Growth and protein turnover during acclimation to acid and aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*)

Rod W. Wilson, Chris M. Wood, and Dominic F. Houlihan

Abstract: Growth, feeding, and protein synthesis (k_s) and degradation (k_d) in gill, liver, and whole body were measured in juvenile rainbow trout, *Oncorhynchus mykiss*, during 32 days exposure to sublethal acid (pH 5.2) and acid + aluminum (Al) (30 μg·L^{-1}) in soft water. The only effects observed for exposure to acid alone were depressions of gill k_s (17%) and k_d (27%) after 15 days (data not available after day 15). Exposure to acid + Al caused a loss of appetite, a 73% reduction in whole body growth rate, and a 36% reduction in whole body k_s during the first 7 days; all of these subsequently recovered, although mean body weight was still significantly depressed after 32 days. Gill k_s and k_d were greatly stimulated after 7 days and gill k_s remained elevated after 32 days, suggesting a chronic cost of gill repair and (or) acclimatory processes even after physiological recovery was achieved. However, this elevated cost was small relative to the whole animal protein synthesis budget. Other chronic effects included suppressed liver k_s and k_d, reduced whole body translational efficiency, and enlarged liver size. Conversion of food into growth was paradoxically increased throughout all stages of acid + Al exposure but may have been the result of reduced routine activity.

Résumé : On a mesuré la croissance, la quantité de nourriture absorbée, ainsi que la synthèse (k_s) et la dégradation (k_d) des protéines dans les branchies, le foie et tout l'organisme de jeunes truites arc-en-ciel, *Oncorhynchus mykiss*, au cours d'une exposition d'une durée de 32 jours à une concentration sublétale d'acide (pH 5,2) et d'acide + d'aluminum (Al) (30 μg·L^{-1}) dans de l'eau douce. La diminution de k_s (17%) et de k_d (27%) après 15 jours (aucune donnée disponible après la 15^e journée) était le seul effet observé dans le cas de l'exposition à l'acide seulement. L'exposition à l'acide + Al entraînait une perte d'appétit, une réduction de 73% du taux de croissance pour tout l'organisme et une réduction de 36% de la valeur de k_s dans tout l'organisme au cours des 7 premiers jours; tous ces paramètres sont redevenus normaux ultérieurement, mais la masse corporelle moyenne était encore assez faible après 32 jours. La k_s et la k_d dans les branchies étaient considérablement stimulées après 7 jours et la k_s dans les branchies était encore élevée après 32 jours, ce qui laisse supposer qu'il y a un coût chronique aux processus de réparation et (ou) d'acclimatation des branchies, même après récupération physiologique. Toutefois, ce coût élevé était faible si l'on tient compte du bilan de synthèse protéique pour tout l'organisme. Parmi les autres effets chroniques, on compte une diminution de la k_s et de la k_d dans le foie, une diminution de l'efficacité de transport dans tout l'organisme et une hypertrophie du foie. La conversion de la nourriture en croissance était paradoxalement accrue à toutes les étapes de l'exposition à l'acide + Al, mais cette observation était peut-être le résultat d'une réduction des activités habituelles.
[Traduit par la Rédaction]

Introduction

Chronic exposure of freshwater fish to sublethal aluminum (Al) concentrations is frequently encountered in low pH soft water owing 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 the gills are the primary target organ of acute Al toxicity and that initial ionoregulatory and respiratory disturbances are 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 (usually within 5–17 days; Orr et al. 1986; Wood et al. 1988*a*, 1988*b*; McDonald et al. 1991; Wilson and Wood 1992; Wilson et al. 1994*a*). This may explain the continued presence of fish populations in acidified softwater lakes and rivers containing levels of Al in excess of thresholds suggested by laboratory studies (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 addition to acclimation,

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R.W. Wilson[1] and C.M. Wood. Department of Biology, McMaster University, Hamilton, ON L8S 4K1, Canada.
D.F. Houlihan. Department of Zoology, University of Aberdeen, Aberdeen, U.K.

[1] Author to whom all correspondence should be sent at the following address: Department of Biological Sciences, Hatherly Laboratories, University of Exeter, Prince of Wales Road, Exeter EX4 4PS, U.K. e-mail: r.w.wilson@exeter.ac.uk

Table 1. Measured water chemistry variables 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	64.6±3.0 (32)	31.4±1.6 (32)	6.52±0.02 (32)	2.6±0.3 (32)
5.2/0	59.0±3.0 (17)	32.1±1.8 (17)	5.21±0.03 (17)	2.3±0.7 (17)
5.2/Al	58.8±1.9 (32)	31.9±1.8 (31)	5.17±0.03 (32)	29.6±0.5 (31)

Note: Values are given as the mean ± SE, with *n* in parentheses.

some physiological disturbances such as whole body ion content may completely recover with prolonged exposure (Wilson et al. 1994*a*). However, although these gill changes result in increased resistance and reduced physiological disturbances during subsequent Al pulses, certain costs such as reduced growth and chronically impaired aerobic scope and swimming performance are simultaneously incurred (Wilson and Wood 1992; Wilson et al. 1994*b*).

Increased metabolic rate during aerobic swimming in fish acclimated to Al was suggested (though not statistically confirmed) when these fish were compared with controls previously held at circumneutral pH (Wilson et al. 1994*b*). Growth is a more sensitive indicator of subtle increases in energy demand, and impaired growth has indeed been documented for fish exposed to sublethal levels of acid and Al (Sadler and Lynam 1987, 1988; Reader et al. 1988; Mount et al. 1988*a*, 1988*b*; Ingersoll et al. 1990*a*, 1990*b*; Wilson and Wood 1992; Wilson et al. 1994*a*). However, the role of appetite in reduced growth under these stressors has been addressed only rarely (e.g., Wilson et al. 1994*a*), and no studies to date have examined the effects of acid and (or) Al exposure on protein turnover, even though this may provide a sensitive indicator of environmental stresses in general (Houlihan et al. 1994).

