9 ± 3.3 mm (8).

After just 24 h of exposure, U_{crit} was reduced by 13% and 28% in the 5.2/0 and 5.2/Al groups respectively (Fig. 3). From days 2 to 7 U_{crit} remained depressed by 9–15% in the 5.2/0 group and by 28–31% in the 5.2/Al group. In both these groups some recovery of U_{crit} occurred after day 7. However, even on day 22 U_{crit} was still 5% lower in the 5.2/0 group and 17% lower in the 5.2/Al group when compared with the day 0 value for the 6.5/0 control fish. Long term sublethal exposure to acid plus aluminium therefore has a 2–3 fold greater effect on swimming performance than does long term exposure to the same acid level alone.

Growth

The mean weight of 6.5/0 control fish was 4.85 ± 0.23 g (46) at the start, and 7.88 ± 0.66 g (18) at the end of the study, representing a 62% increase in wet weight over the 24 day experimental period. Fig. 4 shows the above in addition to the mean wet weights of fish sampled from the 5.2/0 and 5.2/Al tanks (n = 16) on each sampling day. Although the mean weights of the fish sampled during the study varied considerably, certain general points can be made. Firstly, the 5.2/0 fish were approximately the same size as the 6.5/0 control fish at the end of the experiment (7.43 ± 0.39 g on day 22). Thus exposure to pH 5.2 in the absence of aluminium did not appear to influence growth when fish were fed a restricted diet (1% body weight/day). Secondly, and in contrast, the mean weight of Al-exposed fish became significantly lower than the 5.2/0 group as the exposure progressed. On day 22 the mean weight of the 5.2/Al group was 6.14 ± 0.53 g, representing a net increase of 27% from day 0, less than half the growth rate observed in the 6.5/0 and 5.2/0 groups.

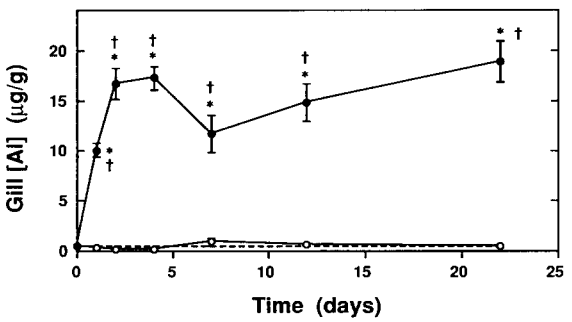

Fig. 5. Gill aluminium content (µg Al/g wet weight of gill tissue) of fish exposed for 22 days to pH 5.2 (open circles), or pH 5.2 plus 30 µg Al/l (filled circles). Mean values for control fish maintained in pH 6.5 (zero Al) are shown on days 0 and 24 (joined by a dashed line). Asterisks indicate means significantly different ($p < 0.05$) from the 6.5/0 control group, and daggers indicate means significantly different from the 5.2/0 group.

Gill Al content

Total gill Al concentrations in the 5.2/Al fish rose in a linear fashion over the first 2 days and reached an initial peak of 17.25 ± 1.16 µg/g (8) at day 4 (Fig. 5). By day 7, gill Al levels had declined significantly to 11.72 ± 1.88 µg/g ($p = 0.025$). However, contrary to previous findings with rainbow trout (Reid *et al.* 1991) and brook trout (McDonald *et al.* 1991), no lasting recovery of the gill Al burden was seen. Indeed the subsequent changes could be perceived as a second phase of the gill Al accumulation since, after day 7, gill Al levels increased steadily reaching a new peak of 18.84 ± 1.99 µg/g (8) by day 22.

Discussion

As in previous studies we have demonstrated that acclimation can be induced by continued exposure to a sublethal level of aluminium (for rainbow trout see Orr *et al.* 1986; Reid *et al.* 1991; for brook trout see Wood *et al.* 1988a,b; McDonald and Milligan 1988; Walker *et al.* 1991; McDonald *et al.* 1991). In agreement with several of these studies, the initial ionoregulatory disturbances induced by Al exposure were overcome with time. However, for the first time, we have also shown that another physiological disturbance, specifically a reduction in the critical swimming speed, persists for a much longer period. We have also found that Al exposure significantly depresses growth of rainbow trout on a restricted diet. These long-lived metabolic effects appear to be exclusively associated with Al, and not with the presence of moderately low pH (5.2) alone. They may well be unavoidable costs of the acclimatory process, and would undoubtedly impinge on the animal's fitness in the wild.

