

# The effects of iron, humic acids and low pH on the gills and physiology of Brown Trout (*Salmo trutta*)

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One-summer-old brown trout (*Salmo trutta*) were exposed to iron and humic acids at pHs 5 and 6 for two to three days in the laboratory. The oxygen consumption of the fish was measured after the exposure whereafter the gill and blood samples were taken. Brown trout gills were damaged in the exposure to iron at both pHs, more so at pH 5. Morphometric analysis showed that the fusion of the lamellae and hypertrophy of the epithelial cells significantly increased the diffusion distance across the gills. The precipitation of iron on the gills may also have increased the diffusion distance which had a negative effect on the oxygen uptake of the fish. Gill damage also impaired ion regulation seen as low  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations in the plasma. The blood glucose concentration increased, indicating stress in the fish. Humic acids in the water containing iron had an ameliorating effect on the ion regulation as well as the oxygen uptake but they did not completely prevent hypertrophy in the gills.

## 1. Introduction

Clear-cutting and subsequent scarification of the soil may cause the leaching of organic material, nutrients and metals which affect water quality in the drainage area (Ahtiainen 1992). In the most heavily manipulated sites the total iron concentration of the water can reach even  $30 \text{ mg l}^{-1}$  (Ahvonen et al. 1993). The effects of iron on fish have been previously studied mainly concerning the effects of mining (for references, see Steffens et al. 1992) and, the role of iron as a toxicant in rivers has been suspected. Iron is known to precipitate on the gills and eggs of fish (Larson & Olsen 1950, Smith et al. 1973, Bagge & Ilus 1975, Lehtonen 1976, von Lukowicz 1976, Vuorinen 1984, Oulasvirta 1990)

which most likely hinders oxygen uptake. In an acidic environment, iron dissolves as ferrous hydrogen carbonate but in the presence of oxygen and at a higher pH it is oxidised to  $\text{Fe}^{3+}$  which becomes precipitated as ferric hydroxide (Stumm & Lee 1960). Ferrous iron ( $\text{Fe}^{2+}$ ) is considered to be more toxic to fish than the ferric ( $\text{Fe}^{3+}$ ) form (Decker & Menendez 1974). Because the pH at the gill micro environment may considerably differ from that of the surrounding body of water (Playle & Wood 1989), the precipitation of metals on gills is possible.

Using inductively coupled plasma spectrophotometry, Weatherley et al. (1991) detected iron and aluminium in the gills of brown trout which had been placed in cages downstream from a liming

station in an acidic river. They thought that iron was a contributory factor in the mortalities of the caged brown trout along with aluminium. Andersson & Nyberg (1984) also caged brown trout in rivers during the snow melt. Fish started to die straight after the appearance of the melt water in the river when the pH was still above 5.5. The metal concentrations ( $\mu\text{g l}^{-1}$ ) in the water were Al 90–160; Fe 550–1200, and Mn 80–180. Iron may have contributed to the mortalities in that experiment, too. Despite its possible role in fish kills, there are practically no studies on iron-induced gill damage. Brenner et al. (1976) exposed common shiner (*Notropus cornutus*) to  $3 \text{ mg l}^{-1}$  ferric hydroxide for eight weeks and observed no mortalities or gill damage. In banded tilapia (*Tilapia sparrmanii*), iron caused hyperplasia and necrosis of the secondary lamellae (Wepener 1990). The changes in the haematological indices of tilapia suggested difficulties in the oxygen uptake (Wepener et al. 1992). We studied the effects of iron on the gills of brown trout in an acute laboratory experiment at two pH values with and without added organic material (humic acids). The iron concentration and pH in the experiment are common in the regions of timber management operations in Finland (Ahvonen et al. 1993).

## 2. Material and Methods

### 2.1. Exposure

One-summer-old brown trout (*Salmo trutta*) (weight  $22.7 \pm 1.4 \text{ g}$ ; six fish in each group) were exposed to iron at a concentration of  $2 \text{ mg l}^{-1}$  ( $\text{FeCl}_3 : \text{FeSO}_4 = 1 : 1$ ) with and without humic acids (Fluka 53680;  $15 \text{ mg l}^{-1}$ , abbreviated as

'HA' in the text) at pHs 5 and 6 (Table 1). The test waters were made with ion-exchanged water to the final ion concentrations ( $\text{mmol l}^{-1}$ ) of  $\text{Na}^+$ , 0.064;  $\text{K}^+$ , 0.011;  $\text{Cl}^-$ , 0.072;  $\text{Ca}^{2+}$ , 0.050;  $\text{Mg}^{2+}$ , 0.032, and  $\text{SO}_4^{2-}$ , 0.052 by adding appropriate salts (*p.a.* quality). The water volume in the exposure tanks was 60 litres, 80% of which was replaced daily and continuously aerated during the experiment. The duration of the exposure was three days but only two at "pH 5 + Fe" due to fish mortalities. Tests were carried out at  $10^\circ\text{C}$  with a 12 : 12 hour light/dark cycle. Fish were acclimated to that temperature and light rhythm one month prior to the experiment. Water samples were analysed according to the SFS standards.

