



Swimming performance and blood chemistry in Atlantic salmon spawners exposed to acid river water with elevated aluminium concentrations

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Exposure of sexually mature pre-spawning Atlantic salmon *Salmo salar* to Fossbekk water (pH 5*2) for 7 days led to a significant reduction in critical swimming speed (U-crit) in females but not in males. Exposure to Fossbekk water +Al (as AlCl₃) for 24 h led to a significant reduction in U-crit in both males and females. In contrast to fish exposed for 7 days to Fossbekk water, fish exposed to Fossbekk+Al had accumulated much more aluminium and mucus on their gills. Losses of plasma ions were similar in both groups exposed to acid water. Blood glucose was twice as high in fish exposed to Fossbekk water for 7 days compared with fish exposed to Fossbekk+Al for 24 h. Plasma cortisol was still elevated compared with controls after exposure to Fossbekk water for 7 days. Possible mechanisms for the observed decrease in U-crit at the different exposures are discussed.

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Key words: Atlantic salmon; spawners; acid water; aluminium; swimming performance; blood chemistry.

INTRODUCTION

Acidification of lakes and rivers due to long-range transport of acidifying components has led to dramatic consequences for freshwater fish populations both in Scandinavia and eastern North America (Beamish & Harvey, 1972; Hesthagen *et al.*, 1999). Acid deposition causes a mobilization of aluminium from soil and sediments into lakes and rivers (Cronan & Schofield, 1979). At pH values between 4*7 and 5.5, toxicity is due primarily to the presence of aluminium rather than the H⁺ concentration (Baker & Schofield, 1982) and it is the inorganic monomeric (labile) forms of aluminium that are most toxic to fish (Driscoll *et al.*, 1980).

The toxicity is caused by aluminium binding to the gill epithelium thereby triggering structural and biochemical changes making the gill epithelium more permeable to ions (Exley *et al.*, 1991), increasing the blood water diffusion distance and reducing gill surface area (Wilson *et al.*, 1994). Exposure to acid water with or without elevated aluminium concentrations causes loss of plasma ions resulting in a reduced plasma volume and increased blood viscosity (Milligan & Wood, 1982; Witters *et al.*, 1990) leading to cardiovascular disturbances (Milligan & Wood, 1982; Brodeur *et al.*, 1999). Also a reduced

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blood oxygen content and an increase in metabolic rate have been reported after acid and aluminium exposure (Rosseland, 1980; Neville, 1985; Malte, 1986). Each of these factors has the potential to have negative effects on aerobic swimming performance as demonstrated in several studies (Graham & Wood, 1981; Hunter & Scherer, 1988; Ye & Randall, 1991; Butler *et al.*, 1992).

Despite the fact that toxicity of moderately low pH is dependent mainly on water aluminium concentration, only two studies have evaluated the effects of aluminium on swimming performance (Wilson & Wood, 1992; Wilson *et al.*, 1994). Juvenile rainbow trout *Oncorhynchus mykiss* (Walbaum) experienced a larger reduction in critical swimming speed (U-crit) when exposed to acid water with elevated aluminium concentration compared with exposure to acid water alone. Also, sexual maturation affects osmoregulation and calcium homeostasis in salmonids (Persson *et al.*, 1998). Calcium is important in regulating gill permeability thereby preventing loss of plasma ions in freshwater. Possible changes in gill calcium content combined with a reduced Na⁺-K⁺-ATPase activity (Persson *et al.*, 1998) could make sexually mature salmonids more sensitive to acid water compared with immature fish and Atlantic salmon spawners are sensitive to acid water with elevated aluminium levels (Skogheim *et al.*, 1984).

The purpose of the study was to examine the effect of acid river water with natural and elevated aluminium concentrations on swimming performance and osmoregulation in sexually mature Atlantic salmon. Sexual differences in hypoosmoregulatory capacity, haematology and prolactin secretion after transfer to freshwater have been reported in other salmonids (Hirano *et al.*, 1985; McCormick & Naiman, 1985; Miguel *et al.*, 1988) so possible differences were examined between males and females in acid tolerance.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Two-year-old adult Atlantic salmon of the Suldal strain (40*0 - 55*5 cm *Lt*, 726-2060 g), first generation derived from wild ancestors, were cultured at NINA'S research station in south-western Norway. The fish had been kept in sea water since the smolt stage and were moved to freshwater (River Imsa) at the beginning of September. The fish were not fed after transfer to freshwater. Both males and females showed breeding colouration and males had developed breeding teeth and the characteristic kype. The presence of eggs in females and sperm in males indicated that these fish would have spawned in the autumn if given the opportunity. The swimming performance tests were carried out from late September until the middle of November.