Ionoregulatory disturbances and recovery in Al-exposed fish

In trout, exposure to H^+ or Al causes ionoregulatory problems due to an increase in the passive loss of electrolytes and to a lesser extent a reduction in the active uptake of ions at the gills (McDonald 1983; Wood and McDonald 1987; Booth *et al.* 1988; McDonald *et al.* 1991). These studies suggest that increased gill permeability results from the displacement of membrane-bound Ca^{2+} by H^+ and Al. From the work of Reid *et al.* (1991) on metal binding affinities of the rainbow trout gill *in vitro*, it is apparent that at the concentrations used in the present study Al would successfully compete with Ca^{2+} for sites on the gill whereas competition between Ca^{2+} and H^+ would only be slight. It is not surprising then that Al-exposed fish suffered a two-fold greater loss of whole body Na^+ and Cl^- compared to the 5.2/0 group during the initial damage phase of Al exposure (Fig. 2). Reid *et al.* (1991) further demonstrated that during chronic exposure (21 days) modifications of the gill cation binding sites occur, specifically a reduction in the affinity of the gill for Al (relative to Ca^{2+}). This would undoubtedly contribute to the recovery of branchial ionoregulation seen during Al exposure (Wood *et al.* 1988a,b; McDonald and Milligan 1988; Reid *et al.* 1991; McDonald *et al.* 1991) and hence the restoration of whole body Na^+ and Cl^- in the present 5.2/Al group. In contrast, the 5.2/0 group did not fully recover their whole body ion status but merely stabilised at a new reduced level. This is similar to the findings of Audet *et al.* (1988) with rainbow trout, and Wood *et al.* (1988b) and McDonald *et al.* (1991) with brook trout, and may be related to the

observation that sublethal acid exposure alone does not appear to elicit an acclimatory response (Audet and Wood 1988; McDonald and Wood 1992).

Clearly, rainbow trout are capable of a full recovery of their branchial ionoregulatory capacity and hence whole body ion status during acclimation to sublethal Al, whereas fish exposed to acid alone are not. However, gill alterations necessary for the restoration of ionic balance may well be distinct from gill changes involved in true acclimation (*i.e.*, increased tolerance to Al) as there is considerable discrepancy between the time courses of the two responses (*cf.* Figs. 1,2).

Swimming performance

Previous investigations into the effects of low pH on swimming performance in rainbow trout were performed at water hardnesses 3–80 fold greater than used here (Hargis 1976; Waiwood and Beamish 1978; Graham and Wood 1981; Ye and Randall 1991). Of these, only Graham and Wood (1981) and Ye and Randall (1991) used pH values below 6.0, and in none of these studies was Al present during the swim trials. For comparison, the depression of U_{crit} we observed after one day at pH 5.2 (a 13% drop; Fig. 3 – water $[Ca^{2+}]$ = 25 μEq/l) was equivalent to that observed by Graham and Wood (1981) in similarly sized rainbow trout (juveniles) acutely exposed to pH 4.4 ($[Ca^{2+}]$ = 200–400 μEq/l), but less than that (a 33% drop) reported by Ye and Randall (1991) for adult trout after one day at pH 5.0 ($[Ca^{2+}]$ = 70–150 μEq/l). These discrepancies may result from differences in hardness, size, or the presence of aluminium.

In both 5.2/0 and 5.2/Al fish, the effect of exposure on U_{crit} was rapid and sustained, the depression of U_{crit} being at least two-fold greater in the 5.2/Al group. Since both groups were exercised simultaneously in pH 5.2 water with 30 μg Al/l present, this persistent difference must have been due to the physiological injury experienced during the previous 1–22 days exposure, rather than to the effects of Al or low pH during the swim test itself. However, in contrast to ionoregulatory status, the recovery of U_{crit} in the 5.2/Al group was only minimal within the 22 day exposure. Obviously, acclimation does not necessitate a complete return to the pre-exposure physiological condition. Indeed it could be argued that a long-lasting reduction in U_{crit} may be an inescapable cost of acclimation to aluminium. Meuller *et al.* (1991) demonstrated that the severe *acute* gill damage caused by Al exposure (see Introduction) was followed by pronounced *chronic* changes in the gill histology which persisted long after the time at which acclimation was first observed. Youson and Neville (1987) and Evans *et al.* (1988) observed comparable gill histopathologies in rainbow trout exposed to aluminium for 12 and 14 days respectively, and longer term studies have demonstrated similar results (Karlsson-Norrgren *et al.* 1986a,b; Tietge *et al.* 1988). The reported long term changes included decreases in respiratory surface area (lamellar fusion, clubbing) and increases in respiratory diffusion distance (mucous and chloride cell hypertrophy and hyperplasia, general epithelial thickening). Such changes likely restrict the capacity for oxygen transfer and therefore limit the scope for aerobic activity.

If these gill changes are a necessary part of acclimation, and concurrently reduce the maximum rate of oxygen uptake (M_{O_2max}), then a reduction in the U_{crit} may be an unavoidable consequence of an increased tolerance to Al. This would be termed a 'limiting' stress by the definition of Brett (1958), since the scope for active metabolism has been reduced by limiting the uptake of oxygen from the environment. In addition, an increase in the energy expenditure at subcritical speeds, for example due to an increased demand on the ionoregulatory system, would also cause a reduction in U_{crit} by utilising more of the oxygen for non-activity related metabolism. Al would therefore act as both a limiting stressor and a 'loading' stressor due to an increase in the cost of routine maintenance (Brett 1958), as found by Waiwood and Beamish (1978) in rainbow trout exposed to copper.