### 2.2. Oxygen uptake

At the end of the exposure, the oxygen uptake of the fish was measured in an acrylic chamber ( $V = 514 \text{ ml}$ ) the water of which was mixed with a magnetic stirrer. The oxygen partial pressure inside the chamber was monitored with a polarographic oxygen electrode (Radiometer 1302 oxygen electrode) connected to the oxygen meter (Strathkelvin Instruments Oxygen meter model 781). The change in the oxygen partial pressure was monitored for about fifteen minutes after the water flow into the chamber was closed off. During that time the fish used at most 24% of the oxygen in the water. The oxygen uptake of the fish was calculated by the formula:

$$V_{\text{O}_2} (\text{mlO}_2 \text{ kg}^{-1} \text{ h}^{-1}) = (\alpha * \Delta P_{\text{O}_2}) * (1/t) * V / M,$$

where  $\alpha$  = oxygen solubility ( $\text{mlO}_2 \text{ l}^{-1} \text{ torr}^{-1}$ ),  $\Delta P_{\text{O}_2}$  = oxygen partial pressure difference in the chamber (torr),  $t$  = measuring time (hours),  $V$  = water

Table 1. Water qualities in the exposure. 'Start' = measured pH in the stock solution, 'End' = measured mean test-water pH after 24 hours of exposure ( $n = 4$ ). HA =  $15 \text{ mg l}^{-1}$  humic acids. Iron concentrations and colour were measured after 24 hours of exposure, before the water replacement ( $n = 4$ ).

	Measured pH		Fe, $\text{mg l}^{-1}$		Colour $\text{mg Pt l}^{-1}$
	Start	End	Nominal	Measured	
"pH 5 + Fe"	5.00	5.56	2.0	1.7	17
"pH 5 + Fe + HA"	5.00	5.46	2.0	1.7	160
"pH 6 + Fe"	6.00	5.99	2.0	0.8	10
"pH 6 + Fe + HA"	6.00	5.81	2.0	1.7	175
Control	6.33	6.33	0	0	0

volume inside the chamber (l), M = fish mass (kg).

The fish were not allowed to recover in the chamber before the oxygen uptake measurement. Therefore, the results from the oxygen measurements should represent the "submaximal" oxygen uptake of a stressed fish. The rationale of this procedure was to emphasize the effects of possible gill damage in the oxygen uptake. The oxygen consumption of the electrode itself was negligible.

### 2.3. Blood and muscle sampling

After the oxygen uptake measurements, trout were anaesthetised with MS-222 (0.13 g l<sup>-1</sup>). The pH in the anaesthetising bath was previously adjusted with NaHCO<sub>3</sub> for the same value as the exposure water. The blood sample was immediately taken with a heparinized syringe by caudal puncture. The blood haematocrit value, haemoglobin and mean cellular haemoglobin concentrations, glucose and lactate concentrations, as well as plasma chloride, sodium, potassium and total protein concentrations were measured as in Vuorinen et al. (1990). An epaxial muscle sample was extracted with nitric acid according to Loenn & Oikari (1982), and sodium, chloride and potassium concentrations were measured from the extract after the centrifugation. The muscle water content was determined by drying at 105°C for 24 hours.

### 2.4. Gills

The second and third gill arch from the left side of the fish were excised immediately after the caudal puncture. To detect accumulated iron on the gill surface, one gill arch was immersed into a haematoxylin sodium iodate solution for twenty minutes, rinsed with distilled water and stored in 70% ethanol. The other gill arch was fixed in 4% buffered formaldehyde, dehydrated, embedded in paraffin and sectioned in 8 µm slices. The slices were stained with periodic acid Schiff (PAS) reagent or haematoxylin sodium iodate. The mean arithmetic distance ( $d_A$ ) from the water to the gill capillaries was measured by superimposing a transparent grid on the imaging screen

of the microscope and calculated as (see Weibel & Knight 1964):

$$d_A = (P_{EPI,SLR} * 12.9)/(2 * I_O)$$

where  $P_{EPI,SLR}$  is the number of grid points falling on epithelial structures in the secondary lamellae, and  $I_O$  is the number of intersections with the outer surface of the secondary lamellae and test grid lines (the grid constant was 12.9 µm).