EXPOSURE CONDITIONS

Fish were exposed to three different water qualities. A control group (20 fish) was exposed to River Imsa water (pH 6*5-6*7). A second group (20) was exposed for 7 days to water from the moderately acidified brook Fossbekkten (pH 5*0-5*2). A third group (20) was exposed for 24 h to water from Fossbekken where extra aluminium had been added in the form of AlCl₃. This water was prepared at least 20 h before use to avoid the presence of highly toxic unstable Al polymers (Rosseland *et al.*, 1992; Poleo *et al.*, 1994). The exposure tanks were 1000l. Fifteen fish from each group were tested in the swim speed chamber. In addition, blood and gill samples were taken from three males and two females of each group that had not undergone swim-speed trials (non-exercised fish). The fish were tested for swimming ability in the same water quality to which they had been

TABLE 1. Water chemistry and aluminium fractions of water from Imsa River, Fossbekk River and Fossbekk River with extra aluminium added. Values are means +/- S.E. (n=8, oxygen: n = 30)

	Imsa	Fossbekk	Fossbekk+Al
pH	6*71+/-0*04	5*15+/-0*02	5*24+/-0*09
Oxygen content (mg l ⁻¹)	9*47+/-0*35	11*20+/-1*07	10*53+/-0*86
Temperature (°C)	10*4+/-0*95	9*9+/-0*18	8*9+/-0*60
Turbidity (FTU)	0*69+/-0*14	1*21+/-0*07	1*9+/-0*10
Colour (mg Pt l ⁻¹)	13*50+/-0*80	32*75+/-1*03	30*23+/-2*27
Conductivity (uS cm ⁻¹)	72*46+/-0*16	48*08+/-0*41	51*37+/-1*56
Alkalinity (uekv l ⁻¹)	142*86+/-2*02	3*32+/-0*45	8*50+/-3*18
Ca (mg l ⁻¹)	3*66+/-0*03	1*07+/-0*01	1*09+/-0*04
Nitrate (ug l ⁻¹)	658*3+/-16*23	47*0+/-3*22	60*5+/-7*18
T-Al (ug l ⁻¹)	50*0+/-12*37	237*1+/-7*07	310*8+/-12*72
Tm-Al (ug l ⁻¹)	13*4+/-0*67	111*8+/-2*47	142*5+/-14*8
Om-Al (ug l ⁻¹)	13*0+/-0*71	78*6+/-2*09	86*3+/-5*02
Im-Al (ug l ⁻¹)	0*4+/-0*39	33*1+/-1*06	56*2+/-11*0
Pc-Al (ug l ⁻¹)	36*6+/-12*06	125*4+/-6*36	162*2+/-13*82

T-Al, Total aluminium content; Tm-Al, total monomeric aluminium; Om-Al, organic monomeric aluminium; Im-Al inorganic monomeric aluminium; Pc-Al, polymeric colloidal aluminium.

exposed. For Imsa and Fossbekk water this was done by connecting the tubes on the swim speed chamber to a tap. It was a flow-through open system, with water coming in at one end of the chamber and being discharged at the other, so avoiding problems with depletion of oxygen and accumulation of waste products. For the water with extra Al added, a closed system was used. Water was supplied to the chamber from a reservoir of 2500l by an external pump and led back to the reservoir in tubes connected to the chamber. This circulation of water through the system helped to aerate the water. Three fish were tested with the same water before the reservoir tank was drained and refilled. Dissolved oxygen concentration and pH were measured at regular intervals. Oxygen levels ranged from 8 to 11 mg l⁻¹, which is much higher than the critical oxygen concentration of 4*8 mg O₂ l⁻¹ that induces a reduction in U-crit in Atlantic salmon (Kutty & Saunders, 1973).

Throughout the experimentation period, water samples were collected both from the exposure tanks and the reservoir, as well as from pure Fossbekk and Imsa water (Table I). Different forms of aluminium were identified using Driscoll's method for fractionating total water aluminium into organic-monomeric, inorganic-monomeric and colloidal Al (Driscoll, 1984). All samples were analysed by pyrecathechol violet (PVC) method. The fractionation was done, using Amberlite 120 mesh size, with a mixture of 1% H+ and 99% Na+. The monomeric fractions were analysed on untreated samples, while T-Al was analysed on samples after 48 hours acidification to 0*1 M with HCl. The analyses were performed at NINA'S laboratories.

SWIMMING SPEED TESTS

The swimming performance trials were conducted using a Blazka-type respirometer characterized by a tube within a tube design (Booth *et al.*, 1997). The total volume of the chamber was 120l. No corrections for the blocking effect that arises when fish are swimming in a narrow water channel (Smith *et al.*, 1971) were made because none of the fish used in the experiments had a cross sectional area >10% of the area of the inner tube.