Gill structural changes are much less marked during exposure to low pH alone (Evans *et al.* 1988; Meuller *et al.* 1991). In fact Evans *et al.* (1988) found that respiratory diffusion distances were not significantly altered by exposure to pH 5.2 in the absence of aluminium. Without obvious morpho-

logical changes at the respiratory surface, it is unlikely that U_{crit} would be impaired because of a reduction in M_{O_2max}. It is more probable that the reduction of U_{crit} in the 5.2/0 fish is simply due to the loading stresses of low pH exposure (*e.g.*, the additional costs of ionoregulation) causing a reduction in the energy available for highly aerobic activity.

Growth effects

Low pH alone did not affect growth (Fig. 4). Any increased energy demand caused by the ionoregulatory problems encountered by 5.2/0 fish were therefore too small to have an impact on growth rate when fed on a 1% body wt/day diet. In contrast growth over the 22 day exposure period was reduced by 50% in the 5.2/Al group. Similar observations have been documented for brown trout (Sadler and Lynam 1987, 1988; Reader *et al.* 1988) and brook trout (Mount *et al.* 1988) exposed to sublethal Al. The reason for impaired growth cannot be determined since in all these studies fish were fed a restricted diet and feeding rates were not assessed. However, recovery from severe ion losses and repair of the gill damage (Meuller *et al.* 1991) would undoubtedly have been more energetically expensive in the 5.2/Al group. With a limited energy input (*i.e.*, diet) these may have been sufficient to reduce the energy invested in growth. Al-exposed fish may also suffer from a reduction in appetite as suggested by Sadler and Lynam (1988), which may also have contributed to the impaired growth rates observed. However, we made no attempt to measure the amount of food actually eaten due to the difficulty in separating any uneaten pellets from debris which accumulates at the bottom of tanks after a feed. Without an accurate estimate of actual food intake we can only speculate as to the role of appetite in the impairment of growth in Al-exposed fish.

Gill Al burden

The time course of the changes in gill [Al] in the 5.2/Al group (Fig. 5) suggest a biphasic response: a rapid accumulation and partial recovery during the first week, followed by a slower and more steady build-up to day 22. This could of course simply represent random variation of gill Al concentrations over the course of the exposure. However, a remarkably similar pattern was observed in a second study with rainbow trout using almost identical exposure conditions (R.W. Wilson, H.L. Bergman, and C.M. Wood, unpublished results).

A subcellular analysis of gill Al in rainbow trout revealed that almost all gill Al was found on the surface of gill lamellae following 3 days exposure to sublethal Al (Goossenaerts *et al.* 1988). However, following longer exposures (1 week to 1 year) accumulation of intracellular Al precipitates have been found in brown trout and rainbow trout (Karlsson-Norrgren *et al.* 1986a,b; Youson and Neville 1987; Evans *et al.* 1988). It is therefore tempting to speculate that the pattern observed in the present study represents two separate accumulations of Al in the gills: one external (on the gill surface and within gill mucus) which peaks during the first few days, and the other internal (within the gill cells themselves) where Al would take longer to penetrate.

McDonald *et al.* (1991) and Meuller *et al.* (1991) found that acclimation was associated with a progressive reduction of gill Al in brook trout. Clearly this was not the case in the present study with rainbow trout. This may reflect real differences in the mechanism of acclimation between the two species. However, the two studies are not strictly comparable; higher external levels of Al were used in the brook trout study (150 and 75 μg/l) and the initial peak of gill [Al] observed by McDonald *et al.* (1991) after just one day was 250 μg/g, more than 10 fold higher than the maximum value noted in our study with rainbow trout (Fig. 5). Gill Al levels were considered 'recovered' once they returned to a stable value of around 40 μg/g (McDonald *et al.* 1991), which is still twice the peak level found in our rainbow trout. Perhaps the higher ambient Al levels employed in the above brook trout study may have masked any subtle changes which would reflect a gradual relocation of gill Al. The partial reduction of total gill Al observed at day 7 in the present study may be analogous to the reduction of

surface Al proposed as a key process in the mechanism of acclimation in brook trout (McDonald *et al.* 1991). Any further reduction in surface Al would be masked by the gradual increase in internal Al. Obviously actual measurements of surface and intracellular Al concentrations are required to clarify the situation.

Fish acclimated to Al are obviously at an advantage in terms of survival during subsequent exposures to lethal Al levels. Although sublethal Al substantially increases the short-term severity of ion losses at low pH, an additional advantage may be conferred by the ability of Al to elicit a complete recovery of ionic status in the longer term, something not found in fish exposed to acid alone. There are, however, costs involved. Both the critical swimming speed and growth rate are reduced during Al exposure. In contrast to ionoregulatory status, no recovery was apparent in either of these variables, at least during the first 3 weeks of exposure. In the wild, reductions in U_{crit} and growth would impair the ability of fish to feed, avoid predation, and reproduce (Little and Finger 1990). Viewed in this light, the benefits of acclimation in terms of overall fitness are equivocal.

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