The effects of the treatments were tested with one-way ANOVA and the differences between the means with Scheffe's test at the 95% confidence level (SAS 1988).

## 3. Results

Three of the six fish died at "pH 5 + Fe" after one day of exposure and three after two days of exposure at "pH 6 + Fe". The gills of the surviving fish were clearly damaged at "pH 5 + Fe", and to a lesser extent at "pH 6 + Fe", with the fusion of the lamellae, separation of the outer epithelial layer, hypertrophy and necrosis of the lamellar epithelium (Fig. 1). At "pH 5 + Fe" and "pH 6 + Fe" the epithelium was significantly thicker than in the control (Fig. 2). At "pH 6 + Fe + HA" the epithelium was also significantly thicker although the mean did not differ from the control conspicuously (Fig. 2). Organic material in the water reduced the damage by preventing most of the accumulation of the metal on the gills (Fig. 3). In the staining of the whole gill arch with haematoxylin sodium iodate, the colour on the gills at "pH 5 + Fe" and "pH 6 + Fe" was a very dark blue, almost black. At "pH 5 + Fe + HA" the colour was deep or light blue but at "pH 6 + Fe + HA" or in the control there was no staining. The oxygen uptake of the fish was reduced in all exposure groups compared to the control, the most in the groups where no humic acids were added (Fig. 4). Damage to the epithelium led to a negative ion balance and changes in the Hct and MCHC values of the blood (Table 2). The blood lactate values did not differ between the exposure groups, nor did the muscle ion concentrations or water content (Table 2). Compared to the control, the blood glucose concentrations were significantly higher in all exposure groups except for "pH 6 + Fe + HA" (Table 2).

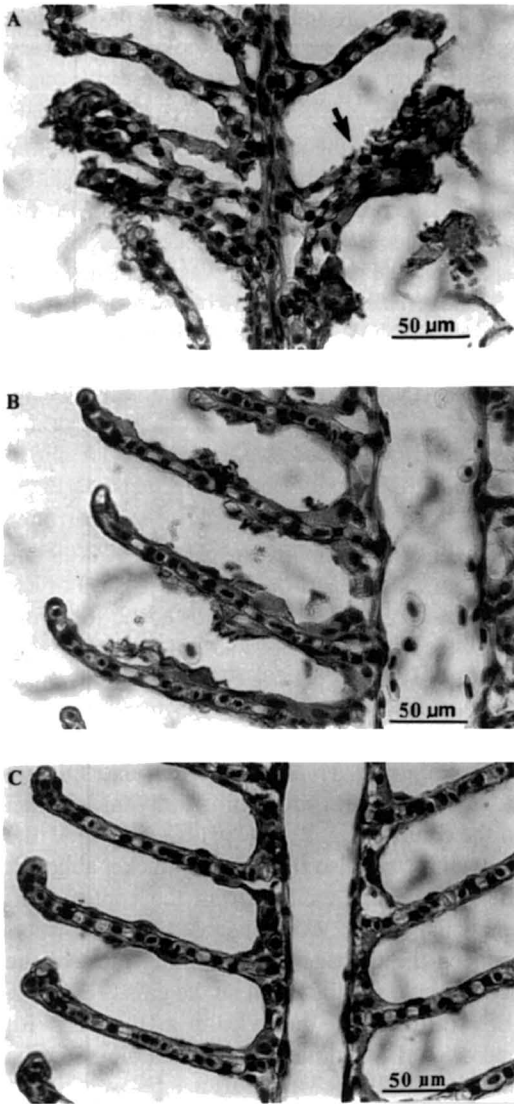


Fig. 1. The lamellae of the gills of brown trout were fused after two days of exposure to Fe at pH 5. The epithelium of the lamellae was necrotic (arrow) (A). In the exposure to Fe with humic acids at pH 5 (two days of exposure), the damage was limited to hypertrophy in the lamellae (B). In the exposure to Fe with humic acids at pH 6, the lamellae looked normal (C) but the morphometric analysis indicated an increased diffusion distance (see Fig. 2). The nominal concentrations of iron and humic acids were 2 and 15 mg l<sup>-1</sup>, respectively.