Before the swim speed tests, the fish were acclimated to the swim speed chamber for 2 h at a resting speed of c. 1 L s⁻¹. The swim speed test was an increased velocity test. Velocity increments were 0*2 ms⁻¹ and the time interval between velocity increments was

10 min as recommended by Dahlenberg *et al.* (1968) when U-crit alone is needed (Beamish, 1980; Hunter & Scherer, 1988). The increments were continued until the fish were unable to hold their position in the water channel. When the side of a fish touched the grid at the back of the chamber, the velocity was lowered and then increased rapidly again. When this happened twice during a period of 4 min, the fish was considered fatigued and the swim trial was stopped. Critical swimming speed (U-crit) was calculated according to Brett (1964). Differing body length was corrected for by expressing U-crit as bodylengths per second ($L s^{-1}$).

BLOOD AND GILL SAMPLES

After the swim trials, fish were anaesthetized using a 5 mg l^{-1} solution of methomidate (Marinil™, Wildlife labs., Fort Collins, CO, U.S.A.). A blood sample was taken by caudal puncture with a heparinized syringe. Gill samples were taken from the second gill arch on the left side. Blood samples were analysed for content of glucose, chloride, sodium and cortisol. Immediately after sampling the glucose concentration was measured on whole blood using a Medisense Precision Q.I.D. sensor. Haematocrit was determined using a compur microspin centrifuge (5 min, 1400 g). Then the sample was centrifuged in a Hettich EBA 85 blood centrifuge (5 min, 3000 g) and the plasma frozen. Plasma chloride concentrations were measured by coulometric titration using a CMT 10 chloride titrator (Radiometer, Copenhagen). Plasma sodium concentrations were determined using a FLM 3 Flame Photometer (Radiometer, Copenhagen). Plasma cortisol concentrations were estimated by radioimmunoassay using a liquid scintillation counter from Packard (TRI-CARB 1900 TR). The method is described by Simensen *et al.* (1978) and modified for fish by Olsen *et al.* (1992). Detection limit was 1.33 ± 0.73 nM. Intra-assay variation was $<4.2\%$ and inter-assay variation was $<8.3\%$. Non-specific binding varied from 0.6 to 2.0% of the total activity. Recovery was 93, 96, 100 and 97% at 4, 17, 34 and 69 nM of cortisol, respectively.

The gill samples were frozen immediately and analysed for total aluminium content. The gills were freeze-dried, weighed (0.02 g gill mass) and digested in a solution of 14 molar HNO_3 , (1 ml) and concentrated H_2O_2 (2%) before dilution 1:9 with Millipore water. The aluminium content in the digests was measured by inductive coupled plasma emission spectroscopy (ICP-AES).

STATISTICAL ANALYSIS

A two-tailed Mann-Whitney U-test for non parametric data (SPSS for Windows, version 8.0) was used to test for difference among the groups. Spearman's rank correlation coefficient was used to determine the linear relationship between variables. A P value <0.05 was considered a significant difference in all statistical tests.

RESULTS

WATER CHEMISTRY

Imsa River water contained $50 \mu g l^{-1}$ aluminium, but due to the high pH, practically none of it was present as inorganic monomeric aluminium (Table I). Fossbekk River water had a total aluminium content of $237 \mu g l^{-1}$ of which $33 \mu g$ was inorganic monomeric aluminium. Adding extra aluminium to Fossbekk water increased the total aluminium concentration to $311 \mu g l^{-1}$ and the inorganic monomeric fraction to $56 \mu g l^{-1}$.

BLOOD CHEMISTRY

Imsa water

Exercise results from male and female Atlantic salmon exposed to Imsa water showed that females had a higher blood glucose level [$P=0.039$; Fig. 1(a)] and a lower haematocrit than males [$P=0.038$; Fig. 1(b)]. Plasma chloride and sodium

and cortisol were at the same level for males and females [Fig. 1(c), (d), (e), respectively]. Comparisons between exercised and non-exercised fish were made with pooled data from males and females in both groups at all three exposures. Exercise did not cause any significant changes in the parameters measured.

Fossbekk water

Fish exposed to Fossbekk water for 7 days and exercised, experienced several changes in blood chemistry compared with exercised fish exposed to Imsa water. Blood glucose increased by 158% in females ($P=0*004$) and by 187% in males ($p=0*001$) [Fig. 1(a)]. Blood glucose was still higher in females ($P=0*032$). Plasma cortisol [Fig. 1(e)] increased by 10 and 28% in exercised males and females, respectively, but was not significantly higher than in the control group. As a result females had a significantly higher cortisol level in their blood than males ($P=0*037$). Plasma cortisol was correlated positively with glucose ($r=0*52$, $P=0*05$, $n=15$). Levels of plasma chloride and sodium [Fig. 1(c), (d)] were lower than in exercised fish exposed to Imsa water. However, the decrease in sodium and chloride compared with fish in Imsa water was significant only for females ($P=0*004$ and $0*016$). Losses of sodium and chloride were correlated negatively with the observed increase in glucose ($r = -0*72$, $P = 0*003$, $n = 15$ and $r = -0*75$, $P=0*001$, $n=15$, respectively). Haematocrit [Fig. 1(b)] was almost identical for exercised males and females due to a slight increase in haematocrit in females. Exercise did not cause any significant changes in blood chemistry. Compared with non-exercised fish exposed to Imsa water there was an increase in blood glucose and a reduction in plasma sodium ($P=0*015$).

