Metabolic Costs and Physiological Consequences of Acclimation to Aluminum in Juvenile Rainbow Trout (*Oncorhynchus mykiss*). 2: Gill Morphology, Swimming Performance, and Aerobic Scope

Rod W. Wilson[1]

Department of Biology, McMaster University, Hamilton, ON L8S 4K1, Canada

Harold L. Bergman

Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, WY 82071-3166, USA

and Chris M. Wood

Department of Biology, McMaster University, Hamilton, ON L8S 4K1, Canada

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Juvenile rainbow trout (*Oncorhynchus mykiss*, 5–13 g) were chronically 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 5.2–5.4) for 36 d. Acclimation (increased resistance to challenge with 162 $\mu g\ Al \cdot L^{-1}$ Al at pH 5.2) occurred after 5 d and was associated with a fourfold increase in gill mucous cell density and reduction in apparent lamellar surface area; initially elevated blood–water diffusion distances returned to normal after 34 d, but the reduction in apparent surface area persisted. Chronic exposure to acid alone (pH 5.2, same water chemistry) caused no morphometric changes but resulted in persistent impairment of U_{crit} (critical aerobic swimming speed) by about 10%. This was due to increased oxygen requirements at subcritical swimming speeds (loading stress) and was alleviated when trout were swum at pH 6.5 (zero Al) on day 36. In trout preexposed to sublethal Al, U_{crit} was chronically impaired by approximately 16% due to loading stresses and reduction in the maximum rate of oxygen uptake, $\dot{M}O_{2\ max}$ (limiting stress); U_{crit} and $\dot{M}O_{2\ max}$ remained depressed even when fish were swum at pH 6.5 (zero Al). Reduced gill area compromises the aerobic scope for activity but may be an unavoidable cost of acclimation to Al.

Des truites arc-en-ciel (*Oncorhynchus mykiss*) juvéniles (5–13 g) ont été exposées de façon chronique à une dose sublétale 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 5,2–5,4) pendant 36 jours. L'acclimatement (résistance accrue à une concentration de 162 $\mu g\ Al \cdot L^{-1}$ à pH 5,2) s'est produire après 5 jours et elle était associée à une augmentation par un facteur de 4 de la densité des cellules muqueuses des branchies et à une réduction de la superficie apparente des lamelles branchiales; après 34 jours, les distances de diffusion sang–eau, élevées au départ, sont revenues à la normale, mais la réduction de la superficie apparente a persisté. Une exposition chronique à l'acide seulement (pH 5,2, mêmes propriétés chimiques de l'eau) n'a provoqué aucune modification morphométrique, mais une altération persistante de la valeur U_{crit} (vitesse de nage aérobique critique) d'environ 10 %. Cette modification est due à des besoins accrus en oxygène à des vitesses de nage sous-critiques (stress de charge) et a été atténuée lorsque les truites se trouvaient dans une eau à pH 6,5 (Al nul) le trente-sixième jour. Dans le cas de truites exposées au préalable à une dose sublétale d'Al, la valeur U_{crit} subissait une modification chronique d'environ 16 % en raison du stress de charge et une réduction du taux maximal de fixation de l'oxygène, $\dot{M}O_{2\ max}$ (stress limitant); les paramètres U_{crit} et $\dot{M}O_{2\ max}$ demeuraient réduits même lorsque les poissons étaient placés dans une eau à pH 6,5 (Al nul). Une surface branchiale réduite compromet la portée aérobique de l'activité, mais peut être un coût inévitable de l'acclimatement à l'Al.

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Acclimation to aluminum (i.e., increased resistance to acutely toxic levels established through *continued* sublethal exposure) has been well documented (Orr et al. 1986; McDonald et al. 1991; Wilson et al. 1994). It has also been demonstrated that 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 inital damage phase (lasting 4–5 d) was accompanied by severe gill histopathologies and impared oxygen delivery to tissues (whole-body lactate levels were elevated (McDonald et al. 1991; Mueller et al. 1991)). Acclimation (increased tolerance

[1]Author to whom correspondence should be addressed. Present address: School of Biological Sciences, G.38 Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom.

to lethal Al) was observed from day 10 onwards and was accompanied by at least partial recovery of whole-body lactate levels. However, many gill histopathologies remained even after acclimation had been established. Indeed, they may well be an unavoidable component of acclimation. Although the resting aerobic metabolism was restored with time (Wood et al. 1988; Walker et al. 1991), the persistence of structural abnormalities at the gills suggests that the *capacity* of the respiratory gas exchange system may remain compromised during prolonged exposure to Al. This would only be apparent during increased aerobic activity such as sustained swimming.

Previous studies have shown that low environmental pH reduces swimming performance (Hargis 1976; Waiwood and Beamish 1978; Graham and Wood 1981; Ye and Randall 1991; Butler et al. 1992; Wilson and Wood 1992). However, only Wilson and Wood (1992) have addressed the problems of chronic acid exposure in combination with sublethal Al levels. From that study, it was apparent that one of the costs of acclimation to Al was a prolonged depression of swiming performance approximately twofold greater than that found in fish exposed to acid alone. The main objective of the present study was to assess whether the impairment of swimming performance is related to chronic changes in gill morphology and ultimately aerobic scope. To this end, aerobic swimming metabolism was assessed in juvenile rainbow trout (*Oncorhynchus mykiss*) at intervals during a 36-d exposure to either pH 6.5 (controls) or pH 5.2 in the presence and absence of sublethal Al (38 $\mu g \cdot L^{-1}$) in synthetic soft water. Morphometric analysis of the gills from these three groups was performed near the beginning ("damage phase") and at the end of the exposure regime. Water pH, Ca, and Na levels relevant to the acid-threatened watersheds in eastern North America and Europe were used. The present study was run in parallel with that of Wilson et al. (1994) using fish kept under identical exposure conditions.