#### 4. Discussion

Iron was detected only on the gill epithelium, not inside, which indicates that the acute metal tox-

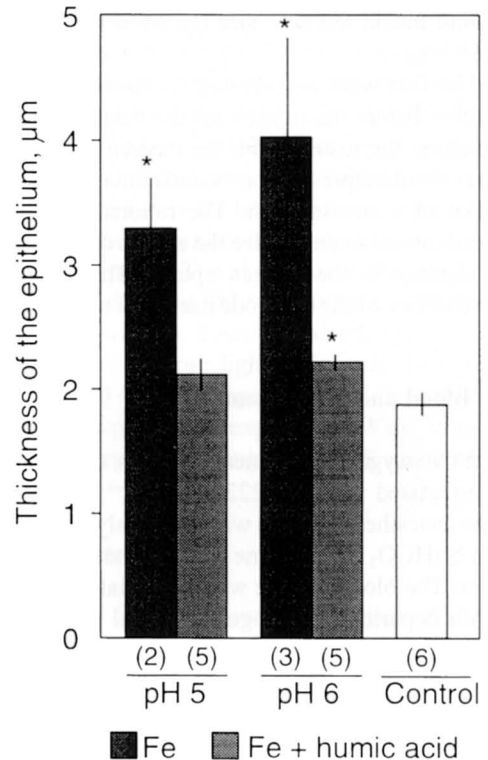


Fig. 2. The thickness of the epithelium of the lamellae (mean ± SE) was significantly bigger in the exposure to Fe at pH 5 (after two days of exposure) and pH 6 (three days of exposure) than in the control. The number of fish is shown under the columns. An asterisk above the column indicates a significant difference to the control ( $P < 0.05$ ). The nominal concentrations of iron and humic acids were 2 and 15 mg l<sup>-1</sup>, respectively.

icity was mediated through the action on the gill surface (see McDonald et al. 1989). The fusion of the lamellae together with the accumulation of iron on the gills in the exposure to Fe at pH 5 and 6 were apparently the main causes for the low oxygen uptake and the concomitant asphyxiation of the fish. The rupture of the epithelium was possibly the reason for the ion loss from the plasma. The rupture and necrosis of the epithelial cells is commonly reported to occur in lethal conditions in fish (Mallat 1985).

Complexation with organic material tends to lower the toxicity of the metals (Witters et al. 1990). The same was true in the present experiment where the toxicity of iron was reduced by humic acids. The damage of the gills was less

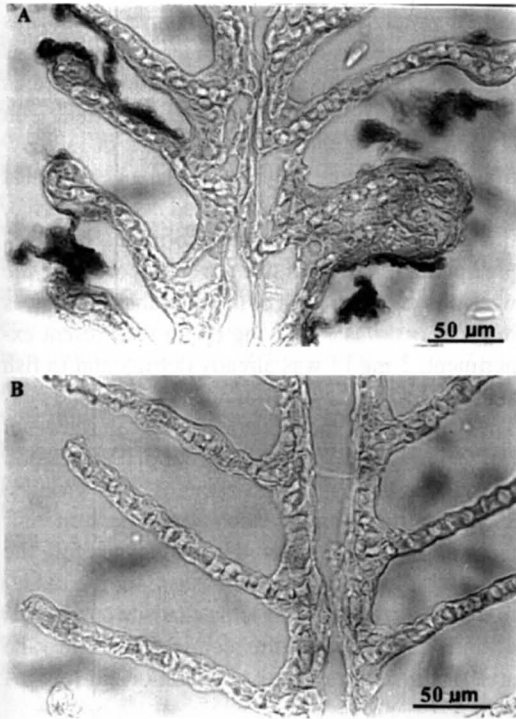


Fig. 3. Iron accumulated conspicuously on the lamellae in the exposure to Fe at pH 5 (A) and pH 6 (not shown) without the addition of humic acids. The addition of humic acids reduced the metal accumulation at pH 5 (not shown), and prevented accumulation at pH 6 (B). The nominal concentrations of iron and humic acids were 2 and 15 mg l<sup>-1</sup>, respectively.

severe and the oxygen consumption less decreased when humic acids were added to the water. In the exposure to Fe at pH 6 with humic acids, the plasma ion concentrations did not differ from the control suggesting the protective role of humic acids, but in the exposure to Fe at pH 5 with humic acid, the low plasma Na<sup>+</sup> and Cl<sup>-</sup> concentrations may have been caused by low pH alone (McDonald et al. 1989). Despite the ameliorating effect of the humic acid, the staining of the whole gill arches showed that Fe was able to bind on the gills at the lower pH indicating that the availability of the metal was not completely prevented by the humic acids. One hypothesis is that there may have been an exchange reaction of iron between humic acids and the gill surface at pH 5 but not at higher pHs. It should be pointed out, however, that the commercially available