Fossbekk water + Al

Exposure for 24 h to Fossbekk water with extra aluminium added (Fossbekk+Al) also led to an elevation of blood glucose in exercised fish [Fig. 1(a)] compared with the control group ($P=0*002$), but not as much an elevation as exposure to Fossbekk water. Blood glucose was lower than after 7 days exposure to Fossbekk water for both males and females ($P=0*021$ and $0*012$, respectively). Levels of plasma sodium and chloride in exercised fish [Fig. 1(c), (d)] were lower than in the control group in Imsa water ($P=0*003$ and $0*006$), but were not significantly different from the 7 day exposure to Fossbekk water. While males showed no significant increase in haematocrit [Fig. 1(b)], females did have a significantly higher haematocrit (31% increase) compared with the control group to Imsa water ($P=0*022$). Females also experienced a more pronounced loss of plasma ions than males. Plasma chloride and sodium concentrations [Fig. 1(c), (d)] were reduced by 10 and 11% and by 5 and 3% for exercised females and males, respectively, compared with fish in Imsa water. There was a negative correlation between haematocrit and loss of chloride and sodium ($r = -0*87$, $P < 0*001$, $n=15$, $r = -0*62$, $P=0*014$, $n=15$, respectively). Plasma cortisol [Fig. 1(e)] levels were elevated in exercised fish compared with both Imsa and Fossbekk water. Plasma cortisol was correlated negatively with the loss of chloride ($r = -0*61$, $P=0*036$, $n=15$). In males, plasma cortisol was significantly higher compared with both the control group ($P=0*012$) and with the one week exposure to Fossbekk water ($P=0*009$). For females the increase in plasma cortisol compared with the two other exposures was not significant, but