Materials and Methods

Animal Holding

Juvenile rainbow trout (5–13 g) were obtained, held, and adapted to synthetic soft water ($[Ca^{2+}]$ = 28, $[Na^+]$ = 85 $\mu Eq \cdot L^{-1}$, pH 6.5–7.0, 15°C) as described in Wilson et al. (1994). After 4 wk in circumneutral soft water, rainbow trout were exposed for 36 d to one of three conditions: (i) normal soft water at pH 6.5 (6.5/0 group), (ii) low-pH soft water at pH 5.2 (5.2/0 group), and (iii) low-pH soft water at pH 5.2 with 38 μg $Al \cdot L^{-1}$ added (5.2/Al group). Water variables (pH, $[Ca^{2+}]$, $[Na^+]$ and total Al) were measured daily on samples from each tank and are reported in Wilson et al. (1994).

Histological and Morphometric Techniques

On days 5 and 34 the second gill arch (left side only) was rapidly excised from four fish (stunned by a blow on the head) from each group and immediately placed in 10% neutral buffered formalin (NBF; pH 7.4) for 1 h. Each gill arch was then trimmed so as to include only the central portion containing approximately 10 paired filaments, and this was placed in fresh NBF for a further 24 h. Once fixed, gill tissues were washed overnight in tap water, dehydrated in graded ethanol solutions, and embedded in plastic (JB-4, Polysciences). Longitudinal 2-μm sections (parallel to the filaments and perpendicular to the lamellae) were cut using a Sorvall MT-2B microtome, mounted on glass slides, and stained with periodic acid-Schiff's reagent and then haematoxylin. Morphometric measurements were performed only on sections that had been cut along the portion of the filament lying between the efferent artery and the cartilaginous gill ray. This region has the most uniform filament thickness and cell composition and includes the central portion of respiratory lamellae (Leino and McCormick 1984). All gill measurements were made using an inverted miscroscope (Zeiss Axiovert 10) and video camera (Hamamatsu C2400) in conjunction with an image analysis system (Neotech, Image Grabber) on a MAC IIci computer. Calibrations were performed using a stage micrometer (Leitz).

The gill dimensions measured were total filament thickness, filament epithelium thickness, interlamellar distance, lamellar thickness, lamellar height, and water–blood diffusion distance. A transparency of a Mertz grid was fixed to the image analyzer screen in order to randomize the points from which all measurements were initiated (Hughes and Perry 1976). It has been shown that for morphometric measurements the within-individual variation is much greater than the between-individuals variation (Haines et al. 1986). Therefore, we used a relatively large number of measurements per individual from a relatively small number of individuals. For statistical purposes, each individual was assigned a single mean value for each of the above variables from the following number of measurements: (i) 10 measurements per tissue section, three sections per filament, from five different filaments per fish (for filament thickness, filament epithelium thickness, interlamellar distance, and lamellar height) and (ii) three measurements per lamella (basal, middle, and distal portions) on 10 lamellae per section, three sections per filament, from five different filaments (for lamellar thickness and water–blood diffusion distance). In addition the number of filamental and lamellar mucous cells per 200 μm of filament length was counted on five sections of five different filaments per fish.

The percentage differences in "apparent" lamellar surface area were approximated by comparing the average perimeters of lamellae, estimated from the mean values of lamellar height and thickness using

$$\text{Perimeter} = 2(\text{height} - [0.5 \times \text{thickness}] + (0.5 \times \pi \times \text{thickness}).$$

Comparing perimeters is only valid if it is assumed that lamellar length and density per filament remained constant in all groups. Although this is not an accurate evaluation of the actual respiratory surface area of the gills, it is a useful measure of percentage differences in "apparent" lamellar surface area between groups.

Swimming Performance and Metabolism Tests

On days 1, 5, 10, 17, 25, and 34, swimming performance and metabolism tests were carried out on eight fish from each group using small-volume (~3.2 L) Blazka-type respirometers similar to those described by Beamish et al. (1989). The soft water supplied to the respirometers was maintained at pH 5.2 when swimming the two acid-exposed groups (5.2/0 and 5.2/Al) with no Al added to the water. Respirometers were supplied with pH 6.5 soft water when swimming the control group (6.5/0). Water temperatures

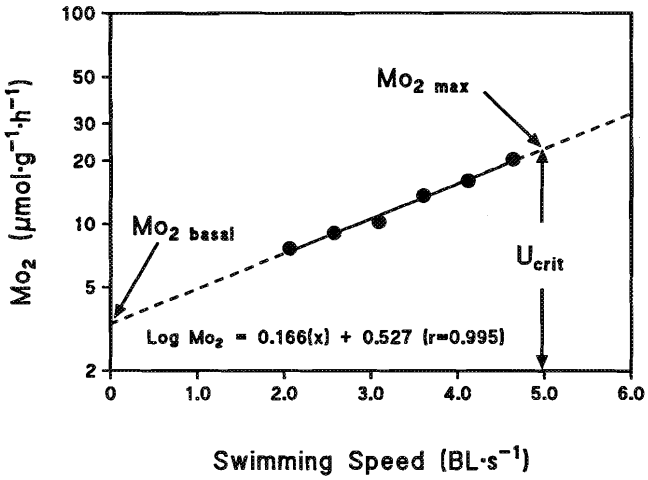

FIG. 1. Relationship between swimming speed and oxygen consumption for a single fish (No. 6, 6.5/0 group on day 5) plotted as a log function of Mo_2. The solid line represents the range over which measurements of Mo_2 were actually made, and the broken lines represents extrapolation to zero speed and U_{crit}, from which the $Mo_{2\ basal}$ and $Mo_{2\ max}$ are derived using the regression equation for log Mo_2 versus speed.

