Metabolic Costs and Physiological Consequences of Acclimation to Aluminum in Juvenile Rainbow Trout (*Oncorhynchus mykiss*). 1: Acclimation Specificity, Resting Physiology, Feeding, and Growth

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Juvenile rainbow trout (*Oncorhynchus mykiss*, 5–13 g) became acclimated (i.e., increased their resistance to lethal Al levels, 162 $\mu g \cdot L^{-1}$, pH 5.2) after only 5 d when exposed to sublethal Al (38 $\mu g \cdot L^{-1}$) in acidified soft water (Na^+ = 85, Ca^{2+} = 28 $\mu Eq \cdot L^{-1}$, pH 5.2). Acclimation was associated with reduced ionoregulatory and respiratory disturbances during lethal Al challenge and was maintained for at least 34 d. Acclimation was relatively specific to Al because no consistently improved resistance to lethal Cu (32 $\mu g \cdot L^{-1}$, pH 5.2) was observed. Exposure to sublethal acid alone (pH 5.2) did not result in acclimation to lethal [H^+] (pH 4.0) and caused a pronounced reduction in whole-body Na^+ and Cl^-. Sublethal acid + Al resulted in a more rapid loss of ions than sublethal acid alone over the first 10 d, but both groups subsequently recovered ionoregulatory status after 34 d. Exposure to sublethal acid alone had a negligible effect on feeding or growth. However, growth was impaired by 29% in Al-exposed trout, primarily the result of reduced appetite during the first 10 d. Decreased growth must be considered one of the costs of acclimation during chronic sublethal exposure to Al.

Des truites arc-en-ciel (*Oncorhynchus mykiss*) juvéniles (5–13 g) se sont acclimatées (c.-à-d. ont accru leur résistance à des concentrations létales d'Al, 162 $\mu g \cdot L^{-1}$, pH 5,2) après 5 jours seulement lorsqu'elles ont été exposées à des doses sublétales d'Al (38 $\mu g \cdot L^{-1}$) dans de l'eau douce acidifiée (Na^+ = 85, Ca^{2+} = 28 $\mu Eq \cdot L^{-1}$, pH 5,2). L'acclimatement était associé à une réduction des troubles de l'ionorégulation et de la respiration pendant l'exposition à des concentrations létales d'Al, et s'est maintenu pendant au moins 34 jours. Cet acclimatement était relativement spécifique à l'Al, car on n'a observé aucune amélioration régulière de la résistance à des concentrations létales de Cu (32 $\mu g \cdot L^{-1}$, pH 5,2). L'exposition à une concentration sublétale d'acide uniquement (pH 5,2) n'a pas produit d'acclimatement à des concentrations létales de [H^+] (pH 4,0) et a causé une réduction prononcée de Na^+ et Cl^- dans l'ensemble de la masse corporelle. L'exposition simultanée à des concentrations sublétales d'acide et d'Al a provoqué une perte plus rapide d'ions que la concentration sublétale d'acide seul pendant les 10 premiers jours, mais les deux groupes ont par la suite retrouvé une ionorégulation normale après 34 jours. L'exposition à une concentration sublétale d'acide seulement avait un effet négligeable sur l'alimentation ou la croissance. Toutefois, la croissance était réduite de 29 % chez les truites exposées à l'Al, ce qui est avant tout causé par une diminution de l'appétit pendant les 10 premiers jours. La réduction de la croissance doit être considérée comme l'un des coûts de l'acclimatement pendant l'exposition chronique à des concentrations sublétales d'Al.

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Chronic exposure of freshwater fish to sublethal aluminum concentrations is frequently encountered in low-pH soft water 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^+ concentation, 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; Booth et al.

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TABLE 1. Measured water chemistry variables (means ± SEM (n)) during the chronic exposure regimes.

Exposure group	$[Na^+]$ ($\mu Eq \cdot L^{-1}$)	$[Ca^{2+}]$ ($\mu Eq \cdot L^{-1}$)	pH	Total [Al] ($\mu g \cdot L^{-1}$)
6.5/0	85.4 ± 4.6 (32)	30.5 ± 1.5 (32)	6.45 ± 0.03 (36)	3.6 ± 0.6 (32)
5.2/0	85.3 ± 5.2 (32)	28.0 ± 1.0 (32)	5.37 ± 0.02 (36)	3.2 ± 0.7 (32)
5.2/Al	85.6 ± 5.3 (32)	26.5 ± 0.9 (32)	5.22 ± 0.01 (36)	38.1 ± 1.2 (35)

1988; Wood et al. 1988a) associated with surface binding and precipitation of Al on the gills (Playle and Wood 1989, 1991; Reid et al. 1991). However, laboratory studies on prolonged exposure to sublethal Al have shown that acclimation (i.e., increased resistance to lethal Al) occurs with time (Orr et al. 1986; Wood et al. 1988b, 1988c; McDonald et al. 1991) which may explain the continued presence of fish populations in acidified softwater lakes and rivers containing levels of Al in excess of 100 $\mu g \cdot L^{-1}$ (Wright and Snekvik 1978; Schofield and Trojnar 1980; Kelso et al. 1986).

The acclimation process results from a "damage/repair" phenomenon involving physiological, biochemical, and structural changes at the gills (McDonald et al. 1991; Mueller et al. 1991; McDonald and Wood 1992). In juvenile brook trout (*Salvelinus fontinalis*), the initial damage phase (lasting 4–5 d) was characterized by the accumulation of gill Al, reduction in whole-body electrolytes, severe gill histopathologies, and impaired oxygen delivery to tissues (whole-body lactate levels were elevated). Acclimation 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 in total gill Al.