The present paper examines some of the potential costs of exposure and acclimation to acid and Al by measuring rates of growth, feeding (appetite), and protein synthesis within the whole body, gills (the key target tissue), and liver (a reference internal tissue). In addition, biochemical correlates of protein turnover (RNA and protein content) have been measured and incorporated with protein synthesis measurements (Garlick et al. 1980) to calculate RNA-translational efficiencies (Millward et al. 1973) in the above tissues. The exposure conditions were created to match as closely as possible our two previous studies (Wilson and Wood 1992; Wilson et al. 1994*a*, 1994*b*). As such, juvenile rainbow trout, *Oncorhynchus mykiss*, were exposed to pH 5.2 in the presence of 30 $\mu g \cdot L^{-1}$ Al for 32 days. All measured variables were compared with two other groups exposed to either pH 5.2 alone, or pH 6.5, both with no Al added. Exposures were carried out in a synthetic soft-water medium relevant to the acid-threatened lakes and rivers of eastern North America and Europe.

Materials and methods

Animal holding and exposure conditions

Juvenile rainbow trout (weight ≈ 6 g) were obtained from Spring Valley Trout Farm and transported to McMaster University, Hamilton, Ontario, where they were initially maintained in continuously flowing dechlorinated tap water ($[Ca^{2+}] \approx 2.0$ meq $\cdot L^{-1}$, $[Na^+] \approx 0.6$ meq $\cdot L^{-1}$, pH ≈ 8) and fed ad libitum. During this holding period fish were divided into three groups of 100 individuals and each given a distinctive freeze brand (Mighell 1969) to allow individuals to be identified during subsequent measurements of growth and protein synthesis.

Following branding the three groups were kept in separate 70-L holding tanks and allowed to recover for 4 days before the transfer to soft water began. Water in the holding tanks was then gradually changed from dechlorinated tap water (11°C) to continuously flowing synthetic soft water (15°C) over 4 days. Flow rates to each tank were approximately 0.6 $L \cdot min^{-1}$ (i.e., 12 volume replacements per day). The synthetic soft-water composition was designed to be similar to that of acid-threatened lakes and streams in eastern North America and Europe (nominal $[Ca^{2+}] \approx 30$ $\mu eq \cdot L^{-1}$, $[Na^+] \approx 60$ $\mu eq \cdot L^{-1}$, $[K^+] \approx 25$ $\mu eq \cdot L^{-1}$, pH 6.5–7.0). A continuous supply of soft water was generated by passing dechlorinated tap water through a reverse osmosis unit (Culligan MP1000), which was occasionally supplemented with product from a deionizing canister (J.W. Anderson Co. Ltd., Dundas, Ont.). The required amounts of reagent-grade $CaCl_2$, NaCl, and KOH solutions were added by peristaltic pump to the main reservoir tank, which supplied all holding tanks, to give the desired ionic composition and pH for the soft-water acclimation period (equivalent to the control group shown in Table 1).

After 8 weeks of acclimation to normal soft water, 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; the 5.2/0 group), and (*iii*) low-pH soft water (nominal pH 5.2) with 30 $\mu g \cdot L^{-1}$ Al added (5.2/Al group). To achieve this, the supply to the 5.2/0 and 5.2/Al exposure tanks was acidified in a secondary head tank using an automatic titration assembly (Radiometer TTT80 titrator, PHM84 pH meter, and GK2401C combination electrode). For acidification a 2:1 equivalent mixture of H_2SO_4 and HNO_3 was used to reflect their proportions in the precipitation of the northeastern United States (Galloway and Likens 1981). Vigorous aeration ensured that P_{CO_2} remained at ambient levels (<0.13 kPa). The [Al] was then elevated in the 5.2/Al holding tank by adding an $AlCl_3 \cdot 6H_2O$ stock solution by peristaltic pump to a mixing vessel at the inlet of this tank. The study was conducted from May to August 1992 at 15°C (range ±0.5°C). Water variables (pH, $[Ca^{2+}]$, $[Na^+]$, and total Al) were measured on samples collected daily from each tank (see Table 1).

Feeding regime and measurement of growth

Fish were fed to satiation once each morning using floating trout pellets (Purina Trout Chow). 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. This feeding protocol was adopted 4 weeks prior to, and continued throughout, the experimental exposure period. Organic debris was removed daily from exposure tanks by siphon.

After 8 weeks of soft-water acclimation, fish had reached a body weight of 12.77 ± 0.35 g (mean ± SEM; $n = 300$). To assess subsequent growth rates, all fish were removed at approximately 2-week intervals, individually identified from their brand marks, weighed in a tared vessel containing the appropriate medium, and then returned to their original tank.

Protein synthesis measurement

On days 7, 15, and 32 of the experimental treatments, 10 fish were sampled from each tank to assess rates of protein synthesis from the incorporation of radiolabelled phenylalanine on the basis of the

method of Garlick et al. (1980). On the above days, feeding was postponed until after the removal of fish for analysis of protein synthesis so that all those used had been starved for approximately 24 h. Fish were individually identified, weighed, and then injected in the caudal vein–artery with a flooding dose of radiolabelled phenylalanine. Successful location of the caudal vein–artery was confirmed by first withdrawing blood into the needle tip. The injection consisted of a 1 mL·100 g^{-1} dose of 150 mmol·L^{-1} phenylalanine (pH 7.5) containing 100 μCi·mL^{-1} (1 Ci = 37 GBq) of L-[2,^{6}–3H]phenylalanine. Following injection fish were transferred to individual darkened tubs containing the appropriate aerated soft-water medium (control, low pH, or low pH + Al). After a 1-h incorporation period, fish were killed by a blow to the head and the whole liver and gills dissected out and weighed. Soft gill tissue was immediately scraped from the cartilaginous filaments and frozen in liquid nitrogen, as were the livers. The remaining carcass was also frozen in liquid nitrogen as quickly as possible. All samples were then stored at –70°C for later analysis.