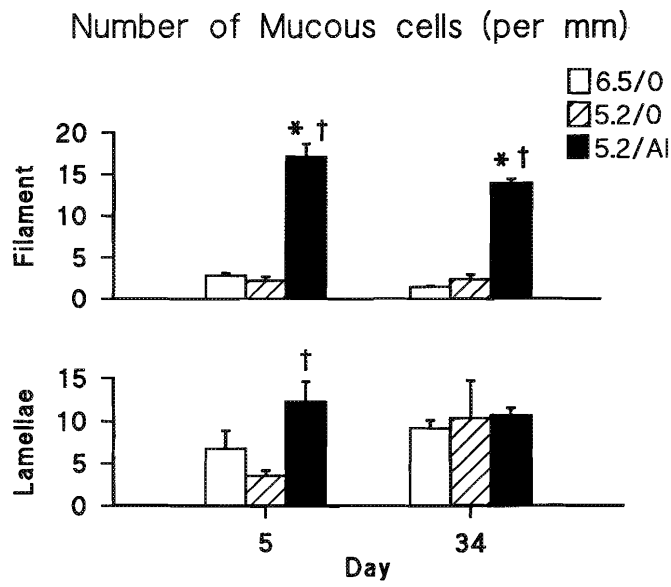

FIG. 2. Gill filamental and lamellar mucous cell densities expressed as number per millimetre along the filaments on days 5 and 34 in the three exposure groups (means ± SEM, $n = 4$). Asterisks indicate means significantly different ($P < 0.05$) from the 6.5/0 control group, and daggers indicate means significantly different from the 5.2/0 group.

within the respirometers were maintained at 15°C (±0.5) by submerging them in a temperature-controlled water table. Fish were transferred to the swimming respirometers the evening before the swim test and maintained at a minimum water velocity (20 cm·s^{-1}) for 12–16 h. Water speed was then increased in 5 cm·s^{-1} stepwise increments every 60 min until fish became exhausted. Fish were considered exhausted once they became impinged on the rear screen, but only if they subsequently refused to swim again when the water velocity was temporarily lowered and then returned to the last speed reached. Critical swimming speed (U_{crit}) was then calculated for each fish according to Brett (1964):

$$U_{crit} = V_f + (T/t \cdot \delta V)$$

where U_{crit} is in centimetres per second, t is time increment (minutes), δV is velocity increment (centimetres per second), and T is the time elapsed at the final velocity (V_f) before exhaustion. Fish were quickly removed following exhaustion, killed with a blow to the head, measured to the nearest 0.1 cm fork length, freeze-clamped between aluminum plates in liquid nitrogen, weighed to the nearest 0.01 g, and stored at −80°C for later analysis for postexercise whole-body lactate content (see Wilson et al. 1994). Swimming velocities were not corrected for solid blocking effects because fish cross-sectional areas did not exceed 10% of the cross-sectional area of the swimming tube (Jones et al. 1974; Webb 1975). Individual U_{crit} values were then transformed to body lengths (BL) per second.

At each swimming speed, oxygen consumption was determined in a flow-through water system from measurements of water flow rate, respirometer volume, fish mass, and the oxygen partial pressure difference across the respirometers ($Po_{2\ in} - Po_{2\ out}$) at the beginning and end of each 1-h period at the new speed. Water Po_2 was measured using Radiometer oxygen electrodes (E5047) and a Cameron Instruments oxygen meter (OM-200) and coverted to oxygen content using tabulated values for the oxygen solubility coefficient in water of zero salinity (Boutilier et al. 1984). The equation given by Fry (1971) was used to account for the time lag between changing the water velocity and reaching a new steady-state outflow Po_2, and corrections were made for the oxygen consumption of individual respirometers in the absence of fish.

For each fish, a regression equation was established for the logarithm of oxygen consumption versus swimming speed. Only data from fish that gave a significant regression correlation coefficient ($P < 0.05$) were used. Using these regression equations, predicted values for Mo_2 at 0 cm·s^{-1} (basal Mo_2) and U_{crit} (maximum Mo_2) were calculated for individual fish (Fig. 1). Aerobic scope was then calculated as the difference between basal and maximum Mo_2 for each fish.

On day 36, an additional test was performed in which U_{crit} and aerobic scope were again measured using fish from the two experimental groups (5.2/0 and 5.2/Al), but this time whilst swimming in soft water maintained at pH 6.5 instead of 5.2. The objective here was to differentiate between the effects caused by the acidity of the water during the swim test versus the exposure histories of fish prior to the test. The results from these tests were then compared with the control group (6.5/0) also swum at pH 6.5 on day 34. Throughout all the swimming tests, fish were exposed to continuous light in order to reduce diurnal fluctuations in metabolic rate (Chakraborty et al. 1992).