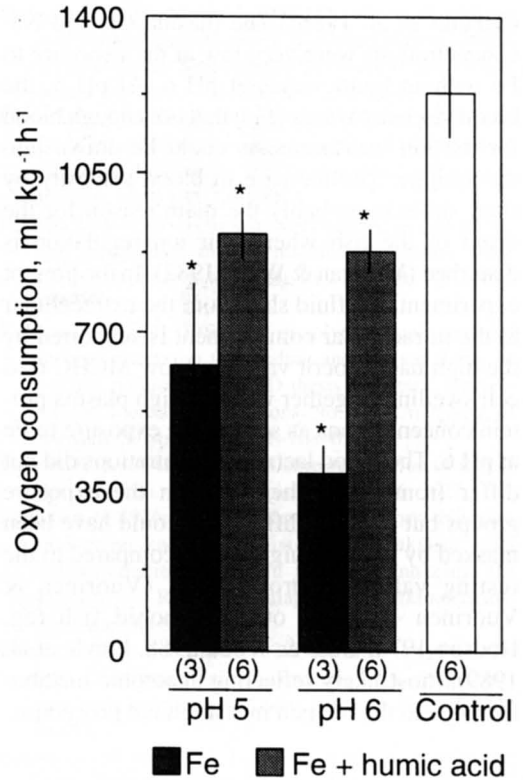


Fig. 4. Oxygen uptake (ml kg<sup>-1</sup> h<sup>-1</sup>; mean ± SE) of brown trout was significantly smaller in all iron-exposed groups than in the control. The number of fish is shown under the columns. An asterisk above the column indicates a significant difference to the control ( $P < 0.05$ ). The nominal concentrations of iron and humic acids were 2 and 15 mg l<sup>-1</sup>, respectively.

humic acid may differ from the dissolved organic materials in Finnish waters and therefore have different metal complexation characteristics (see Kukkonen 1991). It is worth noting that minor damage to the gills is not sufficient to activate repair mechanisms in the epithelium and may thus sensitize fish to acidity as was recently pointed out by Audet & Wood (1993).

The high blood glucose concentration in brown trout when iron was added to the water without humic acids is consistent with the occurrence of gill damage. The increase in the glucose concentration can be considered as a general response to stress (Wedemeyer & McLeay 1981), and also as a compensating factor against the depletion of the monovalent ions from the plasma

(Scherer et al. 1986). The plasma  $\text{Cl}^-$  and  $\text{Na}^+$  concentrations were very low at the exposure to Fe without humic acids at pH 6. At pH 5, the blood viscosity was so high that not enough blood for the ion measurements could be drawn into the syringe. The increase in blood viscosity by fluid shifts is probably the main reason for the death of the fish when their ion regulation is disturbed (Milligan & Wood 1982). In the present experiment, the fluid shift from the extracellular to the intracellular compartment is supported by the high haematocrit value and low MCHC (red cell swelling) together with the high plasma protein concentrations as seen in the exposure to Fe at pH 6. The blood lactate concentrations did not differ from each other between the exposure groups but any real differences could have been masked by the very high values compared to the resting values in brown trout (Vuorinen & Vuorinen 1985) or other salmonid fish (eg. Hodson 1976, Goss & Wood 1988, Playle et al. 1989), most likely reflecting anaerobic metabolism due to the oxygen measurement procedure.

This study suggests that iron may contribute as a toxic factor to fish when the water quality changes following timber management operations. Steffens et al. (1992) concluded that the safe total iron concentration of the inflowing water in rainbow trout hatcheries may well be 5 to 10  $\text{mg l}^{-1}$  taken that the water pH is close to neutral and is well oxidised, i.e. iron is oxidised to a ferric form and becomes precipitated. They did not state the colour of the water but concentration of the filterable substances in the water was 17–19  $\text{mg l}^{-1}$ . In the present experiment, 2  $\text{mg l}^{-1}$  was already detrimental to fish at low pH when the iron was added as  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ . The half time for the oxidation of  $\text{Fe}^{2+}$  in sea water ( $10^\circ\text{C}$ ) is eight days at pH 6 but at pH 5 oxidation takes over two years (Roekens & van Griegen 1983). In the present experiment, the iron-containing water was prepared a few days before the exposure. Because of the time scale for the oxidation of the iron, it is possible that ferrous iron is available to the gills in the recipient water near the area of timber management operations at low pH values.