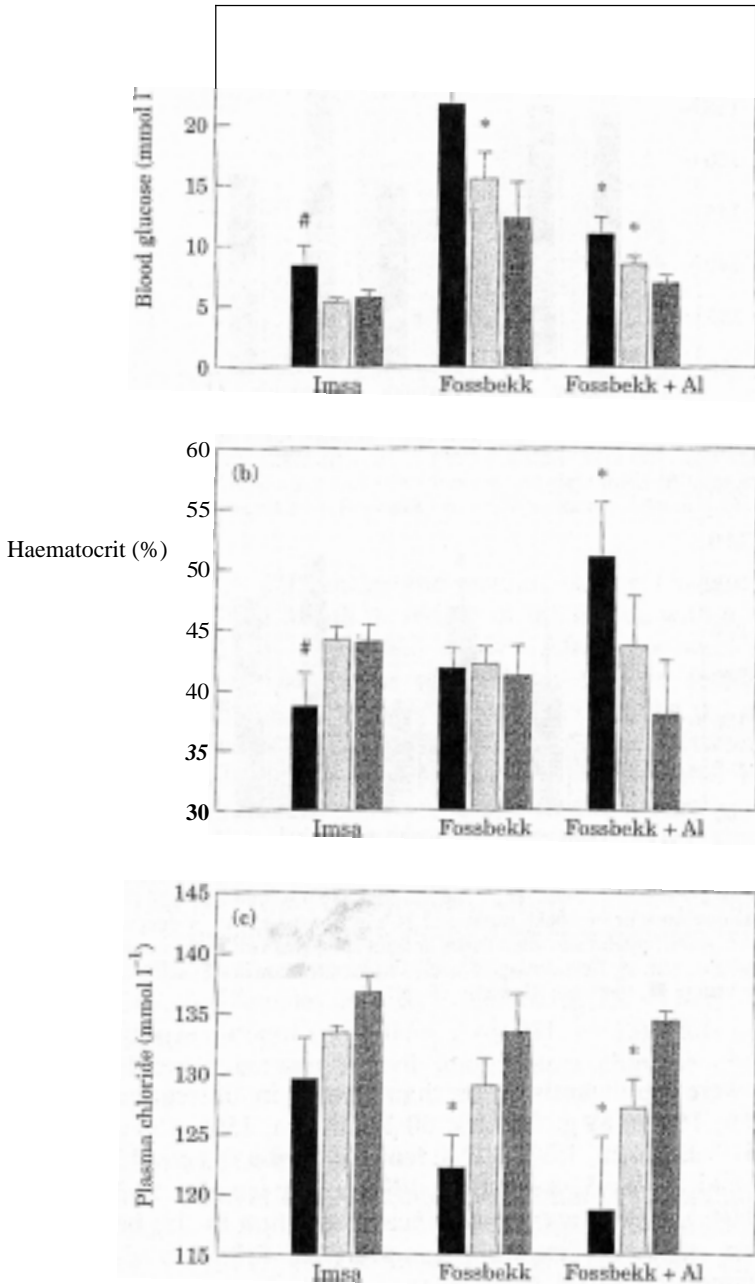


FIG. 1. (a-c).

females had a higher plasma cortisol level than males in Imsa water. In contrast to the two previous exposures, there were no longer any significant differences in blood chemistry between males and females. Exercise caused a significant decrease in plasma chloride ($P=0.009$). Non-exercised fish exposed to Fossbekk + Al had lower plasma sodium ($P=0.008$) compared with fish in Imsa water.

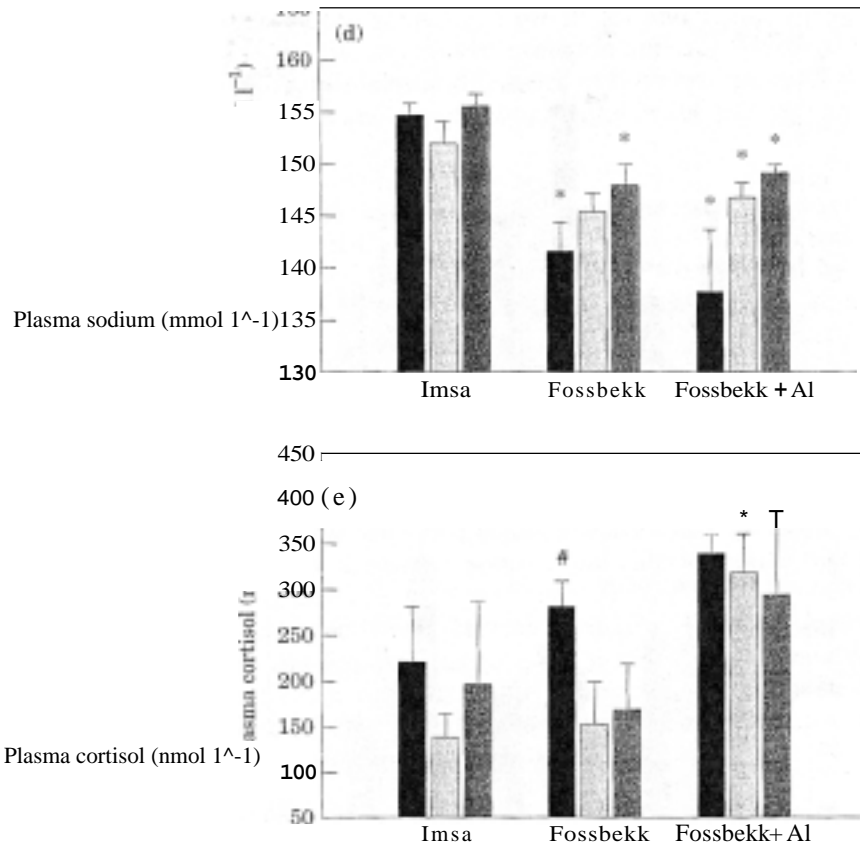


FIG. 1. Blood glucose (a), haematocrit (b), plasma chloride (c), sodium (d) and cortisol (e) for salmon (males and females) that had been swim tested and fish that had not been exercised (NE, $n=5$). The exposure time was one week in Fossbekk water and 24 h in Fossbekk+Al. Values are means+S.E. The sample size was seven females and eight males in Imsa and Fossbekk water and six females and nine males in Fossbekk+Al. #, Females significantly different from males; *, significantly different from fish in Imsa water. g Females; 1 males; ■, NE.

SIZE

Exercised males were significantly larger than females in all treatments (Imsa: males: 52.2 ± 0.7 cm, 1654 ± 89 g; females: 50.2 ± 0.5 cm, 1566 ± 93 g; $P=0.05$; Fossbekk: males: 51.3 ± 0.6 cm, 1597 ± 79 g; females: 49.9 ± 0.3 cm, 1562 ± 83 g; $P=0.05$; Fossbekk + Al: males: 52.8 ± 0.6 cm, 1796 ± 62 g; females: 50.3 ± 0.8 cm, 1519 ± 128 g; $P=0.041$). There was no significant variation in size between the exposures for either males or females. Non-exercised fish were significantly smaller than exercised fish in all three treatments (40.0 ± 1.3 cm, 790 ± 26 g; $P=0.001$; 48.4 ± 2.8 cm, 1387 ± 177 g; $P=0.049$; and 41.9 ± 7.4 cm, 839 ± 228 g; $P=0.025$, respectively). No significant correlations were observed between length and weight and any of the parameters measured in the present study.