Statistical Analysis

Data are expressed as means ± SEM (n) where n refers to the number of fish. Regression analyses for individual swimming fish were performed by the method of least squares and the significance of the correlation coefficient assessed ($P < 0.05$). Multiple comparison and Tukey's statistic (Zar 1984) were used to test for differences between the group regression equations on day 34. For all other measured variables, one-way analysis of variance was used to assess significant differences between groups on each sam-

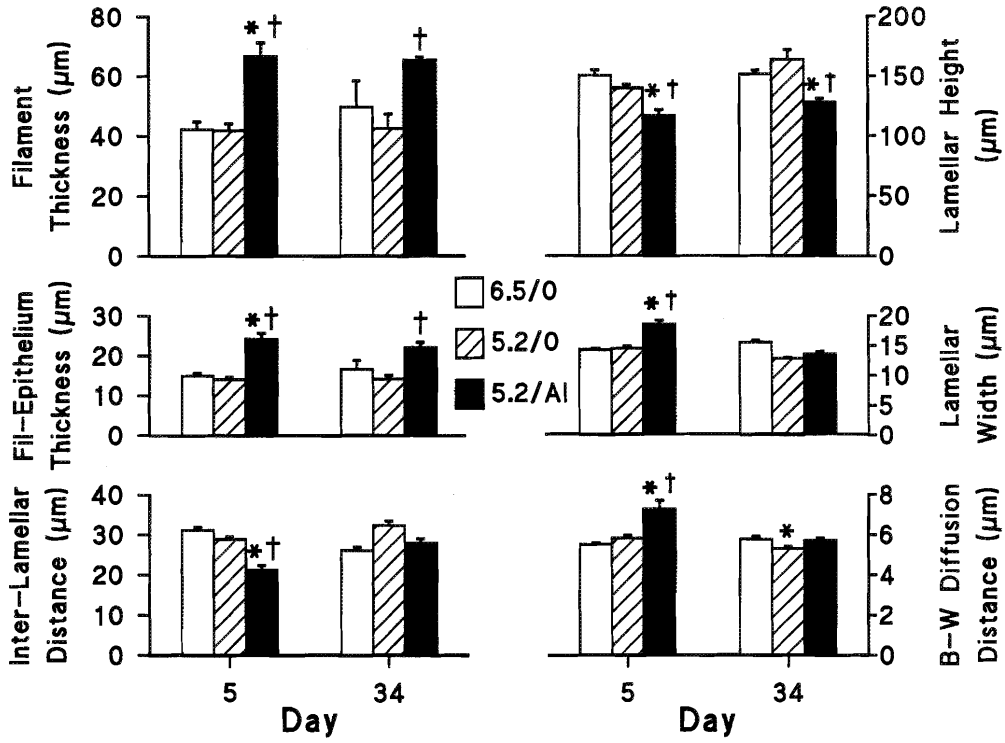

FIG. 3. Gill morphometric measurements of filament thickness, filamental epithelium thickness, interlamellar distance, lamellar height, lamellar thickness, and blood–water diffusion distance on days 5 and 34 in the three exposure groups (means ± SEM, $n = 4$). Asterisks indicate means significantly different ($P < 0.05$) from the 6.5/0 control group, and daggers indicate means significantly different from the 5.2/0 group.

pling day. Where appropriate, Fisher's protected least significant difference (PLSD) was then applied to establish specific differences (at the 5% level of significance).

Results

Gill Morphology

After 5 and 34 d of exposure to sublethal Al at pH 5.2 the number of mucous cells present in the filamental epithelium was five- and fourfold greater, respectively, than the corresponding mucous cell densities of the 5.2/0 and 6.5/0 trout (Fig. 2). The number of lamellar mucous cells in 5.2/Al trout on day 5 was twice the number found in the 5.2/0 trout, but not significantly different from the 6.5/0 trout. In fish exposed to Al the mucous cell densities remained at these elevated levels until day 34. However, on day 34 the density of mucous cells within the lamellar epithelium of both 5.2/0 and 6.5/0 trout was greater such that there were no longer significant differences among the three groups. However, the total number of mucous cells (lamellar plus filamental) in 5.2/Al trout was still at least twofold higher than in either the 5.2/0 or 6.5/0 controls, even after 34 d.

Morphometric differences between the gills of control (6.5/0) and 5.2/0 trout were limited to a small but significant reduction in the blood–water diffusion distance in the acid-exposed fish on day 34 (Fig. 3). In contrast, significant changes in the branchial morphology of Al-exposed fish were found in all variables measured on day 5 when compared with both the control and 5.2/0 groups (Fig. 3). These changes amounted to a 59% increase in filament thickness, a 71% increase in the filamental epithelium thickness, a 22% reduction in lamellar height, a 30% increase in lamellar thickness, a 32% decrease in the interlamellar space, and a 33% increase in the blood–water diffusion distance compared with the 6.5/0 control group on day 5 (Fig. 3). By day 34 the blood–water diffusion distance, lamellar thickness, and interlamellar space had returned to values similar to the control and 5.2/0 groups. Nevertheless, lamellae remained shorter and filaments thicker than those of the 5.2/0 fish (but not the 6.5/0 group) even after 34 d. Apparent lamellar surface area was approximately 22% lower than in the controls on both days 5 and 34 (Table 1).