A 1-h incorporation period was chosen in the present study because we were specifically interested in protein synthesis rates in gill and liver. Preliminary experiments had shown that this was a suitable time for these synthetically active tissues, as specific activities of [^{3}H]phenylalanine within the free pool of these tissues were as predicted for homogeneous distribution. To achieve uniform samples of the whole body, individual frozen carcasses were first ground to a very fine powder in a temperature-controlled mill using a dry ice – methanol mixture as coolant. Whole body and tissue contents of protein and RNA and fractional rates of protein synthesis (k_s) were analysed as detailed in Houlihan et al. (1986). Briefly, tissue samples (approximately 100 mg for gills and livers, 200 mg for powdered whole body) were homogenized in ice-cold 0.2 M perchloric acid (PCA) and the denatured proteins separated by centrifugation. The PCA in the supernatant was precipitated with tripotassium citrate and centrifuged, leaving the free phenylalanine in solution (free pool). Protein in the PCA-extracted tissue pellet was resuspended in 0.3 M NaOH and aliquots were taken for analysis of protein (Lowry et al. 1951) and [^{3}H]phenylalanine content (by liquid scintillation counting). The remaining suspension was acidified with PCA and centrifuged, and the resultant supernatant was analysed for total RNA using the Orcinol method (Munro and Fleck 1966). The pellet, now containing reprecipitated protein and DNA, was washed twice with PCA and then hydrolysed in 6 M HCl for 18 h at 90°C. Following hydrolysis a dry air flow was used to evaporate all traces of the acid and the residues of free amino acids were resuspended in sodium citrate buffer. Phenylalanine in both these protein hydrolysates and the matching free-pool samples was then converted to β-phenylethylamine (using L-tyrosine decarboxylase), extracted using *n*-heptane, and analysed by a ninhydrin reaction. The content of [^{3}H]phenylalanine (now as β-[^{3}H]phenylethylamine) was measured by liquid scintillation counting.

Calculations

Specific growth rates (SGR) for individual fish were calculated from the following equation (Ricker 1979): $SGR = (W_2 - W_1)/((W_1 + W_2)/2) \times 100/t$, where SGR is measured as percentage per day, W_1 is the initial weight (grams), W_2 is the final weight (grams), and t is the length of the growing period (days). Protein growth rates (k_g) of gills and liver were also calculated in the fish sampled for protein synthesis analysis using the same formula (substituting protein content for weight). For these fish, allometric relationships between wet body weight and the whole wet tissue weight were used to estimate initial tissue protein contents for the occasion on which they were weighed 2 weeks previously (corresponding to W_1 in the above equation). The tissue protein contents actually measured at the time of sampling were used as the final values (corresponding to W_2 in the above equation). These allometric relationships were determined from control group fish used during protein synthesis measurement. Additional dissections were carried out on 13 of the remaining fish in the control group at the end of the experiment to increase the range for these allometric relationships (11–83 g). The allometry of the tissues was described using the $\log_e$ transformation of the power relationship $Y = aX^b$ and an exponent of –0.2 (Houlihan et al. 1988), where Y is the wet weight of the tissue (grams) and X is the total wet body weight (grams).

Gross food conversion efficiencies were calculated as described by Brett and Groves (1979), using the dry weight gained (grams) divided by the total weight of dry food consumed for the average fish in each group over a given period (assuming a 73% body water content; Wilson et al. 1994*a*).

The rates of fractional protein synthesis were calculated as $k_s = (SA_b/SA_{fp}) \times (1440/t) \times 100$, where k_s is measured as percentage per day, SA_b and SA_{fp} are the specific activities of phenylalanine (dpm per nanomole) in the protein-bound and free-pool fractions, respectively, and t is the incorporation period (minutes). In practice, true k_s was not measured in all of the tissue samples prepared because of the expense of the assay. However, true k_s was determined in a random selection of liver, gill, and whole body samples (n = 79). Estimated k_s values were obtained for these same samples and all other samples from analysis of the [^{3}H]phenylalanine and total protein content of the pellets resuspended in NaOH (see above), assuming a constant phenylalanine content of fish proteins (275 nmol phenylalanine·g protein^{-1}). By comparing the true with the estimated k_s in the paired samples, a simple regression relationship was obtained (r = 0.903) that was subsequently used to transform all of the estimated k_s to apparent true k_s values. A weight correction exponent of –0.2 was used to convert these individual values of k_s and RNA content to that of a standard-sized 25-g fish (Houlihan et al. 1988). RNA concentrations have been expressed as micrograms of RNA per milligram of tissue protein (capacity for protein synthesis) and the translational efficiency of RNA (k_{RNA}; grams protein synthesized per gram RNA per day) calculated as $k_{RNA} = k_s/[RNA]$ as in Millward et al. (1973).

For liver and gill tissues, protein degradation rates (k_d) were calculated as the difference between k_s and k_g, the specific growth rate for tissue protein.

Acute toxicity tests

On days 7 and 32, 10 fish from each tank were transferred to a single challenge tank supplied with pH 5.2 water containing 200 μg Al·L^{-1}, to assess LT_{50} values (time to 50% mortality) as a measure of changing tolerance to Al. Cumulative mortalities were recorded over the following 96 h or until all fish were dead. LT_{50} values were estimated by log–probit analysis for each group exposed to the challenge. The individual brand marks allowed us to use the same challenge tank for all groups at the same time. Therefore, all groups were exposed to exactly the same challenge conditions.

