Effects of iron, aluminium, dissolved humic material and acidity on grayling (*Thymallus thymallus*) in laboratory exposures, and a comparison of sensitivity with brown trout (*Salmo trutta*)

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Iron alone, as well as aluminium, can be acutely lethal in humus-free acidic water. In a simultaneous laboratory exposure to both Fe and Al the toxic effects on grayling were even more pronounced. Water acidity increased and dissolved humic material reduced the toxicity of Fe and Al. As toxic effects, the ionoregulation of yolk-sac fry was disturbed, swimming activity decreased and mortality increased. Based on mortality and swimming activity, brown trout yolk-sac fry tolerated, depending on the Al concentration, nearly half a pH unit more of acidity than those of grayling. The gills of the affected one-summer-old grayling were damaged, leading to decreased oxygen uptake and disturbed ionoregulation. In cold water (3 °C), one-summer-old grayling did not recover completely from the sublethal exposure. Some tributary waters of Isojoki, a river in West-Central Finland, were toxic to grayling yolk-sac fry. These waters had rather high Al and Fe concentrations but were humic and only slightly acidic. It is concluded that increased concentrations of Fe and Al increases the harmfulness of waters in forestry land use or peat production areas even in humic and slightly acidic waters.


**Introduction**

Forestry land use often drastically changes the water quality of river systems in the treatment area. The leaching of inorganic and organic suspended solids and concentrations of dissolved organic matter and nutrients may increase as may water flow and temperature (e.g., Ahtiainen 1992). Aluminium, iron and other metals are also leached from the soil and rinsed into river systems (Ahtiainen 1992, Reynolds et al. 1992, see also Vuori 1995). Metals are at least partially bound by the dissolved organic carbon, i.e. humic material (Kullberg et al. 1993). Changes in water pH depend greatly on the soil type (Urho et al. 1990, Ahtiainen 1992, Ahti et al. 1995, Rees and Ribbens 1995).

The primary toxic effects of metals on fish can mostly be explained by the surface activity of metals as free cations, i.e. metal binding to the respiratory epithelium damages it and its normal functions (McDonald et al. 1989, McDonald and Wood 1993). The most important water quality variables affecting metal activity are pH, the calcium concentration and the content of complex forming substances. Humic substances form quite stable complexes with metals decreasing the proportion of ionic metals in the water (McDonald et al. 1989, Kullberg et al. 1993). On the other hand, humus inhibits the oxidation of toxic ferrous iron to the less toxic ferric form (Suzuki et al. 1992). Compared to aluminium, little is known about the toxicity of iron to fish. The effects of iron on fish have been studied primarily in connection with effluent discharges from industry and mining (e.g., Sykora et al. 1972, Smith et al. 1973, Lehtonen 1976, Smith and Sykora 1976, Vuorinen 1984, Grippo and Dunson 1996a, 1996b).

Aluminium has harmful effects on the different developmental stages of fish in acidic water (e.g., Vuorinen et al. 1990, Weatherley et al. 1990, Wood et al. 1990, Vuorinen et al. 1992, 1993, 1994a, 1994b). During long-term exposures in aluminium-containing acidic water, the spawning of fish can be delayed (Beanish et al. 1975, Tam and Payson 1986, Rask et al. 1990, Vuorinen et al. 1990, Vuorinen and Vuorinen 1991, Vuorinen et al. 1992). Growth may be slowed if competition for food does not decrease due to lowered numbers of fish species or specimens (Tam and Payson 1986, Gunn et al. 1987, Rask et al. 1988, Vuorinen et al. 1990, Rask et al. 1992, Vuorinen et al. 1992). Newly-hatched fry are usually sensitive to acidic and aluminium-containing water, although the yolk-sac fry of some species can survive even days in such aluminium concentrations that would be lethal during a prolonged exposure (Vuorinen et al. 1993, 1994b). When gills are fully developed the acute toxicity of aluminium to fish is primarily due to its effects on the gill epithelium disturbing gas exchange and body ionic balance (Neville 1985, Waring and Brown 1995). Changes in the ionic balance have been observed during short and long-term exposures (Weiner et al. 1986, Audet et al. 1988, Tuunanen et al. 1990, Vuorinen et al. 1990, McDonald et al. 1991, Vuorinen et al. 1992). The ionic balance is disturbed and oxygen consumption changed in yolk-sac fry, as well, when exposed to aluminium (Keinänen et al. 1998, M. Keinänen, S. Peuranen, M. Nikinmaa and P.J. Vuorinen, unpubl.). There are differences in tolerance between fish species to aluminium (Grande et al. 1978, Holtze and Hutchinson 1989, Vuorinen et al. 1993, Poléo et al. 1997). Nothing was known about the aluminium tolerance of grayling (Thymallus thymallus), until recently Poléo et al. (1997) reported the median lethal time (410 h) in an aluminium exposure (399 μg Al l⁻¹, pH 5.08) for grayling parr.