TABLE 1. Calculated values (means ± SEM (n)) for mean lamellae perimeters (μm), used as an index of apparent lamellar surface area. 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.

	6.5/0	5.2/0	5.2/Al
Day 5	323.1 ± 11.7 (4)	302.2 ± 8.7 (4)	253.1 ± 12.9*† (4)
Day 34	351.5 ± 10.2 (4)	347.1 ± 14.8 (4)	274.5 ± 15.2*† (4)

Swimming Performance

The U_{crit} of control fish swum in pH 6.5 soft water did not vary significantly over the time course of the study (U_{crit} ranged from 4.13 ± 0.21 BL·s^{-1} on day 0 to 4.48 ± 0.12 BL·s^{-1} on day 25; Fig. 4). There were no significant differences in body mass of swimming fish among the three

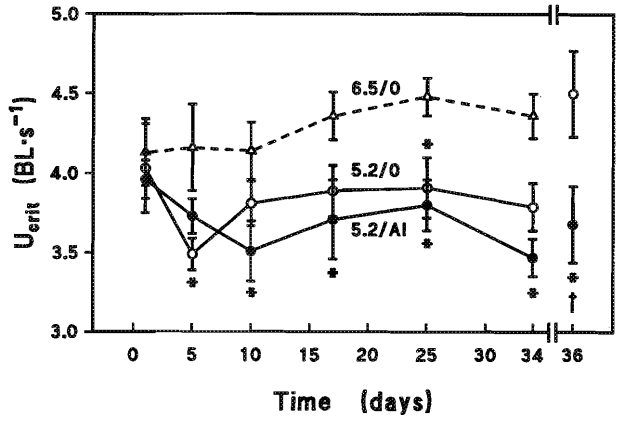

FIG. 4. Critical swimming velocities in juvenile rainbow trout at various intervals during the 36-d exposure regimes. All the control group (6.5/0) swim tests were performed at pH 6.5. From day 1 to 34 the two experimental groups (5.2/0 and 5.2/Al) were swum at pH 5.2 with *no* Al added. On day 36 the two experimental groups were swum at pH 6.5 (no Al added) and compared with the control group swum in the same medium on day 34. Means values ± SEM (n = 8) are shown. 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.

groups on any particular day, but the averages did increase slightly in all groups with time (mean values increased from 5.85 ± 0.83 to 8.59 ± 1.23 g). In contrast, fork length was relatively constant in all groups throughout the study (mean values increased from 9.0 ± 0.3 to 9.7 ± 0.3 cm).

In general, there was a gradual reduction in U_{crit} from day 1 to 10 in the 5.2/Al group when swum at pH 5.2, following which U_{crit} stabilized (Fig. 4). The 5.2/0 group showed the greatest inhibition at day 5. With the exception of day 5, the 5.2/Al fish had the lowest U_{crit}. When swum in pH 5.2 soft water, neither group showed any sign of recovery of U_{crit} during the study.

When compared with that of the control group, the U_{crit} of 5.2/0 fish was reduced by 9–16% (significant on days 5, 25, and 34), except on day 1 when the reduction was only 3%. When fish from the 5.2/0 group were subsequently swum at circumneutral pH (6.5) on day 36, their U_{crit} was found to be almost identical to the U_{crit} found for control 6.5/0 fish on day 34 (Fig. 4).

Trout previously exposed to sublethal acid plus Al (5.2/Al) tended to have (not significant) greater reductions in U_{crit} than the 5.2/0 fish (Fig. 4). Compared with the control group, the inhibition increased progressively from day 1 (5%) to day 10 (17%), thereafter stabilizing at 15–21%. In contrast with the 5.2/0 group, U_{crit} did not recover when 5.2/Al fish were swum at pH 6.5 on day 36.

There was some variation in the mean whole-body [lactate] measured postexercise over the course of the study, but no differences were observed between groups at any time. The overall mean value for all groups, from all days, for postexercise [lactate] was 8.82 ± 0.51 μmol·g^{-1} (n = 157).

Oxygen Consumption

There was a trend for basal oxygen consumption to be highest in the 5.2/Al group and lowest in the control group (Fig. 5), but none of the differences between groups were statistically significant. $Mo_{2\ max}$ was always lowest in the