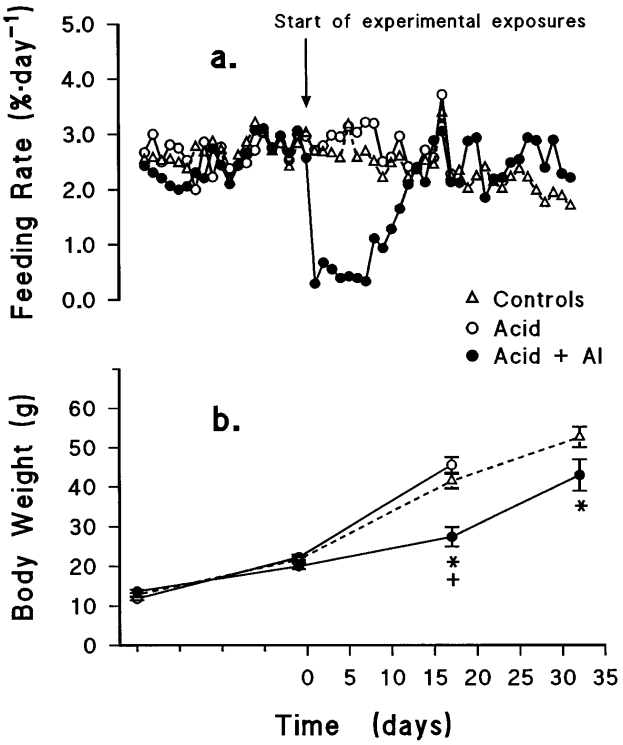
Analytical techniques

Water samples were analysed for total Al content using an atomic absorption spectrophotometer (Varian AA–1275) with graphite furnace attachment (GTA–95). Atomizer parameters were as described previously (Wilson and Wood 1992). Water [Ca^{2+}] and [Na^+] were analysed on the same machine by flame atomic absorption spectrophotometry.

Statistical analysis

Values are generally reported as the mean ± SEM throughout the text. LT_{50} 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 one-way analysis of variance followed by the Scheffé post hoc test (Scheffé 1953) at the 5% level of significance.

Fig. 1. Group feeding rates (*a*) expressed as a percentage of the average body weight per day in the 6.5/0 group (open triangles, broken line), 5.2/0 group (open circles, solid line), and 5.2/Al group (solid circles, solid line), prior to and during 32 days of the chronic sublethal exposure regimes. (*b*) Mean group wet body weights (±SE, n = 30–100) with time during the same chronic sublethal exposure regimes as above. Asterisks indicate significant difference from the 6.5/0 control group, and crosses indicate significant difference from the 5.2/0 group, for the same exposure day.



Results

Mortality and acclimation

No mortality was observed in the control group during the 32-day experiment or in the 5.2/0 group during the first 17 days. Unfortunately, a laboratory mishap caused the loss of all fish in the 5.2/0 tank on day 18. Data are therefore not available for this group following day 17. Exposure to low pH in the presence of 30 $\mu g \cdot L^{-1}$ Al resulted in 30% cumulative mortality over the 32-day experiment.

Increased resistance to lethal Al (200.3 ± 6.9 $\mu g \cdot L^{-1}$ (n = 6) at pH 5.24 ± 0.03 (n = 8)) was tested on days 7 and 32. As in our previous acclimation studies (Wilson and Wood 1992; Wilson et al. 1994*a*), 5.2/Al trout survived approximately twice as long as the 6.5/0 and 5.2/0 trout during both these acclimation tests (e.g., LT_{50} values on day 7 were 25.2, 12.6, and 15.0 h, respectively). LT_{50} values in the 5.2/0 group were not significantly different from those in the control group on day 7.

Whole tank feeding, growth, and food conversion efficiency

Prior to starting the experiment, the three groups had similar feeding rates of between 2 and 3% body weight·day^{-1} (Fig. 1*a*). From day 0 onwards, feeding rates were similar in the 5.2/0 and 6.5/0 groups with a tendency for daily feeding rates to decline over the course of the study as body weight increased (Fig. 1*b*). Exposure to sublethal Al caused an immediate reduction in feeding rate, reaching a minimum of 0.3%·day^{-1} on day 1. Food intake remained low for the 1st week but recovered during the 2nd week. Thereafter, feeding rates in the 5.2/Al group were comparable with those in the 6.5/0 control group.

Whole tank mean body weights were similar in all three groups (range 19.9–22.2 g) prior to starting the experimental regime (Fig. 1*b*). Net growth was observed in all three groups during the study. However, mean body weight was significantly lower in the 5.2/Al on both days 17 and 32, whereas exposure to acid alone (5.2/0) had no significant effect on body weight when compared with the control group on day 17. Gross food conversion efficiencies were almost identical in the control and acid-exposed fish during the first half of the study (Table 2). However, in the 5.2/Al trout, gross food conversion efficiency was 23% higher than in the other two groups during the first half and 76% greater than in the control group during the second half of the experimental period (Table 2).

Table 2. Calculated gross food conversion efficiencies (%) over the first and second halves of the three exposure regimes, and overall values for the whole study.

Exposure group	Days 0–17	Days 17–32	Days 0–32
6.5/0	32.6	20.4	27.1
5.2/0	32.7	na	na
5.2/Al	40.1	36.0	37.5

Note: Data are not available (na) for the 5.2/0 group after day 17.

Whole body weight-specific growth and protein synthesis rates

SGR and whole body protein synthesis rates were measured in the same 10 fish sampled from each tank on days 7, 15, and 32. Mean SGR in these subsamples of fish mirrored the observed changes in whole tank mean body weights but revealed that the reduced weight in the 5.2/Al group on days 17 and 32 was specifically due to a 73% reduction in SGR during the 1st week of exposure (Fig. 2). During the second and third measurement periods (days 7–15 and 17–32), there were no statistically significant differences in SGR between any of the groups (Fig. 2).

Whole body k_s in the 5.2/Al fish followed the same pattern as SGR, being reduced by 36% on day 7, but was similar to that in the other available groups on days 15 and 32. Table 3 shows that this initial fall in k_s on day 7 was associated with a 25% reduction in whole body RNA content but not translational efficiency (k_{RNA}) or percent protein content. However, on day 32 when SGR and whole body k_s where not significantly different from the values in the control fish, a small but highly significant (P = 0.005) depression of k_{RNA} was apparent but without any significant changes in either RNA or percent protein content (Table 3).