CRITICAL SWIMMING SPEED

There was no significant difference in critical swimming speed (U-crit) ($L s^{-1}$) between males and females in Imsa water ($P=0.81$, Fig. 2). Mean U-crit \pm S.E.

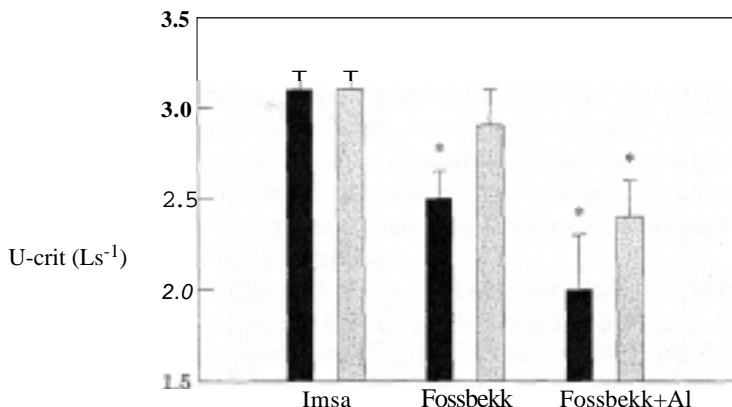


FIG. 2. Critical swimming speed for males (♂) and females (♀) in Imsa water, after 7 days' exposure to Fossbekk water and after 24 h exposure to Fossbekk water with extra aluminium added. Values are means \pm S.E. The sample size was seven females and eight males in Imsa and Fossbekk water and six females and nine males in Fossbekk+Al. *, Significantly different from fish in Imsa water.

was $3.1 \pm 0.1 \text{ L s}^{-1}$ for both males and females. Critical swimming speed was not correlated with either length or weight of the fish or with any of the blood parameters measured in the present study. After one week's exposure to Fossbekk water mean U-crit was reduced by 19% in females ($P=0.038$), compared with fish in Imsa water. Mean U-crit was 2.9 ± 0.2 and $2.5 \pm 0.2 \text{ L s}^{-1}$ for males and females, respectively, but the difference between the sexes was not statistically significant ($P=0.203$). U-crit was correlated negatively with plasma cortisol ($r = -0.67$, $P=0.007$, $n=15$) and glucose ($r = -0.70$, $P=0.004$, $n=15$). Exposure for 24 h to Fossbekk water with extra aluminium added (Fossbekk+Al) led to a further reduction in U-crit for both males and females. Again males performed better than females, with mean U-crit being 2.4 ± 0.2 and $2.0 \pm 0.3 \text{ L s}^{-1}$, respectively, although the difference between the sexes was not significant ($P=0.32$). Compared with the control group, mean U-crit was reduced by 34% for females and by 23% for males. U-crit was correlated negatively with haematocrit ($r = -0.56$, $P=0.031$, $n=15$) while a positive correlation was observed between U-crit and plasma chloride ($r=0.65$, $P=0.01$, $n=15$).

GILL ALUMINIUM CONTENT

Gill aluminium content was related to the amount of aluminium present in the water (total Al: $r=0.81$, $P<0.001$, $n=45$, inorganic monomeric Al: $r=0.58$, $P=0.006$, $n=45$). Fish exposed to Imsa water had practically no aluminium on their gills (Fig. 3). Fish exposed to Fossbekk water for 7 days had significantly more aluminium on their gills ($P=0.003$), mean aluminium content being $78.0 \pm 26.9 \mu\text{g g}^{-1}$ dry weight in exercised fish. Non-exercised fish had less aluminium on their gills than exercised fish ($P=0.045$). Fish exposed for 24 h to Fossbekk+Al had more aluminium on their gills than fish exposed one week to Fossbekk water ($P=0.001$). Mean aluminium content was $217.7 \pm 112.3 \mu\text{g g}^{-1}$ dry weight. Mucus was clearly visible on the gills of these fish and coughing was observed frequently in the group exposed to Fossbekk+Al in contrast to fish