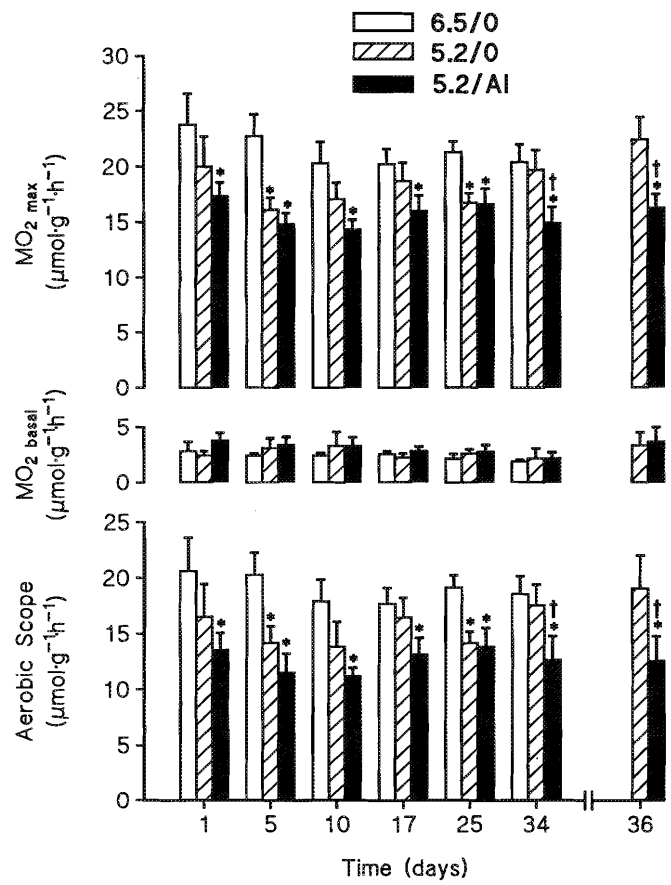

FIG. 5. Maximum and basal rates of oxygen consumption (means ± SEM, n = 8) and aerobic scope ($Mo_{2\ max} - Mo_{2\ basal}$) measured during swimming metabolism tests over the 36-d exposure regimes. See legend to Fig. 1 for further details. Asterisks indicate means significantly different ($P < 0.05$) from the 6.5/0 control group, and daggers indicate means significantly different from the 5.2/0 group.

5.2/Al group, being significantly lower than in the control group on all days measured and lower than in the 5.2/0 group on days 34 and 36. Relative to the control group, the maximum reduction of $Mo_{2\ max}$ (35%) in the 5.2/Al trout was on day 5. $Mo_{2\ max}$ was also reduced in the 5.2/0 trout on days 5 and 25, but on day 34 the $Mo_{2\ max}$ of 5.2/0 trout (swum at pH 5.2) recovered to that of the control group (swum at pH 6.5), and on day 36 when the 5.2/0 group was also swum at pH 6.5, their $Mo_{2\ max}$ was similar to that of the control group swum on day 34. In contrast, $Mo_{2\ max}$ of 5.2/Al trout remained significantly depressed when swum at this circumneutral pH on day 36.

Aerobic scope ($Mo_{2\ max} - Mo_{2\ basal}$) followed the same trend as found for the $Mo_{2\ max}$ values, but the differences between groups were more pronounced (Fig. 5). For example, when compared with the control group, the aerobic scope of the 5.2/Al trout was reduced by 43% on day 5, and even when swum in pH 6.5 soft water on day 36, it was still 36 and 34% lower than in either the control (on day 34) or the 5.2/0 group, respectively.

Discussion

Gill Morphology

The gill changes in response to pH 5.2 alone were con-

sistent with the general trend found in rainbow trout (Youson and Neville 1987; Evans et al. 1988; Audet and Wood 1993), brook trout (Tietge et al. 1988; Mueller et al. 1991), brown trout (*Salmo trutta*) (Karlsson-Norrgren et al. 1986a, 1986b), minnow (*Phoxinus phoxinus*) (Norrgren et al. 1991), and fathead minnow (*Pimephales promelas*) (Leino et al. 1990) in which histopathologies due to sublethal acid were generally mild. However, in agreement with many of these same studies, sublethal Al combined with acid caused thickening of the respiratory epithelium, hyperplasia of the filamental epithelium, and a proliferation of mucous cells (Fig. 2 and 3). In the present study the changes caused by Al were not as severe or extensive as the damage observed in brook trout exposed to higher Al (75–150 $\mu g \cdot L^{-1}$) at pH 5.2 (Mueller et al. 1991). This was also consistent with the much lower mortality we observed. Although severe abnormalities such as lamellar fusion were not prevalent, we did find substantial changes in gill morphometrics that would be expected to have a significant impact on gas exchange capacity.

All the changes observed in the 5.2/Al trout after 5 d of exposure would increase the blood–water diffusion distance and/or reduce the total gill surface area available for gas exchange. Experimentally induced reductions in functional gill area are known to cause a proportional reduction in $\dot{M}O_{2\,max}$ (Duthie and Hughes 1987). We estimated a reduction in "apparent" lamellar surface area of approximately 22% on day 34. Accordingly, the corresponding reduction in $\dot{M}O_{2\,max}$ on day 34 was very similar (26%).

The hyperplasia of mucous cells seen by day 5 in 5.2/Al trout coincided with the first positive test for acclimation to Al (see Wilson et al. 1994) and persisted throughout the study. We consider this mucous cell response to be compensatory or protective rather than pathological and to be directly related to the acclimation to Al (McDonald et al. 1991; Mueller et al. 1991). The purpose of mucocyte hyperplasia is presumably to accelerate the secretion of mucus that preferentially bind and carries away Al from the sensitive gill surface (Playle and Wood 1989, 1991; McDonald et al. 1991; Mueller et al. 1991). Conversely, the increase in blood–water diffusion distance and the reduction in "apparent" gill area after 5 d of Al exposure are more likely to be the direct result of physical damage to epithelial cells typically encountered during the acute phase of sublethal metal exposures (Mallatt 1985; McDonald an Wood 1992). The subsequent recovery of diffusion distances we observed contrasts with previous morphological studies (e.g., Tietge et al. 1988; Mueller et al. 1991; Norrgren et al. 1991). This likely reflects the small amount of initial damage inflicted by the low level of Al we used and suggests that elevated diffusion distances may not be an obligatory part of the acclimation process for Al. However, apparent gill area did not return to normal, indicating that this may be an important part of acclimation to Al. Reduced gill area may offer some advantage by minimizing the amount of branchial epithelium susceptible to the surface binding of toxic metals (Mallatt 1985; McDonald and Wood 1992), but corresponding limitations to branchial gas exchange and ultimately aerobic scope are inevitable (see below).