The specificity of acclimation to Al is unknown, i.e., whether it will simultaneously increase a fish's resistance to other metals. Because the mechanism of acclimation is probably designed to combat the mechanism of toxicity of a metal, it is possible that metals with similar modes of toxic action will elicit similar acclimatory responses and thus may exhibit crossover resistance (McDonald and Wood 1992). Cu has similar effects to Al in freshwater fish; both can impair respiratory gas exchange during acute exposure and both interfere with the active influx and passive efflux components of branchial ion transport (Laurén and McDonald 1985, 1986; Booth et al. 1988; Playle et al. 1989; Wilson and Taylor 1993). Therefore, Cu is a likely candidate for testing crossover resistance during acclimation to Al.

Estimates of metabolic rate in fish acclimated to Al showed increased energy requirements compared with control fish when swimming aerobically (Wilson et al. 1994). However, growth is a more sensitive indicator of subtle increases in energy demand, and impaired growth has indeed been documented for fish exposed to sublethal acid and Al (Sadler and Lynam 1987, 1988; Reader et al. 1988; Mount et al. 1988a, 1988b; Ingersoll et al. 1990a, 1990b; Wilson and Wood 1992). However, the relationship between energetic costs and growth cannot be defined without the quantification of energy input (feeding rate), in which appetite represents an equally important factor.

The present paper deals with three elements: (i) the specificity of acclimation to Al tested by challenge with lethal levels of Cu, (ii) the resting ionoregulatory, respiratory, and gill Al status during both chronic exposure to sublethal Al and acute challenge with lethal Al, and (iii) some of the potential costs of acclimation assessed by measuring growth and feeding rates and the relevance of appetite. The study was conducted in parallel with that described in our companion paper (Wilson et al. 1994), using juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to pH 5.2 in the presence of 38 μg Al$\cdot L^{-1}$ for a period of 36 d. All measured variables were compared with two other groups exposed to either pH 5.2 or pH 6.5, both with no Al added. Exposures were carried out in a synthetic software medium relevant to the acid-threatened lakes and rivers of eastern North America and Europe.

Materials and Methods

Animal Holding

Juvenile rainbow trout (5–13 g) were obtained from the hatchery of the Wyoming Game and Fish Department, Wyoming, and transported to the Red Buttes Fish Physiology and Toxicology Laboratory, Laramie, Wyoming, where they were initially maintained in continuously flowing well water ($[Ca^{2+}] \approx 2.6$, $([Na^+] \approx 0.3$ mEq$\cdot L^{-1}$) and fed ad libitum. During this holding period in hard water, fish were divided into three groups of approximately 400 individuals. At this stage, each individual was given a freeze-brand distinctive of its group (Mighell 1969) to allow identification during subsequent tests (see below).

Four weeks before starting the experimental regime the holding water was switched from hard well water to continuously flowing synthetic soft water (nominal $[Ca^{2+}] \approx 25$, ($[Na^+] \approx 80$ $\mu Eq \cdot L^{-1}$, pH 6.7–7.0, $P_{CO_2} < 0.13$ kPa) designed to be similar to the composition of acid-threatened lakes and streams in eastern North America and Europe. Soft water was generated by treating the hard well water with reverse osmosis followed by separate bed anion and cation deionization. The required amounts of reagent-grade $CaCl_2$ and NaCl were added via Mariotte bottles to the main head tank and pH adjusted to the appropriate level in secondary head tanks with either KOH or H_2SO_4 using Leeds and Northrup pH controllers. Fish were kept in circular 400-L tanks supplied with water at 3.5 L$\cdot$min^{-1} (i.e., 12.6 volume addition per day). The study was conducted from January to March 1991 at 15°C (range ±0.5°C) under a photoperiod adjusted biweekly to follow the natural cycle for Laramie, Wyoming.

After softwater acclimation, rainbow trout were exposed to one of three conditions: (i) normal soft water at pH 6.5 (6.5/0 group), (ii) low-pH soft water (nominal pH 5.2)

(5.2/0 group), and (iii) low-pH soft water (nominal pH 5.2) with 38 μg $Al \cdot L^{-1}$ added (5.2/Al group). The elevated level of Al was achieved by adding an $AlCl_3 \cdot 6H_6O$ stock solution from Mariotte bottles into the appropriate softwater supply. Water variables (pH, $[Ca^{2+}]$, $[Na^+]$, and total Al) were measured on samples collected daily from each tank (Table 1).

Sampling Protocol

Thirty fish were sampled from each tank after 1, 5, 10, 17, 25, and 34 d of exposure to test for changes in tolerance to Al and Cu (by LT50 tests) and to determine whole-body ions and lactate and gill Al content. For Al LT50 tests, 10 fish from each group were transferred to a 400-L challenge tank receiving pH 5.2 soft water at 3.5 $L \cdot min^{-1}$ with 162 μg $Al \cdot L^{-1}$ added. For Cu LT50 tests, another 10 fish were transferred to an identical tank with 32 μg $Cu \cdot L^{-1}$ added (no added Al). Cumulative mortalities in each tank were recorded over the following 96 h or until all fish were dead. Fish were removed and weighed as soon as mortalities were observed. Using a single challenge tank for the Al LT50 tests and one tank for the Cu LT50 tests ensured identical "challenge" conditions for all three groups. Individual freeze-brand marks allowed identification of the origin of each fish. At the end of each test, LT50 values (time to 50% mortality) were estimated by log/probit analysis for each group. The background level of Cu in chronic exposure tanks was 1.45 ± 0.20 μg $\cdot L^{-1}$ (mean ± SEM of 34 water samples).