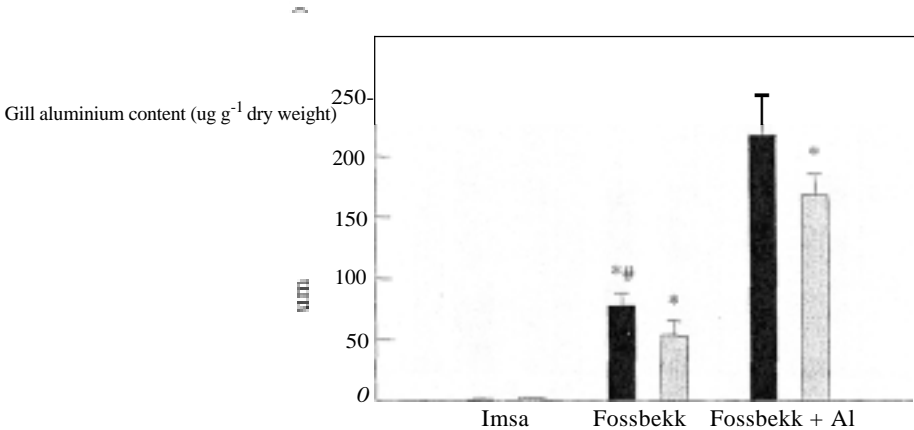


FIG. 3. Gill aluminium content in exercised fish (males+females, $n=5$ in Imsa water, $n=6$ in Fossbekk water, $n=10$ in Fossbekk+Al) and in fish that had not been exercised (NE, $n=5$ at all three exposures). The exposure time was one week in Fossbekk water and 24 h in Fossbekk+Al. Values are means+S.E. #, Exercised fish significantly different from fish that had not been exercised (NE); *, significantly different from fish in Imsa water. \bar{g} , Males+females; 1 NE.

exposed to Fossbekk water for one week. Gill aluminium content was slightly but not significantly higher for exercised fish compared with non-exercised fish. There were no significant differences in gill aluminium content between exercised males and females at any of the exposures.

DISCUSSION

EFFECTS ON U-CRIT AND BLOOD CHEMISTRY

In the present study, exposure of sexually mature Atlantic salmon to acid water with elevated aluminium concentrations led to a reduction in swimming performance. Aerobic swimming performance in fish is affected by muscle contractility and the amount of oxygen available for aerobic muscle activity. Any environmental factor that reduces the aerobic scope for activity either by reducing gas uptake or by increasing the costs of routine maintenance can lead to a reduced swimming ability. The increased levels of blood and muscle ammonia, reduced muscle glycogen stores and reduced muscle ion concentrations after exposure to acid water can also have a negative effect on swimming performance (Day & Butler, 1996). An increase in metabolic rate has been reported after exposure to acid water with or without aluminium (Rosseland, 1980; Malte, 1986; Butler *et al.*, 1992). In contrast, Wilson *et al.* (1994) found that aerobic scope for activity was reduced due to a reduction in the maximum rate of oxygen consumption (MO_{2max}) while basal metabolic rate was not significantly elevated. However, basal metabolic rate was not measured directly but estimated by extrapolation beyond the measured range. The decrease in oxygen phase uptake was due most likely to morphometric changes in the gill epithelium resulting in a 30% reduction in gill surface area. The magnitude of the decrease in U-crit in the present study was similar, while losses of plasma ions were « the 30% reduction reported by Wilson & Wood (1994). Fish exposed for

one week to Fossbekk water had very high levels of glucose in their blood, indicating a severe stress response. The negative correlation between blood glucose and losses of sodium and chloride could indicate that energy reserves were mobilized to satisfy increased metabolic costs associated with osmoregulation. An increased basal metabolism would limit the aerobic scope for activity by reducing the amount of oxygen available for activity. The negative correlation observed between critical swimming speed and plasma cortisol and glucose also supports the idea that an increased metabolic rate could have contributed to the decrease in U-crit at this exposure.

Both the aluminium concentration in the water and on the gills were much higher at both acid exposures in this study than in the study by Wilson *et al.* (1994). Therefore, one can assume that oxygen uptake was compromised in the present study contributing to the decrease in U-crit. Probably, impairment of oxygen uptake was of greater importance in fish exposed to Fossbekk+Al because gill aluminium content was higher and mucus was clearly visible on the gills. Mucus acts as a diffusion barrier for oxygen uptake (Ultsch & Gros, 1979).

The decrease in critical swimming speed was larger after exposure to Fossbekk water with extra aluminium added for 24 h (Fossbekk+Al) than after one week's exposure to pure Fossbekk water. The most probable explanation is the higher aluminium concentration in the water, but the shorter exposure time in Fossbekk+Al could be of importance also. Acclimation to aluminium has been accompanied by some recovery of U-crit after 7–10 days (Wilson & Wood, 1992; Wilson *et al.*, 1994). Therefore it is possible that a 24 h exposure to Fossbekk water would result in a larger depression of U-crit than the 7 days' exposure. Even if the fish had not restored their ionoregulatory status after 7 days, there could have been a reduction in gill aluminium and some repair of gill damage resulting in some recovery of U-crit. A 24 h exposure to Fossbekk water or a 7 day exposure to Fossbekk+Al would have quantified the effect of the extra aluminium, but the limited number of fish made this impossible.