Gill protein turnover

None of the variables associated with gill protein turnover was affected by 7 days exposure to acid alone (Fig. 3, Table 4). In contrast to this, and despite a one-third reduction in whole

Fig. 2. Weight-specific growth rates (SGR; %·day^{-1}) and fractional rates of protein synthesis (k_s; %·day^{-1}) for whole bodies (minus gills and liver) in the three groups (open bars, 6.5/0 group; hatched bars, 5.2/0 group; solid bars, 5.2/Al group) after 7, 15, and 32 days of their respective exposure regimes. Asterisks indicate significant difference from the 6.5/0 control group, and crosses indicate significant difference from the 5.2/0 group, for the same exposure day.

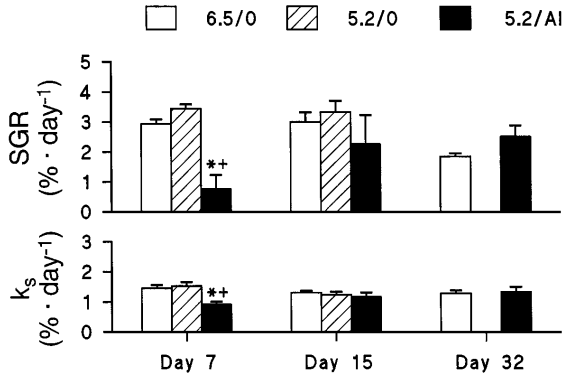
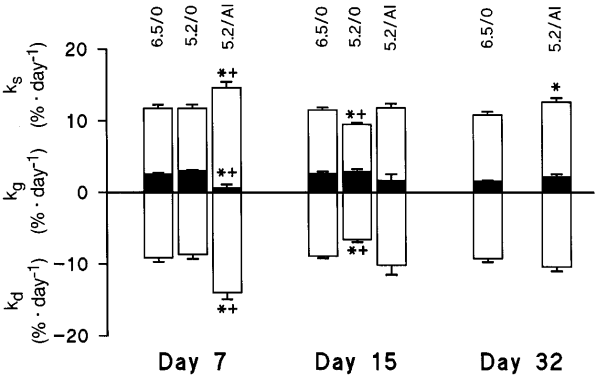


Fig. 3. Fractional rates of protein synthesis (k_s; open bars above the *x* axis), protein degradation (k_d, calculated from $k_g - k_s$; open bars below the *x* axis), and net protein growth (k_g; solid bars) in the soft gill tissue of trout after 7, 15, and 32 days of their respective exposure regimes. Asterisks indicate significant difference from the 6.5/0 control group, and crosses indicate significant difference from the 5.2/0 group, for the same exposure day.



body protein synthesis, gill k_s was significantly elevated (by 24%) after 7 days exposure to acid + Al (Fig. 3). An even greater increase in the fractional rate of protein degradation (k_d) was observed in the gills of 5.2/Al trout (up by 53%; Fig.3). These initial changes in gill protein turnover were not attributable to any significant changes in either RNA content or k_{RNA}. The net effect of these changes in k_s and k_d was a reduction in gill protein accretion (k_g; Fig. 3) similar to that observed in the rest of the body (whole body SGR; see Fig. 2), although actual protein content (expressed as mg protein/100 mg wet tissue weight) was elevated slightly relative to that in the 5.2/0 trout (Table 4).

On day 15, both k_s and k_d were significantly suppressed in the gills of trout exposed to acid alone (Fig. 3) and coincided with a 12% reduction in gill RNA content (significantly different from the 5.2/Al group but not the control group; Table 4). In contrast, gill protein turnover in the 5.2/Al trout had returned to a level similar to the controls by day 15.

After 32 days gill k_s was 16% higher in the 5.2/Al trout than in the controls and was associated with an 11% higher gill RNA content (Table 4).

Table 3. Additional parameters related to protein turnover in whole bodies (minus gills and liver).

Exposure time	Exposure group	[RNA] (μg·mg^{-1})	k_{RNA} (g·g·day^{-1})	Protein content (%)
Day7	6.5/0	28.2±1.7	0.51±0.02	11.2±0.5
	5.2/0	29.9±2.3	0.52±0.04	12.0±0.6
	5.2/Al	21.2±1.3*†	0.44±0.02	12.0±0.6
Day 15	6.5/0	24.6±0.8	0.53±0.02	11.3±0.3
	5.2/0	25.9±1.0	0.48±0.04	11.0±0.2
	5.2/Al	23.2±1.8	0.53±0.04	11.4±0.2
Day 32	6.5/0	25.6±2.6	0.52±0.02	11.6±0.8
	5.2/0	na	na	na
	5.2/Al	31.7±3.2	0.43±0.02*	10.9±0.9

Note: Data are not available (na) for the 5.2/0 group after day 17. $n = 10$ except for the 5.2/Al group on day 7 where $n = 9$.

*Significantly different from the 6.5/0 control group, for the same exposure day.

†Significantly different from the 5.2/0 group, for the same exposure day.

Table 4. Additional parameters related to protein turnover in gill tissue.

Exposure time	Exposure group	[RNA] (μg·mg^{-1})	k_{RNA} (g·g·day^{-1})	Protein content (%)
Day 7	6.5/0	81.2±2.4	1.45±0.06	7.08±0.22
	5.2/0	82.0±1.8	1.43±0.06	6.84±0.21
	5.2/Al	91.0±4.5	1.62±0.09	7.92±0.26†
Day 15	6.5/0	90.6±4.1	1.29±0.04	6.63±0.25
	5.2/0	79.7±2.3	1.20±0.03	6.78±0.23
	5.2/Al	94.7±2.2†	1.25±0.06	7.42±0.15
Day 32	6.5/0	78.3±2.6	1.36±0.03	7.37±0.15
	5.2/0	na	na	na
	5.2/Al	87.1±2.4*	1.45±0.04	7.49±0.16

Note: Data are not available (na) for the 5.2/0 group after day 17. $n = 10$ except for the 5.2/Al group on days 7 and 15 where $n = 9$.

*Significantly different from the 6.5/0 control group, for the same exposure day.