Ten of the 30 fish sampled from each tank were netted individually, killed by decapitation with a sharp knife, and the body portion immediately freeze-clamped between two aluminum plates in liquid nitrogen. The mass of the frozen body plus head was then measured to the nearest 0.01 g and the frozen body stored at −80°C until preparation for measurement of lactate and ion content. Gill baskets were dissected free from the unfrozen head portion, washed for 10 s in pH 5.2 Al-free soft water, and stored at −80°C for later analysis of Al content.

On day 36, two additional challenge tests were performed. First, 10 fish from each group were transferred to the Al challenge tank and then sampled after 10 h (i.e., before any mortality) for analysis of whole-body ions and lactate and gill Al content to assess their physiological status during Al challenge. A second group of 10 fish from each tank was transferred to a low-pH challenge tank (pH 3.99, no Al added) and LT50's determined as above in order to test for acclimation to acid alone. A further 10 fish from each exposure tank were sampled on day 36 to assess whole-body water content. These fish were individually killed, blotted dry in paper towels, weighed to the nearest 0.001 g, and dried at 85°C to a constant weight.

Feeding Regime and Growth

Fish were fed to satiation twice per day at approximately 8-h intervals using floating trout pellets (Purina Trout Chow ground and sieved manually to an average pellet size of 1.2 × 1.5 mm). For each exposure tank, a separate preweighed bag of pellets was used. At the start of each meal, food was offered in small aliquots (equivalent to less than 5% of the daily ration) once every minute. Feeding was stopped when uneaten pellets still remained after a period of 2 min, and the amount of food eaten per day was calculated from the difference in bag weight at the beginning and end of each day. Feeding rates (percent body weight per day) were calculated from the weight of food eaten and the biomass in each tank (equivalent to the product of average weight and total number of fish per tank). This feeding protocol was adopted during the last week of adjustment of softwater conditions and continued throughout the entire experimental exposure period. Exposure tanks were cleaned of organic debris daily by siphon. To assess growth rates, 50 fish from each tank were removed twice per week, weighed individually in a tared vessel containing the appropriate medium, and then returned to their original tank. Gross food conversion efficiencies were calculated as described by Brett and Groves (1979), using the weight of food eaten and the change in whole-body dry weight (assuming 73% water content) for the average fish in each group over the course of the study.

Analytical Techniques

For analysis of whole-body ions and lactate, frozen carcasses were ground to a fine powder under liquid nitrogen with a mortar and pestle. The frozen powder was weighed in 50-mL centrifuge tubes (once all residual liquid nitrogen had gassed off) and deproteinized by adding a precise volume (15–30 mL) of ice-cold 8% (w/v) perchloric acid. Homogenates were shaken thoroughly, allowed to stand for 2 h on ice, and centrifuged at 900*g* for 10 min. The concentration of lactate in the resultant supernatant was then measured on 67-μL aliquots using an enzymatic assay (L-lactate dehydrogenase/NADH method) and Sigma reagents. Supernatant Cl^- was measured directly on duplicate 200-μL aliquots by coulometric titration (Aminco-Cotlove). Supernatant Na was measured by atomic absorption spectophotometry (Perkin Elmer 2380) following 1:100 dilution with deionized water.

In preparation for the analysis of gill Al content, gill baskets were thawed, weighed, and digested with five times their weight of 1.0 N H_2SO_4 in 15-mL capped polypropylene centrifuge tubes at 80°C overnight. Gill digests were vortexed, centrifuged (900*g* for 5 min), and a sample of the supernatant diluted 1:10 in deionized water. Twenty-microlitre aliquots of the diluted supernatant were analyzed for Al content using an atomic absorption spectrophotometer (Perkin Elmer 2380) with graphite furnace attachment (Perkin Elmer HGA-400). Atomiser parameters were 2-s ramp to 120°C, 20 s at 120°C, 2-s ramp to 1700°C, 10 s at 1700°C, 0.1-s ramp to 2700°C, and 5.0 s at 2700°C. Argon was used as the carrier gas, and peak heights were read during the final 5.0 s at λ = 309.3 nm. Total [Cu] and [Al] in water samples from the LT50 tests were similarly analyzed by electrothermal atomic absorption spectrophotometry and water $[Ca^{2+}]$and $[Na^+]$ by flame atomic absorption spectrophotometry.

Statistical Analysis

Mean values ± SEM (*n*) are generally reported throughout the text where *n* represents the number of fish or water samples. LT50 values were compared between groups using the nomographic methods of Litchfield (1949) and Litchfield and Wilcoxon (1949) at the 5% level of significance. Mean values of all other variables were compared between groups on each day using a Student's unpaired *t*-test at the 5% level of significance.