Swimming Performance

With the exceptions of Cleveland et al. (1989), Butler et al. (1992), and Wilson and Wood (1992), studies on the effects of acidity on swimming performance in trout have not used water pH and Ca^{2+} levels relevant to the acid-threatened waters of eastern North America and Europe. Only Wilson and Wood (1992) and the present study have addressed the chronic effects of both low pH and Al on truly aerobic swimming performance. Both studies show that chronic exposure to sublethal low pH results in a sustained impairment of U_{crit}. This tends to be further exacerbated when trout are simultaneously exposed to sublethal Al, although the additional effect of Al was not statistically significant in the present study. As no differences in postexercise [lactate] were found, we have assumed that all groups reached their true aerobic maximum.

The same general pattern of impairment was observed whether the swimming tests were performed at pH 5.2 with 30 $\mu g\ Al \cdot L^{-1}$ either present (Wilson and Wood 1992) or absent (present study), and in both cases, no evidence of recovery was observed. However, the reductions in U_{crit} were smaller (by about one third) and took longer to occur (after 5 d instead of 1 d) in the present study where swimming tests were performed in the absence of Al. Clearly the choice of external medium used during swimming influences these tests. In the natural environment, fish will be swimming in the external milieu to which they have been previously exposed, suggesting that the differences between fish chronically exposed to acid alone and acid plus Al may be greater in the wild than in these laboratory studies.

Aerobic Scope in Relation to Swimming Performance

Aerobic swimming performance in fish can be affected by changes at any level of the oxygen transport system and/or muscle contraction process. Randall and Brauner (1991) demonstrated that disturbances in the ion and water balance in muscle of coho salmon (*Oncorhynchus kisutch*) reduced U_{crit} and suggested that this occurred via decreased muscle contractility. Similar changes could be involved during the initial stages of acid/Al exposure because both experimental groups experienced pronounced whole-body ion losses over the first 5–10 d (see Wilson et al. 1994). However, both groups recovered ionic balance, so it seems unlikely that alterations at the level of the muscle contraction were involved in the lower U_{crit} values after day 25.

In terms of oxygen transport and aerobic metabolism, Brett (1958) divided environmental stresses into two categories: "limiting" stresses that reduce the maximum aerobic capacity ($\dot{M}O_{2\,max}$) and "loading" stresses that increase the cost of routine maintenance ($\dot{M}O_{2\,basal}$). The reduction of U_{crit} in trout exposed to acid alone does not appear to be related to limitations in the branchial gas exchange capacity. For example, on day 34, $\dot{M}O_{2\,max}$ was almost identical to that of the control fish, and yet, U_{crit} was impaired (13%). Because we could detect no gill morphometric changes, this indicates that the reduced U_{crit} must be due to loading rather than limiting factors. The presence of loading factors is shown by the trend for $\dot{M}O_{2\,basal}$ in 5.2/0 trout to be higher than in the controls (although this was never statistically significant). However, this is shown more clearly by comparing the log $\dot{M}O_2$ versus swimming speed regression lines for the three groups on day 34 (Fig. 6). Equating swimming performance to aerobic scope alone on day 34 is most valid because reductions in U_{crit} due to muscle contratility changes would be minimal at this time (see above). For any given swimming speed, the 5.2/0 trout required more oxygen than the control trout (the regression line for 5.2/0 was signifi-