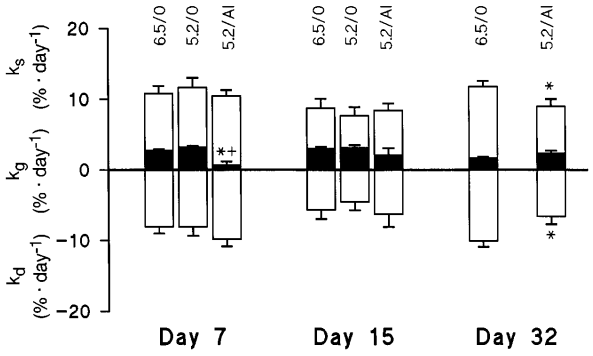
†Significantly different from the 5.2/0 group, for the same exposure day.

Liver protein turnover

Net protein accretion rate (k_g) in the livers of 5.2/Al trout was reduced by 74% on day 7 (Fig. 4), paralleling the almost identical reduction in whole body SGR and gill k_g. However, changes in protein turnover in the liver differed markedly from the responses seen in the gills. The only change in liver protein turnover was observed on day 32 when k_s and k_d were reduced by 24 and 35%, respectively, in 5.2/Al trout compared with the controls. No changes in liver RNA content or k_{RNA} were apparent.

Liver protein content (percent wet weight) was increased by 13% following 7 days exposure to acid + Al, despite a reduction in protein growth in this organ, but had returned to

Fig. 4. Fractional rates of protein synthesis (k_s; open bars above the *x* axis), protein degradation (k_d, calculated from $k_g - k_s$; open bars below the *x* axis), and net protein growth (k_g; solid bars) in the livers of trout after 7, 15, and 32 days of their respective exposure regimes. Asterisks indicate significant difference from the 6.5/0 control group, and crosses indicate significant difference from the 5.2/0 group, for the same exposure day.



normal by day 15. Despite this recovery of liver protein content, disproportionate growth of whole livers was apparent in 5.2/Al trout by day 32 when the liver somatic index was 31% higher than in the control fish (Table 5).

Table 5. Additional parameters related to protein turnover in liver tissue.

Exposure time	Exposure group	[RNA] (μg·mg^{-1})	k_{RNA} (g·g·day^{-1})	Protein content (%)	Somatic index (%)
Day 7	6.5/0	102.7±3.3	1.07±0.12	10.8±0.2	0.92±0.07
	5.2/0	110.9±2.0	1.06±0.13	10.4±0.2	1.12±0.18
	5.2/Al	101.5±1.8	1.03±0.08	12.2±0.3*†	0.74±0.10
Day 15	6.5/0	102.1±3.4	0.88±0.15	12.9±0.2	1.08±0.04
	5.2/0	105.6±1.5	0.74±0.12	12.7±0.3	1.19±0.04
	5.2/Al	102.9±4.4	0.87±0.13	11.2±0.7	1.21±0.10
Day 32	6.5/0	108.8±2.6	1.08±0.06	12.6±0.2	0.93±0.05
	5.2/0	na	na	na	na
	5.2/Al	105.2±5.1	0.87±0.11	11.7±0.5	1.22±0.09*

Note: Data are not available (na) for the 5.2/0 group after day 17. $n = 10$ except for the 5.2/Al group on day 7 ($n = 9$) and the 6.5/0 group on day 15 ($n = 9$).

*Significantly different from the 6.5/0 control group, for the same exposure day.

†Significantly different from the 5.2/0 group, for the same exposure day.

Discussion

Feeding and growth

We have confirmed that, in trout fed to satiation, a dramatic reduction in appetite is one of the first outward manifestations of exposure to acid + Al. However, for the first time we have additionally shown that the effect this has on growth rate is relatively short lived. After an initial period of 7–12 days, appetite and food intake recover, and thereafter, whole body growth rates return to values not different from trout held at neutral or acidic pH in the absence of Al. It would appear that the chronic reductions in growth reported in previous studies on fish exposed to sublethal acid + Al (e.g., brown trout, *Salmo trutta*, Sadler and Lynam 1987, 1988; Reader et al. 1988; brook trout, *Salvelinus fontinalis*, Mount et al. 1988*a*, 1988*b*; Ingersoll et al. 1990*a*, 1990*b*; rainbow trout, Wilson and Wood 1992; Wilson et al. 1994*a*) may be largely, if not entirely, due to the initial effect that acid + Al exposure has on appetite. However, the recovery of feeding may also be dependent on the dose of Al and (or) the sensitivity of the species, as Mount et al. (1988*b*) observed a prolonged reduction of feeding (up to 193 days) with brook trout exposed to higher Al levels (169 μg monomeric Al·L^{-1}) in pH 4.97 soft water. The effects on feeding and growth are clearly attributable to the presence of Al as they are consistently absent in fish exposed to pH 5.2 alone (see also Wilson and Wood 1992; Wilson et al.1994*a*).

The observation of reduced appetite is not unique to Al exposure. Indeed, a complete cessation of feeding has been reported during the first few days of exposure to sublethal copper in both rainbow trout (Lett et al. 1976) and brook trout (Drummond et al. 1973), and in Atlantic salmon, *Salmo salar*, exposed to sublethal zinc (Farmer et al. 1979), although none of these studies was performed in acidic soft water. A reduction in feeding has also been noted in rainbow trout exposed to lower pH values (4.2–4.7) in the absence of added toxic metals (Brown et al. 1984; Tam et al. 1988). The cause of suppressed appetite in all of these cases is poorly understood but may be related to the increased plasma glucose concentraion often seen during acute and chronic acid or Al stress (Goss and Wood 1988; Playle et al. 1989; Brown et al. 1990; Witters et al. 1990). There is evidence that corticosteroids and catecholamines may be involved in elevated plasma glucose levels, although the relationships among the three are complex (Whitehead and Brown 1989), and some believe that some as yet unidentified factors underlie the hyperglycaemia (Tam et al. 1988). Whatever the cause, Waiwood et al.(1992) argued that hyperglycaemia may reduce appetite by acting as a satiation signal that controls the feeding level, as in mammals. If so, then it would be interesting to see if the subsequent return of appetite between days 7 and 12 is associated with, or preceded by, a similar recovery of plasma glucose concentration.