Iron occurs primarily as a free ferrous form, iron(II), in acidic water (pH < 5.8) when the water oxygen concentration is low (Stumm and Lee 1960). This iron form is deleterious to fish (Decker and Menendez 1974, von Luckowitch 1976, Amelung 1982). Newly-hatched rainbow trout (Oncorhynchus mykiss) died in an iron(II) concentration of 1.3 mg l⁻¹ (pH 6–8) (Amelung 1982) and brook trout (Salvelinus fontinalis) at pH 5.5 in one day when 3.2 mg l⁻¹ of iron was added to the water as ferrous sulphate, the 48-hour LC50 value being 0.4 mg l⁻¹ (Decker and Menendez 1974). The numbers of hatched coho salmon (O. kisutch) decreased when the water iron concentration was over 1.0–1.3 mg l⁻¹ (pH > 7.7). In addition, oxidized iron is deposited on eggs and gills whereupon the diffusion of gases becomes more difficult (Larson and Olsen 1950, Smith et al. 1973, Bagge and Ilus 1975, Lehtonen 1976, von Lukowicz 1976, Andersson and Nyberg 1984, Vuorinen 1984, Oulasvirta 1990, Geertz-Hansen
and Rasmussen 1994). The harmful effects of iron will decrease if ferrous iron has time to oxidize into the ferric form before it comes into contact with the fish (Liebmann 1960). In 10 °C sea water, half of the ferrous iron will oxidize into the ferric form within eight days at pH 6, while at pH 5, it may take over two years (Roekens and van Griegen 1983).

When brown trout (Salmo trutta) were caged in an acidic river below a liming station, iron and aluminium were observed on their gills (Weatherley et al. 1991). Iron, along with aluminium, was suspected to be the cause of death of the caged fish. Andersson and Nyberg (1984) caged brown trout in a river during snow-melt. Fish started to die when melt waters flowed into the river although the water pH was still above 5.5. Water aluminium, iron, and manganese concentrations were 90–160, 550–1200, and 80–180 μg l⁻¹, respectively. Iron was presumed to contribute to the deaths in that test as well. In Finland, iron and aluminium concentrations in river systems can be as much as several milligrams per litre (Hudd et al. 1984, Heikkinen 1990, Vuori 1995), though in very high concentrations the greater part of these metals is probably bound to organic matter. The combined effects of aluminium and iron have been studied with lamprey larvae (Lampetra fluviatilis): the oxygen consumption of lamprey larvae decreased when they were exposed simultaneously to iron (1.5 mg l⁻¹) and aluminium (0.3 mg l⁻¹) at pH 5.5 (Saski and Nikinmaa 1990). The toxicity of river water to the newly-hatched larvae of lamprey seemed to be related to the increase in the total iron concentration in the water flowing through sulphide-rich soils (Myllynen et al. 1997). Aluminium is known to accelerate the iron and H₂O₂-induced peroxidation of erythrocyte membranes in vitro (Gutteridge et al. 1985).

This investigation aimed at demonstrating the effects of iron and aluminium on grayling and the modifying effects of water acidity and dissolved humic material. A study of the effects on the gills and physiology of one-summer-old grayling was carried out as in our earlier brown trout experiment (Peuranen et al. 1994). Because the changes in the water quality of river waters can be short-term, the recovery of one-summer-old grayling from a short-term exposure was studied; the effect of temperature was also tested. The sensitivity of grayling and brown trout was compared in yolk-sac fry. In addition to different water quality combinations in artificial water, grayling yolk-sac fry were exposed to natural waters from Isojoki, a river in West-Central Finland, and a peat production area and to mixed waters simulating conditions in early spring and rainy periods. Grayling and brown trout were selected as test species because they are common in small rivers and brooks in areas subject to heavy forest treatment and peat production though their stocks have declined, for instance, in the Isojoki system (Laamanen et al. 1994).