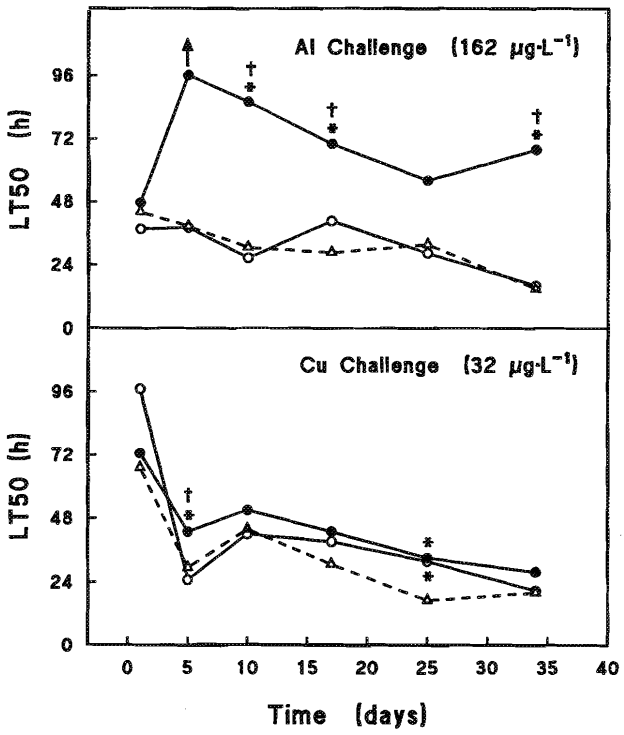

FIG. 1. Estimated times to 50% mortality (LT50's) during challenge with acutely lethal Al (162 $\mu g \cdot L^{-1}$, pH 5.2) or Cu (32 $\mu g \cdot L^{-1}$, at pH 5.2). The vertical arrow on day 5 indicates that less than 50% mortality occurred in the 5.2/Al group within the 96-h test. Asterisks indicate a significant difference from the 6.5/0 control group, and daggers indicate a significant difference from the 5.2/0 group.

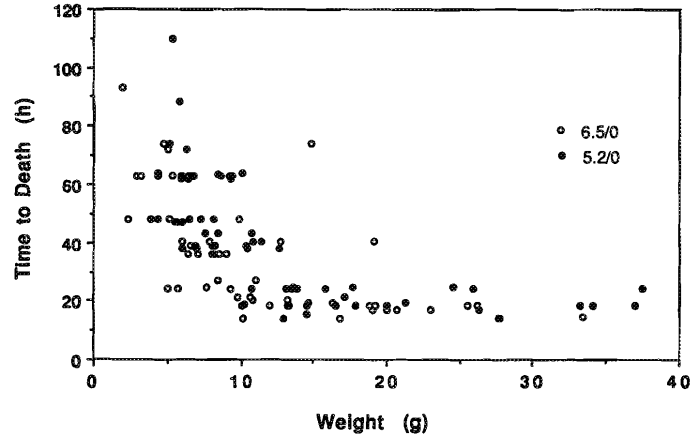

FIG. 2. Relationship between body mass and time to mortality in control (open circles) and 5.2/0 (solid circles) trout challenged with lethal Al (162 $\mu g \cdot L^{-1}$, pH 5.25).

Results

Mortality and Acclimation

Mortality was low in the chronic exposure groups (0.25, 0.5, and 5.5% in the control, 5.2/0, and 5.2/Al groups, respectively). Acclimation to lethal Al (161.7 ± 2.5 $\mu g \cdot L^{-1}$, (n = 35); pH 5.25 ± 0.01, (n = 38)) was first observed in the 5.2/Al group on day 5 and persisted thereafter, with the 5.2/Al fish surviving at least 2.6 times longer than the other two groups (Fig. 1). Preexposure to acid alone did not result in acclimation to Al.

With the exception of day 1 (see Fig. 1), challenge with lethal Cu (32.2 ± 1.3 $\mu g \cdot L^{-1}$ (n = 33); pH ± 5.21 ± 0.01 (n = 37)) resulted in 50% mortality in all groups within about 48 h or less. On day 1 the Cu challenge LT50 values were substantially higher (67–96 h) but were associated with significantly lower measured ambient Cu levels during this particular toxicity test (20.8 ± 0.4 $\mu g \cdot L^{-1}$ (n = 4)). During all subsequent LT50 tests the average measured Cu levels in the Cu challenge tank ranged from 30.7 to 35.9 $\mu g \cdot L^{-1}$ and produced LT50 values comparable with those for unacclimated fish in the Al challenge tests. Thus, the levels of Cu and Al (32 and 162 $\mu g \cdot L^{-1}$) were of approximately equivalent toxicity. On a molar basis ([Cu] = 0.52 and [Al] = 5.55 $\mu mol \cdot L^{-1}$), Cu was therefore approximatly 10 times more toxic than Al at the ambient pH and Ca levels we used.

In Cu LT50 tests, significantly improved survival times were seen on days 5 and 25 for the 5.2/Al trout and on day 25 for the 5.2/0 trout when compared with the control group (Fig. 1). However, these differences were small (relative to the Al LT50 tests) and were inconsistent. On day 36, when challenged with lethal pH alone (pH 3.99 ± 0.04 (n = 4), no Cu or Al added), LT50's were similar in all three groups (14.8, 17.3, and 17.5 h for the control, 5.2/0, and 5.2/Al groups, respectively), indicating no acclimation to low pH itself in the absence of Al.

During the course of LT50 tests, it was noticed that larger fish tended to die earlier than smaller fish. Figure 2 shows this phenomenon using the data accumulated from Al toxicity tests during the 34-d experimental period for the two unacclimated groups (5.2/0 and 6.5/0). It is apparent that no fish larger than about 15 g survived for more than 40 h during the Al challenge. In contrast, 45% of fish smaller than 15 g survived for 40 h or longer, and some of these smaller fish were able to survive the full 96 h of the tests. Thus, size had a substantial effect on survival during lethal Al challenge.

Ionoregulatory and Respiratory Disturbances and Gill Al content

Both experimental groups suffered losses of whole-body Na^+ and Cl^- over the first 5–17 d of exposure (Fig. 3). Initially, the losses were both more rapid and more severe in the 5.2/Al trout, being at least twofold greater on day 5. Whole-body ions subsequently stabilized in the 5.2/Al trout, but continued to fall in the 5.2/0 group until, by day 17, they had reached similar levels to those in the 5.2/Al trout, representing losses of about 24 and 32% of their whole-body Na^+ and Cl^-, respectively. Following day 17, ionic status gradually recovered, and by day 34, whole-body Na^+ and Cl^- of both groups were not significantly different from the control group. In contrast with ionoregularoty status, the whole-body lactate contents of 5.2/0 and 5.2/Al trout were never significantly different from the control group (Fig. 3).