Table 2. The blood haematocrit value (Hct), haemoglobin (Hb), mean cellular haemoglobin (MCHC), glucose (Glc) and lactate (Lact.) concentrations, and plasma chloride, sodium, potassium and total protein concentrations (mean  $\pm$  SE). In the exposure to Fe at pH 5, the exposure time was two days, in the others three. At pH 5 with Fe, the blood viscosity was so high that only a very small amount of blood could be drawn into the syringe. An asterix as a superscript indicates a significant difference compared with the control ( $P < 0.05$ ). – = no observation. The nominal concentrations of iron and humic acids were 2 and 15  $\text{mg l}^{-1}$ , respectively. The number of fish is shown in brackets.

	"pH 5+Fe"	"pH 5+Fe+HA"	"pH 6+Fe"	"pH 6+Fe+HA"	Control
Hct	0.51 $\pm$ 0.03 <sup>*</sup> (3)	0.41 $\pm$ 0.03 (6)	0.54 $\pm$ 0.04 <sup>*</sup> (3)	0.41 $\pm$ 0.02 (6)	0.38 $\pm$ 0.02 (6)
Hb, $\text{g l}^{-1}$	83.6 $\pm$ 0.7 (3)	85.8 $\pm$ 6.7 (6)	92.8 $\pm$ 9.1 (3)	89.2 $\pm$ 5.7 (6)	85.6 $\pm$ 4.1 (6)
MCHC, $\text{g l}^{-1}$	164.1 $\pm$ 10.8 <sup>*</sup> (3)	209.5 $\pm$ 6.9 (6)	170.1 $\pm$ 5.3 <sup>*</sup> (3)	220.3 $\pm$ 7.2 (6)	224.4 $\pm$ 4.2 (6)
Glc, $\text{mmol l}^{-1}$	16.3 $\pm$ 1.7 <sup>*</sup> (3)	11.4 $\pm$ 1.4 <sup>*</sup> (6)	15.1 $\pm$ 3.0 <sup>*</sup> (3)	4.8 $\pm$ 0.2 <sup>*</sup> (6)	7.3 $\pm$ 0.8 (6)
Lact., $\text{mmol l}^{-1}$	6.9 $\pm$ 0.2 (3)	7.5 $\pm$ 1.2 (6)	7.3 $\pm$ 2.4 (3)	6.4 $\pm$ 0.8 (6)	8.1 $\pm$ 0.9 (6)
$\text{Cl}^-$ , $\text{mmol l}^{-1}$	–	117.8 $\pm$ 1.2 <sup>*</sup> (6)	100.0 $\pm$ 5.6 <sup>*</sup> (3)	131.8 $\pm$ 1.2 (6)	130.8 $\pm$ 1.0 (6)
$\text{Na}^+$ , $\text{mmol l}^{-1}$	–	147.0 $\pm$ 3.7 <sup>*</sup> (5)	–	159.0 (1)	164.7 $\pm$ 0.9 (3)
$\text{K}^+$ , $\text{mmol l}^{-1}$	–	3.5 $\pm$ 0.10 (5)	–	2.3 (1)	3.0 $\pm$ 0.3 (3)
Prot., $\text{g l}^{-1}$	–	44.3 $\pm$ 1.4 (5)	54.3 $\pm$ 1.9 <sup>*</sup> (3)	37.5 $\pm$ 1.9 (6)	41.7 $\pm$ 1.2 (6)

Other factors with known or possible harmful effects near timber management areas are aluminium, manganese (Wepener et al. 1992) and suspended solids (Servizi & Martens 1991). Aluminium, for example, is known to accelerate iron-induced peroxidation in the cell membranes (Gutteridge et al. 1985, Stripp & Trombetta 1992) which may contribute to the toxic action of these metals in the gills. In natural waters, the total aluminium concentration can increase up to several hundreds of micrograms per litre after soil manipulation. Studies on the combined effects of metals on the structure and function of fish gills will thus be necessary. Further studies will also be needed on possible gill damage in the natural populations of fish in regions influenced by timber management operations.