Material and methods

In all experiments, aluminium was added as sulphate (Al₂(SO₄)₃ × 16H₂O) and pH was adjusted with sulphuric acid (see Vuorinen et al. 1993). Iron was added as FeCl₃ × 6H₂O and FeSO₄ × 7H₂O, 1:1, and humic acid as a commercial preparation from Fluka (53680) or Aldrich (H1,675-2; in the recovery test).

Newly-hatched fry

The effects of iron (0, 1, 2, and 5 mg l⁻¹), aluminium (0, 100, 200, 400, and 800 μg l⁻¹), dissolved humic material (0 and 10 mg l⁻¹) and acidic water (pHs 5.0, 5.5, and 6.0) on grayling (Thymallus thymallus) yolk-sac fry were tested using an eight-day (192 h) test. The test solutions were prepared in artificial water (described in Peuranen et al. 1994 and Keinänen et al. 1998) containing no organic matter. In addition, grayling yolk-sac fry were exposed to waters taken from the tributaries of Isojoki, to water flowing into the clarification basin of a peat production area (Koirasuo in Pudasjärvi), and to water leaving that basin. The outflowing “peat water” was also mixed (1:1) with Lohiluoma water (a tributary of Isojoki) or with aluminium-containing (400 μg l⁻¹) artificial water, and the water mixtures were acidified to pHs 5.0, 5.5, and 6.0. The aim of these tests was to simulate the situation in spring when peat production waters flow from a clarification basin to a brook, into which acidic aluminium-containing melt waters flow at the same time. Water quality data on the tributary and
Yolk-sac fry were kept in glass jars containing about 800 ml of test water. The temperature was 10 °C. At the beginning, there were 10 fry in each jar. The tests were finished when the control fry had utilized their yolk. During the experiments, the swimming activity and mortality of the fry were observed. The swimming activity was registered as the number of swimming (including those resting temporarily on their bellies) or non-swimming fry (lying on their sides). For the analysis of the exchangeable body sodium concentration, fry were sampled as described in Keinänen et al. 1998 and extracted with diluted nitric acid, applying the method of Loenn and Oikari (1982). The concentration of Na+ in the centrifuged extract was measured with a flame photometer (see Keinänen et al. 1998).

The sensitivity of brown trout (Salmo trutta m. fario L.) from Luutajoki, a river in southern Finland, stock and grayling to acidic water and aluminium was compared in experiments where newly-hatched yolk-sac fry were exposed to different aluminium concentrations (0, 100, 200, 400, 600, and 800 μg l⁻¹) at different pHs (4.00, 4.25, 4.50, 4.75, 5.00, 5.25, and 5.50). The test solutions were prepared with Lake Päijänne water diluted with ion-exchanged water (1:1), so that the final colour was about 10 mg Pt l⁻¹ and the calcium concentration 0.07–0.08 mmol l⁻¹. The fry were kept in polythene bowls containing 1300–1500 ml of test water. At the beginning of the exposures there were 16 grayling or 10 brown trout in each bowl. Grayling were exposed for seven days (168 h) at 12 °C until the yolk was nearly utilized in the control group. Brown trout were exposed for 32 days (768 h) at 5 °C and still had some yolk left. The test temperatures for the species were selected to be the natural ones. The swimming activity and number of dead fry were observed daily. Effective values for 50% of the yolk-sac fry, EC50 (effective concentration) and EL50 (effective pH level), were calculated as in Vuorinen et al. (1993). The values were based on the number of dead and non-swimming fry.

In all yolk-sac fry experiments the test solutions in the exposure vessels were renewed every two to three days. The water renewal inhibited any considerable change in water pH. The tests were carried out similarly to the tests on the effects of aluminium and low pH on the yolk-sac fry of other species (Vuorinen et al. 1993), even though there were some differences in water quality.