At the beginning of the sublethal exposures, total gill Al in the 5.2/Al trout increased almost linearly with time, reaching an initial peak on day 5 of around 29 $\mu g \cdot g^{-1}$ (Fig. 3). Gill Al burden subsequently declined between days 5 and 10, but thereafter increased again reaching a maximum of 37.9 ± 5.0 $\mu g \cdot g^{-1}$ on day 34. The peak gill Al levels on days 5

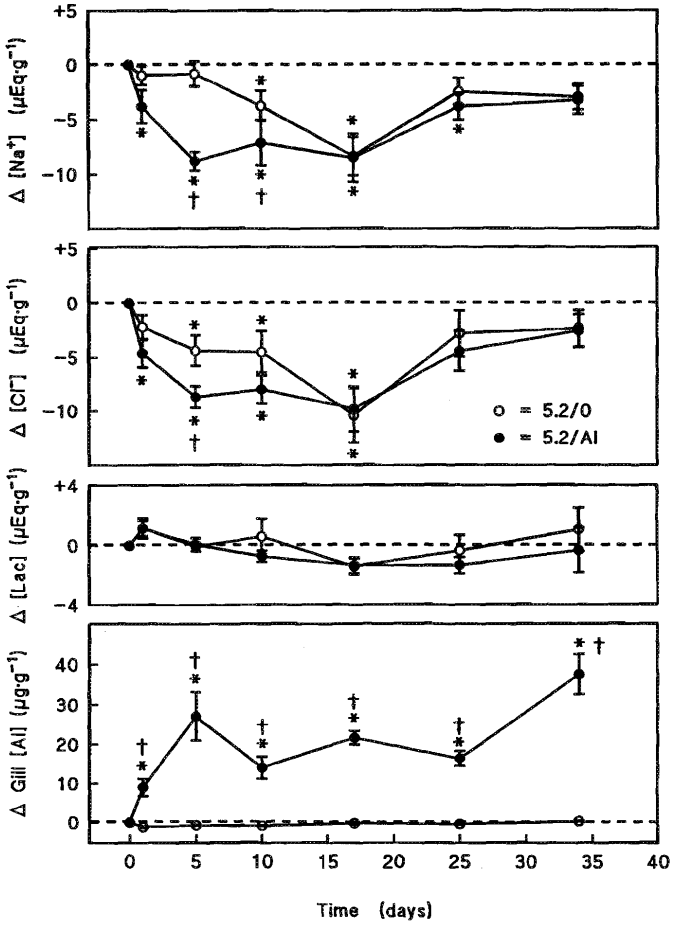

FIG. 3. Whole-body Na^+, Cl^-, and lactate and gill Al concentration (relative to the control group) during 34 d of exposure to pH 5.2 (open circles) or pH 5.2 with sublethal Al present (38 μg $Al \cdot L^{-1}$) (solid circles). The broken line represents the combined mean values for control fish (maintained at pH 6.5, zero Al; $[Na^+] = 32.7 \pm 0.5$ ($n = 60$), $[Cl^-] = 29.0 \pm 0.6$ ($n = 60$), [lactate] = 3.59 ± 0.32 ($n = 59$) μEq·g wet $weight^{-1}$, gill [Al] = 1.09 ± 0.27 ($n = 60$) μg·g wet $weight^{-1}$). 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. The two peaks of gill Al in the 5.2/Al group (days 5 and 34) were also significantly higher than the trough on day 5.

and 34 were both significantly higher ($P < 0.05$) than the trough on day 10 (Student's unpaired t-test). There was no accumulation of Al on the gills of the other two groups throughout the study.

Physiological Responses to Lethal Al Challenge

Figure 4 shows the changes in four variables (whole-body Na, Cl, and lactate and gill Al) in all three groups when subjected to 10 h of exposure to lethal Al at pH 5.2 on day 36. Whole-body losses of Na^+ and Cl^- and increases in lactate were significantly attenuated in the 5.2/Al group compared with the controls. In contrast, the accumulation of gill Al during lethal challenge was not different from that in the control group. In the 5.2/0 group, ion losses and lactate accumulation did not differ from those found in control fish (Fig. 4).