Despite the initial depression of feeding and growth, fish exposed to acid + Al exhibited a substantially higher gross food conversion efficiency both during the initial period of reduced appetite and after the first 12 days when appetite had completely recovered. The same effect was observed in our previous study on acclimation to sublethal acid + Al (Wilson et al. 1994*a*). This conflicts with the a priori assumption that chronic exposure to metals should act as a loading factor to metabolism and contrasts with the trend for trout exposed to acid + Al to have increased basal oxygen consumption rates (Wilson et al. 1994*b*). The latter were estimated by swimming fish in a respirometer at increasing aerobic speeds (from 20 cm·s^{-1} upwards) and making post hoc extrapolations of oxygen consumption rate back to zero speed. However, neither of these approaches takes into account the behavioural response to acid + Al exposure (see below) and, most importantly, the associated routine metabolic rate of fish under these exposure conditions. To our knowledge, direct measurements of routine metabolic rate under these conditions are not available. However, both in our present and previous studies, trout exposed to acid + Al were noticeably less active than the other two exposure groups. Indeed, reduced spontaneous activity

has been quantitatively documented in cutthroat trout, *Oncorhynchus clarkii* (Woodward et al. 1989), and brook trout (Cleveland et al. 1986, 1989) exposed to sublethal levels of acid + Al. If reduced activity is accompanied by a lower overall routine metabolic rate, then it could result in a greater percentage of the dietary protein being retained for growth. An improvement in food conversion efficiency might normally be considered an advantage but should be interpreted with caution in the present case. Enhanced food conversion efficiency may occur in the laboratory when the food supply is not limited, but the ultimate effect of a decrease in appetite and routine activity in the wild would surely be a reduction in an animal's overall fitness to feed, avoid predation, and reproduce (Little and Finger 1990).

Whole body protein turnover

Whole body fractional rates of protein degradation could not be estimated in the present study as the k_s values determined by the [^{3}H]phenylalanine technique were smaller than the corresponding k_g values for whole body, which were measured over the preceding 1–2 weeks (Fig. 2). Because the total amount of protein synthesized in the gills and liver only amounted to approximately 15% of the protein synthesized by the remaining carcass, summing the gill, liver, and carcass k_s values did not change this relationship. There are two possible reasons for this discrepancy. Firstly, in fish accurate values for whole body k_s generally require incorporation periods longer than the 1 h that we used (e.g., 2–4 h; Houlihan et al. 1994). This is probably due to the predominance of the white muscle mass in fish, which is relatively poorly perfused (e.g., Wilson and Egginton 1994) and requires longer than other tissues to ensure free-pool swamping with [^{3}H]phenylalanine (Houlihan et al. 1986). Secondly, k_g represents an average protein growth rate over the previous 1–2 weeks during which fish were fed daily, which may be higher than the k_g value at the precise time when k_s was measured (i.e., following 24 h without food). The whole body k_s values quoted in the present paper should be considered relative values to allow comparison between groups rather than absolute estimates.

Changes in whole body protein synthesis rates followed the same pattern as SGR, although the percent drop in whole body k_s after 7 days was only half the equivalent decrease in SGR during the 1st week of acid + Al exposure. Again, this discrepancy may be due to the different times over which these measurements were made (k_s was measured just as the 5.2/Al trout were beginning to recover their food intake whereas k_g is an average value for the preceding 7 days when food intake was at its lowest). Whatever the reason for this discrepancy, the fall in whole body k_s after 7 days acid + Al appeared to be due to a quantitatively similar decline in the number of ribosomal units (RNA content) rather than any changes in the translational efficiency (k_{RNA}). The recovery of whole body k_s once appetite and feeding rate had returned suggests that exposure to acid + Al does not compromise whole body protein synthesis or growth except through the initial effect on appetite and feeding. However, by the end of the 32-day exposure there was a small but highly significant effect of acid + Al on whole body translational efficiency (k_{RNA}) in the 5.2/Al group. Although this suggests that more ribosomes were required to synthesize the same quantity of new protein, the increase in whole body RNA content (23%) was not statistically significant ($P = 0.152$) on day 32.

Because whole body k_s remained similar to controls after the 1st week of acid + Al exposure and translational efficiency was actually reduced, we may tentatively conclude that a reduction in protein degradation rate (although not measured) was involved in the increased efficiency of converting ingested food into weight gain.

Protein turnover in gill tissue

Rates of protein synthesis in gill tissue vary according to feeding state and, hence, whole body k_s and k_g in fish (Houlihan et al. 1988; McMillan and Houlihan 1988; Houlihan 1991). Therefore, analysis of k_s values in gill tissue must take into consideration any difference in feeding rate and (or) whole body k_s between the 5.2/Al trout and the other two exposure groups. For this reason the increase in gill k_s in 5.2/Al trout after 7 days is more than it at first seems, given the substantially depressed whole body k_s at this time. In fact the ratio of gill k_s to whole body k_s in the 5.2/Al trout was almost double that of the control group on day 7. This trend was predicted to some extent as gill damage generally peaks during the 1st week of exposure to acid + Al (e.g., Mueller et al. 1991; Wilson et al. 1994*b*) and is presumably accompanied by an increase in cellular turnover. However, the increases in gill RNA content and k_{RNA} were not statistically significant on day 7, so we cannot specify the underlying cause(s) of this initial increase in gill protein synthesis. Nevertheless, the even greater increase in protein degradation suggests that breakdown of damaged and (or) necrotized gill tissue predominates over the synthesis of new cellular material during this early damage phase of acid + Al exposure. Because both protein synthesis and degradation involve significant energetic cost (Siems et al. 1984; Hawkins 1991) it seems likely that energy consumption by the gills (contrary to that in the whole body) was greatly intensified at this time when damage was still great yet repair and acclimatory processes had been initiated (McDonald and Wood 1992). The intensification of branchial protein turnover while protein metabolism in the remaining whole body is depressed enhances the view that acute Al toxicity is directed principally at the gills.