### One-summer-old fish

In the first experiment, one-summer-old grayling were exposed to iron and aluminium (Fe 2 mg l⁻¹, Al 250 μg l⁻¹) in artificial water containing dissolved humic material (15 mg l⁻¹) and in non-humic water at pHs 5 and 6. The duration of the exposure was three days and the temperature 10 °C. In the second experiment, grayling were

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**Table 1. Water quality data of test waters from the tributaries of Isojoki, two samples of waters entering the clarification basin of a peat production area (Koirasuo in Pudasjärvi) and two samples of waters leaving that basin in 1993.**

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<thead>
<tr>
<th></th>
<th>pH</th>
<th>Conductivity mS m⁻¹</th>
<th>Colour mg Pt l⁻¹</th>
<th>Ca⁺⁺ mmol l⁻¹</th>
<th>Fe, μg l⁻¹</th>
<th>Al₃⁺, μg l⁻¹</th>
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<td>Lohiluoma, 28 May</td>
<td>7.07</td>
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<td>0.028</td>
<td>509</td>
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<td>Hukanluoma, 28 May</td>
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<td>115</td>
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<td>496</td>
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<td>4.0</td>
<td>280</td>
<td>0.083</td>
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<td>340</td>
<td>0.104</td>
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<td>0.129</td>
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effect of water iron, aluminium, acidity, humus on grayling exposed to lower iron and aluminium concentrations (Fe 1 mg l\(^{-1}\), Al 100 \(\mu g\) l\(^{-1}\), humus 15 mg l\(^{-1}\)) at pH 5.5 at two different temperatures (3 and 13 °C) for six days, after which they were transferred to metal-free water for a seven-day recovery. The water volume in the test tubs was 50–60 l. At the beginning of the exposures, there were 12–13 fish in each tank. At the end of the exposures and at the end of the recovery period in the second test, the oxygen consumption of the fish was measured (as described in Peuranen et al. 1994) and blood samples were taken from dorsal veins (see Vuorinen et al. 1992, Peuranen et al. 1994) for the determination of the plasma chloride concentration (see Vuorinen et al. 1990). Gill samples were processed using the paraffin technique as described in Peuranen et al. (1994) and the gill slices were studied using a light microscope.

The test solutions in the tubs were renewed once or twice a day. The water was also aerated during the experiments. The fish were not fed, and feeding was stopped two days before the beginning of the exposures.

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

**Results**

**Newly-hatched fry**

Dissolved humic material reduced the toxicity of iron and aluminium to the yolk-sac fry of grayling (Figs. 1 and 2). However, iron and aluminium decreased the swimming activity and exchangeable body sodium concentration of grayling yolk-sac fry up to pH 6 in water containing humus as well (Figs. 1 and 2). The ion balance (the exchangeable body Na\(^+\) concentration) of the grayling yolk-sac fry was disturbed at pH 5.0 (Fig. 2) before any decrease in swimming activity was detected (Fig. 1). Dissolved humic material was also beneficial in acidic water which contained no iron or aluminium.

The humic water of Ohriluoma (a tributary of Isojoki) which contained high aluminium and rather high iron concentration (Table 1), was acutely lethal to grayling yolk-sac fry and the ionoregulation of the surviving fry was disturbed (Fig. 3). The swimming activity of the fry decreased in Pajuluoma water (Fig. 3), although the water pH was nearly neutral (Table 1). The exchangeable body Na\(^+\) concentration of the fry exposed to Hanhioja water was also lower than nor-
mal (Fig. 3). These effects were not detected in the water of Kärjenuoma which contained the greatest amount of dissolved humic material of these waters (Table 1 and Fig. 3). When the water leaving the clarification basin of peat production waters was added (1:1) to the non-toxic water of Lohiluoma and the mixture was acidified, the swimming activity of grayling decreased and some deaths occurred. The water leaving the clarification basin of peat production waters was even more harmful when aluminium-containing ion-poor artificial water was added (1:1) to it and the mixture was acidified to simulate melt waters (Fig. 3).

Based on mortality and swimming activity, the newly-hatched fry of grayling were more sensitive to acidity and aluminium than brown trout yolk-sac fry (Figs. 4 and 5). Brown trout seemed to tolerate nearly half a unit lower of pH than grayling. However, near pH 5 an aluminium concentration of 400 μg l⁻¹ or more was very deleterious to brown trout as well. On the other hand, brown trout yolk-sac fry might tolerate these test waters even at pH 4.5 if the water contained only a small amount of aluminium (Fig. 5).