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## References

- Ahtiainen, M. 1992: The effects of forest clear-cutting and scarification on the water quality of small brooks. — *Hydrobiologia* 243/244: 465–473.
- Ahvonen, A., Jutila, E. & Koskiniemi, J. 1993: Metsätalouden vaikutukset kalastoon Isojoen vesistön alueella: tutkimusalue ja kalaston perusselvitys (In Finnish) — *Kalatatkimuksia – Fiskundersökningar* 69: 61–101.
- Andersson, P. & Nyberg, P. 1984: Experiments with brown trout (*Salmo trutta* L.) during spring in mountain streams at low pH and elevated levels of iron, manganese and aluminium. — *Rep. Inst. Freshwater Res. Drottningholm* 61: 34–47.
- Audet, C. & Wood, C. M. 1993: Branchial morphological and endocrine responses of rainbow trout (*Salmo gairdneri*) to a long-term sublethal acid exposure in which acclimation did not occur. — *Can. J. Fish. Aquat. Sci.* 50: 198–209.
- Bagge, P. & Ilus, E. 1975: Ferrosulfaatin ja sitä sisältävien jätevesien akuutista myrkyllisyydestä murtovedessä (In Finnish). — *Meri* 3: 46–64.
- Brenner, F. J., Corbett, S. & Sherzer, R. 1976: Effect of ferric hydroxide suspension on blood chemistry in the common shiner, *Notropus cornutus*. — *Trans. Amer. Fish. Soc.* 105: 450–455.
- Decker, C. & Menendez, R. 1974: Acute toxicity of iron and aluminium to brook trout. — *Proc. W. Virg. Acad. Sci.* 46: 159–167.
- Goss, G. G. & Wood, C. M. 1988: The effects of acid and acid/aluminium exposure on circulating plasma cortisol levels and other blood parameters in the rainbow trout, *Salmo gairdneri*. — *J. Fish Biol.* 32: 63–76.
- Gutteridge, J. M. C., Quinlan, G. J., Clark, I. & Halliwell, B. 1985: Aluminium salts accelerate peroxidation of membrane lipids stimulated by iron salts. — *Biochem. Biophys. Acta* 835: 441–447.
- Hodson, P. V. 1976: Temperature effects on lactate-glycogen metabolism in zinc-intoxicated rainbow trout (*Salmo gairdneri*). — *J. Fish. Res. Bd. Can.* 33: 1393–1397.
- Kukkonen, J. 1991: Effects of dissolved organic material in fresh waters on the binding and bioavailability of organic pollutants. — PhD thesis summary, University of Joensuu Publications No: 22, University of Joensuu, 39 pp. ISBN: 951–708–030–1.
- Larson, K. & Olsen, S. 1950: Ochre suffocation of fish in the river Tim. — *Rep. Danish Biol. Sta.* 6: 3–27.
- Lehtonen, H. 1976: Tutkimus Kemira Oy:n Porin tehtaiden jätevesien kalataloudellisista vaikutuksista sekä kalataloudellisten tarkkailu ja hoitosuunnitelma (In Finnish) — *Riista- ja kalatalouden tutkimuslaitos, Tiedonantoja* 6: 292 p.
- Loenn, B.-E. & Oikari, A. 1982: Determination of muscle ions from trout, *Salmo gairdneri*, by a simple wet extraction technique. — *Comp. Biochem. Physiol.* 72A: 49–53.
- Lukowicz, M. von 1976: Der Eisengehalt im Wasser und seine Wirkung auf den Fisch. — *Fisch Umwelt* 2: 85–92.
- Mallat, J. 1985: Fish gill structural changes induced by toxicants and other irritants: a statistical review. — *Can. J. Fish. Aquat. Sci.* 42: 630–648.
- McDonald, D. G., Reader, J. P. & Dalziel, T. R. K. 1989: The combined effects of pH and trace metals on fish ionoregulation. — In: Morris, R., Taylor, E. W., Brown, D. J. A. & Brown, J. A. (eds.), *Acid toxicity and aquatic animals. Society for experimental biology, seminar series* 34. Cambridge: 221–242.
- Milligan, C. L. & Wood, C. M. 1982: Disturbances in haematology, fluid volume, distribution, and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. — *J. Exp. Biol.* 99: 397–416.
- Oulasvirta, P. 1990: Effects of acid-iron effluents from a titanium dioxide factory on herring eggs in the Gulf of Bothnia. — *Finnish Fish. Res.* 11: 7–15.
- Playle, R., Goss, G. G. & Wood, C. M. 1989: Physiological disturbances in rainbow trout (*Salmo gairdneri*) during acid and aluminium exposures in soft water of two calcium concentrations. — *Can. J. Zool.* 67: 314–324.
- Playle, R. & Wood, C. M. 1989: Water chemistry changes in the gill microenvironment of rainbow trout: experimental observations and theory. — *J. Comp. Physiol. B* 159: 527–537.
- Roekens, E. J. & van Griegen, R. E. 1983: Kinetics of iron(II) oxidation in seawater of various pH. — *Marine Chemistry* 13: 195–202.