By day 15, the rate of protein turnover in the gills of 5.2/Al trout was similar to that in the control fish. Time-course studies have shown that, although gill morphology may never completely recover, the majority of changes that do occur (such as mucous and chloride cell hypertrophy) are well established by the end of the 2nd week of exposure to acid + Al (Mueller et al. 1991). The return of gill protein turnover rates in 5.2/Al trout to control levels after 15 days implies that sustaining the new gill morphological status is not particularly costly in terms of protein synthesis. However, this conclusion does not take into account the suppression of gill protein turnover caused by low pH itself (gill k_s and k_d were significantly reduced in fish exposed to acid alone on day 15). On day 15, therefore, rate of gill protein turnover was elevated in the 5.2/Al trout relative to the 5.2/0 group. We also found that gill k_s in 5.2/Al trout was significantly higher than in controls after 32 days of exposure. There is, therefore, some evidence to suggest that continued exposure to acid + Al does produce chronic elevations in protein synthesis within the gills, which implies some increased metabolic cost. This could be associated

with branchial processes for maintaining increased resistance (acclimation; e.g., increased turnover of mucus), increased cost of combating ionoregulatory problems (e.g., increased production of transport proteins), or simply elevated cell turnover owing to the continuation of Al-induced gill damage. Whatever the reason for this increased protein turnover, the additional metabolic cost will probably be small relative to the fishes' overall energy budget given that the increase in gill k_s only represents about 2.3% of the total protein synthesized by the whole body per day. However, even such a small percent increase could have a significant influence over longer periods of time.

Liver protein turnover

Although protein synthesis in the liver of trout can be influenced by feeding events and ration, McMillan and Houlihan (1988, 1992) showed that after 6 days of either starvation or low ration (0.6% body weight·day^{-1}), liver k_s returned to the same level found in continuously fed trout. Comparison of liver protein synthesis rates between the three exposure groups after 7 days is, unlike the situation in the gills, probably uncomplicated by the lack of feeding in the 5.2/Al group. If this comparison between studies is valid then it would appear that rates of protein synthesis and degradation in the liver are unaffected by 7 days exposure to either acid or acid + Al. Instead, the significant increase in liver protein content of the 5.2/Al trout after 7 days can perhaps be accounted for indirectly by the consumption of liver glycogen during the previous week of near starvation. Glycogen may occupy as much as 7% of the wet weight of trout liver (Waiwood et al. 1992). In addition, glycogen is generally stored with a high water content (Olsson and Saltin 1968). Therefore, a depletion of hepatic glycogen stores would account for most of the increase in liver protein content observed. While a large decline in liver glycogen content has been documented for trout exposed to low pH (Waiwood et al. 1992), this remains to be confirmed for trout exposed to acid + Al.

The liver then does not respond acutely to acid + Al exposure in the same way as the gills. However, continued exposure to acid + Al does eventually lead to a marked suppression of liver protein synthesis and degradation after 32 days. As with the depression of whole body translational efficiency (k_{RNA}) on day 32 this delayed response in the liver may be linked to the very slow accumulation of Al within the internal organs (only detectable after about 10 days; Karlsson-Norrgren et al. 1986; Lee and Harvey 1986; Booth et al. 1988; Witters et al. 1988) compared with the very rapid accumulation of large amounts of Al at the gills (within hours or even minutes; Playle and Wood 1989, 1991; Handy and Eddy 1989). Although the fractional rate of protein synthesis was chronically depressed, the total protein synthesized in the liver per day for a standard-sized 25-g fish (3.3 mg·day^{-1}) was only slightly lower than in the controls (3.5 mg·day^{-1}) because the liver somatic index was substantially higher in the 5.2/Al group on day 32. Therefore, the depressed fractional k_s in the livers of 5.2/Al trout would have had little impact on the overall protein synthetic budget of the whole animal. Liver hypertrophy has also been reported for crucian carp (*Carassius carassius*) exposed to Al for 4 months in an artificially acidified forest pond (Holopainen and Oikari 1992). At present we have no validated explanation for the cause of liver enlargement because there was no statistically significant difference in liver k_g between control and Al-exposed fish on day 32 ($P = 0.083$). However, it is unlikely that changes in either water or glycogen content were involved. Whole body ion status should be fully recovered after about 34 days acid + Al exposure (Wilson et al. 1994*a*), indicating normal fluid distribution, and feeding rates were similar to those in the control fish so we have no reason to invoke different liver glycogen levels.

In summary, we can divide the effects observed in this study into acute (within 7 days) and chronic (after 7 days). The most pertinent acute effect of acid + Al exposure was an almost complete loss of appetite that resulted in depressed whole body protein synthesis during the 1st week. At the same time, gill protein synthesis and degradation were greatly stimulated, an effect we associate with an (assumed) enhancement of gill cell turnover during the early damage phase of acid + Al exposure. Appetite and feeding subsequently recover during continued acid + Al exposure, but some chronic effects persist. These include elevated protein synthesis in the gills, although the cost is small compared with the overall protein synthesis of the animal. Liver protein synthesis and degradation were chronically depressed, as was the whole body translational efficiency. This may indicate a delayed effect of Al on the mechanics of cellular protein synthesis that appears to coincide with the slow accumulation of Al within the internal organs (Lee and Harvey 1986; Karlsson-Norrgren et al. 1986; Booth et al. 1988; Witters et al. 1988; Norrgren et al. 1991). Thus, although a cellular mechanism has not been proposed it is possible that chronic biochemical effects observed within internal tissues are caused directly by elevated tissue Al levels. However, these effects were curiously not observed in the gills where Al levels are probably highest. This could perhaps indicate either a tissue-specific response to Al or perhaps a difference in the nature of Al present within cells, which is dependent on the rate of accumulation. Finally, throughout acid + Al exposure the fish were much more efficient at converting food into whole body growth, a paradox that may be an artifact of our laboratory conditions (feeding to satiation) but that warrants further investigation. We suspect that this may be the result of reduced routine activity and metabolic rate. This behavioural anomaly would be of great consequence to survival and reproduction in the wild where food must be actively sought and predators avoided.

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