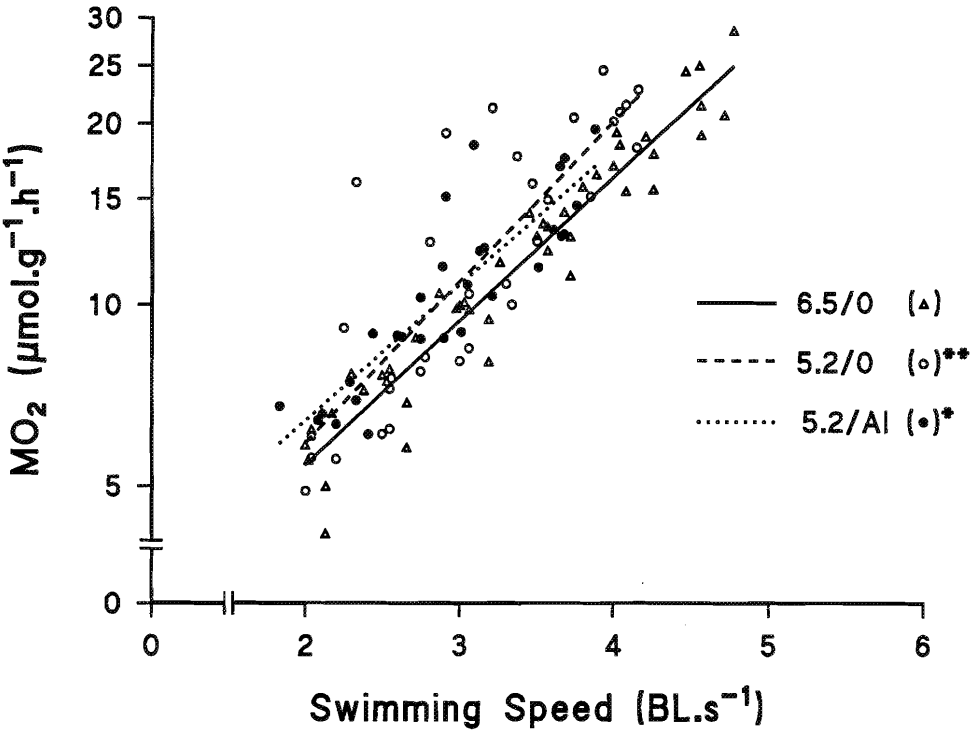

FIG. 6. Regression relationships between oxygen consumption (note log scale) and swimming speed for all three exposure groups on day 34. Group regression equations, where y = log Mo_2 and x = speed, were $y = 0.239x + 0.256$ ($r = 0.962$) in the 6.5/0 group, $y = 0.267x + 0.236$ ($r = 0.836$) in the 5.2/0 group, $y = 0.225x + 0.358$ ($r = 0.874$) in the 5.2/Al group. Multiple comparison revealed that the three slopes were not significantly different, having a common regression coefficient of 0.244. However, regression lines for both the 5.2/0 and 5.2/Al groups were significantly elevated above the control group ($P < 0.005$ and $P < 0.05$, respectively), indicating increased maintenance costs at all subcritical swimming speeds. Note also the additional depression of $Mo_{2\ max}$ in the 5.2/Al group.

cantly elevated ($P < 0.005$) relative to the control group; Fig. 6). We speculate that increased ionoregulatory costs associated with acid exposure act as a loading stress and lead to an increase in the metabolic rate needed for normal homeostatis. This loading stress thereby impairs U_{crit} by reducing the proportion of available oxygen that reaches the working aerobic muscle.

Perhaps the most interesting result with respect to loading stresses in acid-exposed trout was the complete recovery of U_{crit} when fish were swum at circumneutral pH on day 36. This suggests that their reduced swimming performance when swum at pH 5.2 was *entirely* due to the presence of increased [H^+] during the swim test itself rather than any accumulated physiological damage caused by the previous 36 d of exposure to low pH. The implication for fish in the wild is that even after prolonged exposure to acid water (uncontaminated with Al), a rapid restoration of more neutral pH (e.g., by liming) should result in an immediate recovery of aerobic swimming performance.

In contrast with acid alone, the reduction in swimming performance caused by preexposure to Al appears to be caused by a combination of both loading and limiting factors. Al-exposed trout had the highest basal metabolic rates and higher Mo_2 values at all subcritical speeds (Fig. 6), indicative of increased maintenance costs as in the 5.2/0 trout. However, limitations to the rate of oxygen uptake were also very apparent, as shown by the persistent depression of $Mo_{2\ max}$. The reduction in aerobic scope is not surprising considering the extent of the changes in gill morphology caused by preexposure to Al. Initially, limitations to oxygen uptake were probably the result of both reduced surface area and increased blood–water diffusion distances. However, by day 34 the reduced $Mo_{2\ max}$ was apparently the result solely of a smaller surface area for gas exchange, although increased mucus could additionally act as a diffusion barrier (e.g., see Ultsch and Gros 1979). Whatever the relative contributions of surface area and diffusion barrier alterations, the presence of limiting factors appears to be the major difference between 5.2/Al and 5.2/0 trout in terms of aerobic scope.

In addition to the elevated ionoregulatory costs due to acid alone, general gill repair, higher cell turnover, and mucus secretion rates are presumably other sources of loading stress faced by Al-exposed trout. The energetic cost of increased mucus defences may be considerable (Calow 1991), as is (presumably) the cost of gill repair. It is therefore somewhat surprising that their elevation of Mo_2 at subcritical swimming speeds was not substantially higher than for 5.2/0 trout. This observation implies that the increased metabolism associated with these acclimatory changes at the gills is small in comparison with that caused by the ionoregulatory stress induced by acid alone. This may be particularly relevant during swimming, as the cost of mucus secretion and gill repair is probably fixed, whereas Febry and Lutz (1987) have shown that the cost of osmoregulation in freshwater fish increases with swimming speed. Thus,

at the swimming speeds used in our tests (20–50 $cm \cdot s^{-1}$), elevated ionoregulatory costs may have masked any metabolic effects due to gill acclimatory processes.

We observed that the depression of both U_{crit} and $MO_{2\ max}$ persisted even when the 5.2/Al trout were swum at pH 6.5 on day 36. This contrasts with the complete recovery of U_{crit} in 5.2/0 trout and suggests that the morphological limitations to branchial gas exchange capacity are only slowly reversed on return to neutral Al-free water. In support, Norrgren et al. (1991) demonstrated complete recovery of gill morphology only after 36 d in Al-free water at pH 7 following exposure of minnow to sublethal acid and Al for the previous 48 d.

The structural changes at the gill associated with long-term Al exposure, which appear to be an unavoidable consequence of the acclimatory process, probably account for the differences in swimming performance when compared with trout exposed to acid alone. While chronic sublethal background levels of Al may endow endogenous fish species with increased resistance to temporary pulses of potentially lethal Al, the mechanisms involved in acclimation carry a substantial cost in terms of scope for activity. In light of these costs, the overall benefits of acclimation may be slight in terms of the animal's fitness in the wild.

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