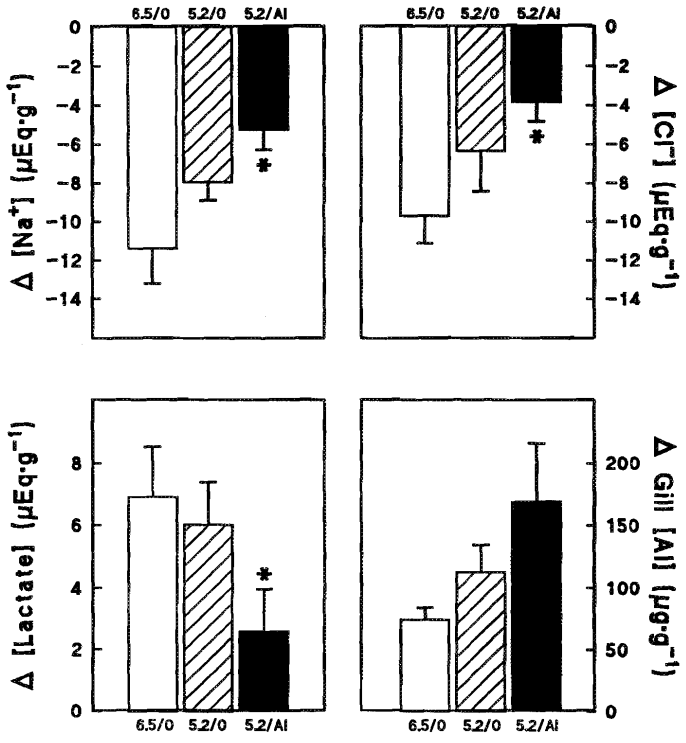

FIG. 4. Physiological changes (whole-body Na^+, Cl^-, and lactate and gill Al) following a 10-h challenge with lethal Al (162 μg·L^{-1}, pH 5.2) on day 36. Δ values ($n = 10$) were obtained by subtracting the mean value observed for each group on day 34 of the chronic sublethal exposure regimes. Open bars represent the control group (6.5/0), hatched bars the 5.2/0 group, and solid bars the 5.2/Al group.

Feeding and Growth

At the start of the exposure regimes, all three groups had feeding rates of around 1.4% body weight per day (Fig. 5). From day 0 onwards, feeding rates were similar in the 5.2/0 and 6.5/0 groups (although the 6.5/0 group was usually slightly higher), and both groups gradually increased their daily food intake over the course of the study, reaching 3.3% by day 36 (Fig. 5). Exposure to sublethal Al caused an immediate reduction in appetite and hence feeding rate, reaching a minimum of 0.4% on day 3. Food intake gradually recovered thereafter, but remained below 1.5% for the first 10 d. After day 10, daily food intake approached, but never really matched, the rates maintained by the 5.2/0 and 6.5/0 groups and was still approximately one full percentage point lower at day 34 (Fig. 5).

At the start of the study, average weights were very similar in the three groups at around 7.5–8.5 g. Growth occurred in all three exposure regimes and followed an exponential curve when plotted as wet weight versus time (Fig. 5). Overall, the control group had the largest increase in mass during the study, almost tripling their average to 21.1 ± 1.1 g on day 35. The 5.2/0 trout were slightly (but not significantly) smaller on day 35 (19.17 ± 1.07 g) whereas the 5.2/Al trout only managed to double their mass by this time (16.91 ± 1.01 g). However, this was the only occasion on which the weight of 5.2/Al trout was significantly lower than that of the control group. Interestingly, from day 21 to 28, the 5.2/0 trout actually appeared to be growing faster than the control trout and were significantly larger than the 5.2/Al trout on

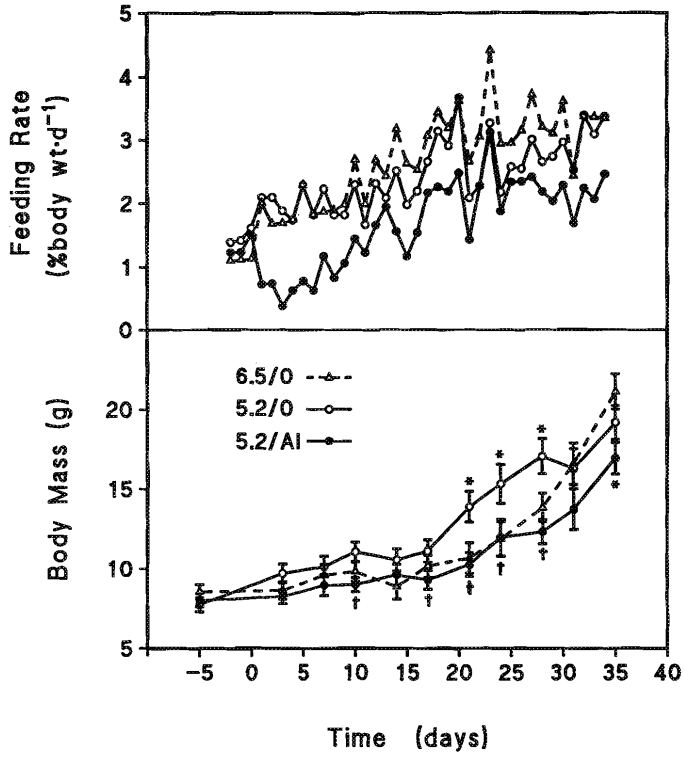

FIG. 5. Feeding rates and growth in the 6.5/0 group (broken line), 5.2/0 group (open symbols), and 5.2/Al group (solid symbols) during 34 d of the chronic sublethal exposyre regimes. The lower panel shows mean wet weight (± SEM, n = 50–90) with time during the chronic sublethal exposure regimes. Asterisks indicate significant difference from the 6.5/0 control group, and daggers indicate significant difference from the 5.2/0 group.

days 10, 17, 21, 24, and 28 (Fig. 5).To test whether these greater increases in wet mass in the 5.2/0 trout could be due to impaired water balance rather than growth, we measured the water contents of 10 fish from each group at the end of the study. On day 36, we found that whole-body water contents ranged from 72.4 ± 0.4% (5.2/0) to 74.6 ± 0.6% (controls), but no differences were found between any of the groups. The 5.2/Al group had the highest gross food conversion efficiency at 38.2% compared with 31.6% for the 6.5/0 control group and 28.3% for the 5.2/0 group.

Discussion

We have confirmed that acclimation is induced when trout are continuously exposed to sublethal Al and that this acclimation is a product of the ambient Al level rather than the simultaneous exposure to increased acidity (Orr et al. 1986; Wood et al. 1988b, 1988c; McDonald et al. 1991; Wilson and Wood 1992). The achievement of acclimation after just 5 d was slightly earlier than in most previous studies (see below).