EFFECTS OF EXERCISE

In accordance with the findings of Butler *et al.* (1992) exercise caused only minor changes in blood chemistry. However, exposure to acid water with elevated aluminium levels did have a greater effect on exercised than on resting fish. This is not surprising because exercise leads to an increase in blood pressure and functional gill surface area (Jones & Randall, 1978). The increased gill permeability caused by acid exposure would escalate ionic losses when fish are exercised compared with when they are resting. Also, exercised fish had a higher gill aluminium content than non-exercised fish after 7 days' exposure to Fossbekk water indicating that additional aluminium accumulated during the swimming trials. This implies that ventilatory volume and level of activity is important for the amount of aluminium deposited on the gill surface. Neville (1985) reported similar findings in rainbow trout, where the most active fish had accumulated more aluminium on their gills and suffered a higher mortality compared with less active fish. This demonstrates the importance of assessing potential effects of environmental pollutants under realistically physiological conditions.

SEX DIFFERENCES

In contrast to previous studies concerning swimming performance in acid water this study has revealed some interesting differences in blood chemistry and physiological performance between male and female Atlantic salmon. Exercised females in Imsa water had a lower haematocrit than males, which supports the findings of Miguel *et al.* (1988) in rainbow trout. Blood glucose was higher in females. After 7 days' exposure to Fossbekk water both glucose and plasma cortisol was significantly higher in females than in males. Losses of plasma sodium and chloride compared with Imsa water were significant only in females. Females also suffered a significant reduction in U-crit compared to fish in Imsa water while this was not the case for males. The higher plasma cortisol and blood glucose levels in females could indicate a more severe stress response in females than in males. If females experienced a greater reduction in aerobic scope compared with males, this would explain the larger effect on U-crit in females.

The elevated haematocrit observed often after exposure to acid conditions (Milligan & Wood, 1982; Butler *et al.*, 1992) is caused by osmotic swelling of red blood cells due to reduced plasma osmolarity, reduced plasma volume and adrenergic release of erythrocytes from the spleen. As a result, blood viscosity increases (Milligan & Wood, 1982; Witters *et al.*, 1990). Butler *et al.* (1992) suggested that the increased blood viscosity could cause a disruption in the local capillary blood supply to the muscles and impair the supply of oxygen and glucose to the working muscle during swimming. The only sign of haemoconcentration in the present study was a 30% increase in haematocrit in females after 24 h exposure to Fossbekk+Al compared with fish in Imsa water. A negative correlation between U-crit and haematocrit could indicate that an increased blood viscosity might be responsible for the more severe effect on U-crit in female fish. An increase in haematocrit from 40 to 50% increases blood viscosity by 50% in rainbow trout (Wells & Weber, 1991).

The reason for the differential response to acid and aluminium exposure between males and females is not known. Due to the low sample size in the non-exercised groups it is not possible to say whether the observed differences in blood chemistry between males and females would be apparent in resting fish or if they were induced by strenuous exercise. Gill aluminium content is an unlikely explanation because no significant differences between the sexes were observed. Sexual differences in osmoregulatory capacity have been observed in brook trout *Salvelinus fontinalis* (Mitchill). Sexually mature males were more sensitive to salinity stress than mature females (McCorinick & Naiman, 1985). The authors suggested that this was due to a difference in gill permeability because no significant difference in Na⁺-K⁺-ATPase activity was found between males and females. Hirano *et al.* (1985) reported a four-fold higher increase in plasma prolactin levels in mature female chum salmon *Oncorhynchus keta* (Walbaum) than in males after transfer from seawater to freshwater. Plasma prolactin levels increase as a response to reduced plasma osmolarity and stress (Avella *et al.*, 1991). The higher prolactin secretion in females could indicate a more permeable gill epithelium in females, which would make females more susceptible to acid and aluminium exposure.

In conclusion, this study has shown that a 7 day exposure to water with a pH of 5.2 and a total aluminium content of 237 $\mu\text{g l}^{-1}$ (inorganic monomeric aluminium was 33 $\mu\text{g l}^{-1}$) led to a significant reduction of U-crit in females but not in males compared with fish exposed to pH 6.7 (total aluminium 50 $\mu\text{g l}^{-1}$ no inorganic aluminium) while a 24 h exposure to pH 5.2 (total aluminium 330 $\mu\text{g l}^{-1}$, inorganic monomeric aluminium 59 $\mu\text{g l}^{-1}$) caused a significant reduction in U-crit in both males and females. There were indications, but no conclusive evidence, of a larger sensitivity to acid water with elevated aluminium in females than in males. However, the sample size in the present study was limited.

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