- SAS Institute Inc. 1988: SAS/STAT User's guide. Release 6.03 Edition. — Gary, NC. 1028 pp.
- Scherer, E., Harrison, S. E. & Brown, S. B. 1986: Locomotor activity and blood plasma parameters of acid-exposed lake whitefish, *Coregonus clupeaformis*. — *Can. J. Fish. Aquat. Sci.* 43: 1556–1561.
- Servizi, J. A. & Martens, D. W. 1991: Effect of temperature, season, and fish size on acute lethality of suspended sediments to coho salmon (*Oncorhynchus kisutch*). — *Can. J. Fish. Aquat. Sci.* 48: 493–497.
- Smith, E. J., Sykora, J. L. & Shapiro, M. A. 1973: Effect of neutralized iron hydroxide suspensions on survival, growth, and reproduction of the fathead minnow (*Pimephales promelas*). — *J. Fish. Res. Board. Can.* 30: 1147–1153.
- Steffens, W., Mattheis, Th. & Riedel, M. 1992: Field observations on the sensitivity of rainbow trout (*Oncorhynchus mykiss*) to high concentrations of water-born iron. — EIFAC/XVII/92/Symp. E39. 11 p.
- Stripp, R. A. & Trombetta, L. D. 1992: Antioxidant enzyme activity as biomarkers of acid stress in natural aquatic systems. — EIFAC/XVII/92/Symp. E26. 16 p.
- Stumm, W. & Lee, G. F. 1960: The chemistry of aqueous iron. — *Schweizerische Zeitschrift für Hydrologie* 22: 295–319.
- Vuorinen, P. J. 1984: Rautaruukki Oy: n Rautuvaaran kaivoksen vaikutuksesta taimenen alkionkehitykseen ja poikasiin (In Finnish) — *Riista- ja kalatalouden tutkimuslaitos, Monistettu julkaisu* 23: 193–206.
- Vuorinen, P. J. & Vuorinen, M. 1985: Effects of kraft mill effluent on reproduction of brown trout (*Salmo trutta*) on a restricted diet. — *Finnish Fisheries Research* 6: 92–105.
- Vuorinen, P. J., Vuorinen, M. & Peuranen, S. 1990: Long-term exposure of adult whitefish (*Coregonus wartmanni*) to low pH/aluminium: effects on reproduction, growth, blood composition and gills. — In: Kauppi, P., Anttila, P. & Kenttämies, K. (eds.), *Acidification in Finland*. Springer-Verlag: 941–961.
- Weatherley, N. S., Rutt, G. P., Thomas, S. P. & Ormerod, S. J. 1991: Liming acid streams: aluminium toxicity to fish in mixing zones. — *Water Air Soil Pollut.* 55: 345–353.
- Wedemeyer, G. A. & McLeay, D. J. 1981: Methods for determining the tolerance of fishes to environmental stressors. — In: Pickering, A. D. (ed.), *Stress and fish*. Academic Press, London: 247–275.
- Weibel, E. R. & Knight, B. G. 1964: A morphometric study on the thickness of the air-blood barrier. — *J. Cell Biol.* 21: 367–384.
- Wepener, V. 1990: The effect of heavy metals at different pH values on the haematology and metabolic enzymes of *Tilapia sparrmanii* (Cichlidae). — M. Sc Thesis (in Afrikaans), Rand Afrikaans University, Johannesburg. (Ref. Wepener, V., Van Vuren, J. H. J. & Du Preez, H. H. 1992).
- Wepener, V., Van Vuren, J. H. J. & Du Preez, H. H. 1992: Effect of manganese and iron at a neutral and acidic pH on the hematology of the banded tilapia (*Tilapia sparrmanii*). — *Bull. Environ. Contam. Toxicol.* 49: 163–619.
- Witters, H. E., Van Puymbroeck, S., Vangenechten, J. H. D. & Vanderborgh, O. L. J. 1990: The effect of humic substances on the toxicity of aluminium to adult rainbow trout, *Oncorhynchus mykiss* (Walbaum). — *J. Fish Biol.* 37: 43–53.