Specificity of Acclimation

Cu and Al act primarly at the gills of fish and appear to have similar modes of toxic action; both disrupt Na^+ and Cl^- balance and cause respiratory disturbances during acutely lethal exposures (e.g., Playle et al. 1989; Wilson and Taylor 1993). The lack of cross-toxicant resistance between these two metals implies that acclimation to Al involves changes that are specific to Al rather than the toxic effects it produces. This is not the case for other toxic metals. For example, Zn and Cd both interfere with branchial control of Ca^{2+} balance, and acclimation to Cd has been shown to increase resistance to Zn and vice versa (Kito et al. 1982; Duncan and Klaverkamp 1983; Thomas et al. 1985; Klaverkamp and Duncan 1987). However, this may simply mean that Cd and Zn inhibit Ca^{2+} transport via very similar mechanisms. Cu and Al inhibit the Na^+ uptake mechanism in a similar if not identical manner, through the blockade of transport enzymes (reviewed by Wood 1992). However, they may disrupt tight junctions and stimulate passive Na^+ losses (J^{Na}_{out}), via subtly different mechanisms because J^{Na}_{out} is independent of the ambient [Ca^{2+}] during Cu exposure (Laurén and McDonald 1985, 1986) but is Ca^{2+} dependent during Al exposure (Booth et al. 1988; Wood et al. 1988a, 1988c; Playle et al. 1989). Perhaps this subtle difference between the mechanisms of Al and Cu toxicity is the basis for the lack of their crossover resistance. Whether this is true or not, it is apparent that the gross symptoms of toxicity are not sufficient in themselves to be able to predict mechanisms of toxicity and acclimation.

Size Effect

The observation that size has a critical effect on the acute toxicity of Al is not new (Baker and Schofield 1982; Brown 1983; Rosseland and Skogheim 1984; Cleveland et al. 1986; Hunn et al. 1987; Ingersoll et al. 1990b; Wood et al. 1990). However, these previous studies demonstrated this effect using different life stages or age groups of fish. All our fish were of the same age, and therefore, differential sensitivity to Al can *only* be attributed to size in this case. This age-independent size effect has not been previously reported and could obviously influence toxicity test results. However, there were no significant differences in the mean weight of fish used in LT50 tests between groups on any given day, so size cannot be considered a complicating factor in the acclimation observed in the 5.2/Al trout. Nevertheless, the general increase in weight in all three groups with time may explain why LT50 values all tended to decrease during the course of the study. One possible physiological explanation for the size effect during lethal Al challenge could be that cutaneous gas exchange is greater (proportionally) in smaller fish, making them less dependent on the gills (where most of the damage through Al precipitation is occurring) and therefore more tolerant to toxic Al concentrations. Some support for this may be the opposite size dependence found during exposure to ambient hypoxia (Shepard 1955) where gas transfer through the skin and gills would be affected equally.

Acclimation and Physiological Recovery

Peak respiratory and ionoregulatory disturbances consistently occur within the first 5 d of sublethal exposure to Al (Booth et al. 1988; Playle et al. 1989; McDonald et al. 1991; Wilson and Wood 1992; present study). In contrast, the time required for the development of acclimation varies considerably (5–17 d). This variability in the time to acclimation is not related to the concentration of Al used or, consequently, the severity of the initial damage caused. However, acclimation usually does precede the recovery of physiological status. The temporal seperation of these processes

suggests that the underlying mechanisms behind acclimation and physiological recovery may be independent of each other. One observation previously linking acclimation to physiological recovery was that fish exposed to acid alone, which does not induce acclimation, did not recover ionoregulatory status but merely stabilized at a new reduced level (Audet et al. 1988; Audet and Wood 1988; Wood et al. 1988c; McDonald et al. 1991; Wilson and Wood 1992). We have now observed that trout exposed to acid alone or acid + Al can recover their ionic status to the same degree given time, even though fish exposed to acid alone still fail to acclimate to low pH itself. Clearly, increased resistance is not required for the recovery of ionoregulatory status, and vice versa. In terms of respiratory disturbances, we observed that resting whole-body [lactate] was unaffected, indicating negligible impairment of oxygen uptake under *routine* conditions. Although a prerequisite of acclimation may be some degree of disturbance to gill morphology and aerobic capacity (McDonald and Wood 1992; Wilson et al. 1994), the threshold for acclimation to Al obviously does not necessitate impaired gas transfer in the nonactive state.

Physiological Correlates of Acclimation

Fish chronically exposed to sublethal Al clearly showed reduced physiological disturbances, in terms of whole-body ion losses and lactate accumulation, when challenged with a potentially lethal Al concentration. This attenuation was similar to that described by McDonald et al. (1991) and Reid et al. (1991) in acclimated brook trout and is to be expected if acclimatory changes are directed at preventing the detrimental action of Al at the gills. Nevertheless, opposite to the expected trend, and in contrast with previous findings (McDonald et al. 1991; Reid et al. 1991), we observed that acclimated fish did not accumulate any less Al on their gills than nonacclimated fish during lethal Al challenge. This disagreement is difficult to explain, but may be a function of the different sampling protocols used; we measured gill Al on all fish after 10 h of exposure, prior to any mortality, whereas McDonald et al. (1991) and Reid et al. (1991) used only the surviving fish remaining after 24 or 40 h of lethal challenge.

A similar anomaly was observed during the sublethal Al exposure in our study, i.e., that acclimation was first observed on day 5 when gill Al content had reached an initial peak, and that acclimation remained despite a secondary, more gradual increase in the gill Al content with time. Earlier, we observed an almost identical, biphasic pattern of gill Al changes in the same species, under very similar conditions (Wilson and Wood 1992). We speculated at the time that this pattern represented two separate accumulations of Al in the gills: one that occurs rapidly and is externally located (on the gill surface and within the gill mucus) but that recovers with acclimation and the other internal (within the gill cells themselves) that is slower but eventually masks the decline of external [Al]. Both these studies with rainbow trout contrast with the findings of McDonald et al. (1991) who showed that brook trout only became acclimated as the gill Al burden was progressively reduced. This could reflect species differences in the mechanism of acclimation; however, the two studies are not directly comparable due to the higher level of Al (75–150 $\mu g \cdot L^{-1}$) used in the brook trout study (McDonald et al. 1991).

A change at the gill surface that prevents the deposition of Al is often cited as a key feature of acclimation to Al (McDonald et al. 1991; Reid et al. 1991; McDonald and Wood 1992). Several components of such acclimatory changes have been suggested. Respiratory problems probably result from precipitation of Al within the gill microenvironment (Playle and Wood 1991). The inflammatory response to this may be reduced when mucus turnover rates are increased such that clearance of insoluble Al is accelerated (McDonald et al. 1991). On the other hand, the disruption of ionoregulation is thought to result from the chemical binding of soluble, charged Al species to structural elements of the gill surface (Playle and Wood 1991). A second component of acclimation appears to be a change in ligand chemistry with a reduction in the affinity of the gill surface for Al, resulting in the restoration of normal ionoregulatory function (Reid et al. 1991).

In support of this "model" was the observation that a substantial hypertrophy of mucous cells coincided with the first apperance of increased resistance (Wilson et al. 1994). However, it is difficult to reconcile these ideas with our results concerning increases in gill Al content during both sublethal exposure and lethal challenge. Clearly, more work is required to provide a model of acclimation that satisfies all the existing observations.

Feeding and Growth

Previous investigations on brown trout (*Salmo trutta*) (Sadler and Lynam 1987, 1988; Reader et al. 1988), brook trout (Mount et al. 1988a, 1988b; Ingersoll et al. 1990a, 1990b), and rainbow trout (Wilson and Wood 1992) have documented reduced growth in Al-exposed fish. However, most studies have either used a fixed maintenance ration or given only anecdotal or semiquantitative reports of feeding behaviour. Here, we have clearly shown that when trout are fed to satiation, reduced growth after 34 d in Al-exposed fish can be at least partially accounted for by a reduction in appetite during the first 10 d. Increased energy expenditure seemed a likely additional factor involved in reduced growth based on our estimates of metabolic rate from forced swimming tests (Wilson et al. 1994). However, trout within the 5.2/Al exposure tanks were noticeably less active, even listless, compared with the other two groups. Indeed, reduced spontaneous activity has been quantitatively documented in cutthroat trout (*Oncorhynchus clarki*, previously *Salmo clarki*) (Woodward et al. 1989) and brook trout (Cleveland et al. 1986, 1989) exposed to sublethal Al. Ironically, this could be important in explaining the greater gross food conversion efficiency in 5.2/Al fish (38.2% compared with 31.6% in the control group). A lower overall *routine* metabolic rate could result in a greater percentage of the dietary protein being retained for growth. This will be true in the laboratory where food is supplied, but a decrease in routine activity in the wild would surely reduce an animal's overall fitness to feed and avoid predation. The apparent increase in food conversion efficiency should therefore be interpreted with caution, and appetite, rather than changes in metabolism, must be considered the principal cause of reduced growth in Al-exposed trout.

Feeding rates in trout exposed to acid alone were similar to those in control fish, indicating that the reduced appetite was solely due to the presence of Al. Acid pH lev-

els lower than 5.2 have been found to impair growth in the absence of Al (Menendez 1976; Sadler and Lynam 1987; Ingersoll et al. 1990a). However, pH 5.2 had no significant impact on overall growth in the present study, similar to our findings in trout fed a constant maintenance ration of 1% body weight per day (Wilson and Wood 1992). Thus, regardless of the feeding regime adopted, it would appear that any increased energy expenditure associated with exposure to pH 5.2 has little impact on the total energy budget of the animal, and specifically the energy invested in growth. This is supported by the fact that the gross food conversion efficiencies of 5.2/0 and 6.5/0 trout were similar.

In summary, acclimation to Al clearly occurs when trout are chronically exposed to sublethal Al at low pH and is entirely due to the presence of Al because acidity alone does not result in increased resistance to either acid or Al. As one might expect, acclimation is associated with reduced physiological disturbances during lethal challenge with Al, but is relatively specific to the offending metal, rather than the symptoms it produces, because increased resistance to Cu was not consistently observed. Contrary to previous studies (Audet et al. 1988; Wood et al. 1988c; McDonald et al. 1991; Wilson and Wood 1992), we have found that trout exposed to acid alone (in addition to acid + Al) can recover their physiological status, given time, even though acclimation is not induced. Finally, while increased resistance to potentially lethal pulses of acid/Al is an obvious benefit of chronic sublethal exposure to acid + Al, there are some costs involved. Reduced feeding and growth rates (in addition to impaired aerobic scope and swimming performance; Wilson et al. 1994) have now been identified as major costs associated with chronic sublethal exposure and in the wild would limit the ability of fish to feed, avoid predation, and reproduce (Little and Finger 1990). These costs must be weighed against the benefits of increased resistance when considering the overall fitness of acclimated fish in real ecological situations.

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