**One-summer-old fish**

In three days, 23% of the one-summer-old grayling exposed to iron (2 mg l⁻¹) and 8% of grayling exposed to aluminium (250 μg l⁻¹) died at pH 5 and none at pH 6 (Table 2). When grayling were exposed simultaneously to iron (2 mg l⁻¹) and aluminium (250 μg l⁻¹), 69% of the fish died at pH 5 and 8% at pH 6. Fifty per cent of the grayling died in six days exposed simultaneously to 1 mg l⁻¹ of iron and 100 μg l⁻¹ of aluminium at pH 5.5.

The gills of the grayling exposed to aluminium and iron had deteriorated, i.e. the gill lamellae were typically adhered and the epithelial cells damaged (Fig. 6). The plasma chloride concentration (Fig. 7) and the oxygen consumption (Fig. 8) of fish were lower compared to the control (p < 0.05).

Adding dissolved humic material (15 mg l⁻¹) to the water reduced the toxic effects of aluminium and iron and no grayling died. Gill damage was reduced (Fig. 6) and ionoregulation was not disturbed (Fig. 7). The decrease in metal toxicity due to dissolved humic material was also seen as improved capacity for oxygen uptake (Fig. 8). How-
Fig. 3. (A) The percentage of affected (dead or non-swimming) newly-hatched grayling fry and (B) the exchangeable body sodium concentration of the fry after an eight-day laboratory exposure in different test waters (water quality data are given in Table 1): (1) the waters from the tributaries of Isojoki taken on 28 May and (2) 11 June; (3) two water lots entering the clarification basin of peat production waters and two water lots leaving that basin; (4) leaving “peat water” mixed 1:1 with acidified Lohiluoma water and (5) leaving “peat water” mixed with artificial acidified water containing aluminium (the final concentration of added aluminium was 200 μg l⁻¹).

Fig. 4. (A) The EC50-values of aluminium at different pHs and (B) the EL50-values of pH in different concentrations of aluminium for grayling after a seven-day exposure at 12 °C and for brown trout from the Luutajoki stock after a 32-day exposure at 5 °C. Effective values for 50% of the yolk-sac fry are based on the number of dead or non-swimming.

ever, oxygen consumption in the presence of humus was still lower than in the control.

After seven days in 13 °C control water, the gills of grayling were almost completely recovered from the six day simultaneous exposure to 100 μg l⁻¹ of aluminium and 1 mg l⁻¹ of iron, even though the epithelial cells were still swollen. At 3 °C, the gills had not recovered and the lamellae remained attached to each other. Ionoregulation in the grayling had also recovered within one week at 13 °C, but not at 3 °C (Fig. 9).

Discussion

The gills of one-summer-old grayling were damaged, leading to decreased oxygen uptake and disturbed ionoregulation, in iron and aluminium
concentrations found in areas influenced by forestry treatments (Ahtiainen 1992). As well, the ionoregulation was disturbed and swimming activity decreased in grayling yolk-sac fry. In natural conditions, the decrease of swimming activity alone increases the susceptibility of fry to predation. The exchangeable body sodium concentrations of grayling yolk-sac fry changed at pH 5 even in humic water before any alteration in swimming activity could be seen. Ionic disturbance is a well-known response in fish exposed to acidic and aluminium containing water (e.g., Muniz and Leivestad 1980, Neville 1985, Vuorinen et al. 1990, Keinänen et al. 1998). In a thirty-day exposure at pH 6.5 in soft water (Reader et al. 1989) both aluminium, 162 μg l⁻¹, and iron, 39 μg l⁻¹, reduced the sodium, potassium and calcium whole body content of brown trout yolk-sac fry. In that study water ion concentrations were very similar to the concentrations of the artificial low ionic strength water used in the present study.

The iron concentration of 2 mg l⁻¹ in humus-free acidic (pH 5) water was acutely nearly as lethal to one-summer-old grayling as it was to brown trout (Peuranen et al. 1994). Toxic effects were more pronounced in simultaneous exposure to aluminium and iron even as lower concentrations. The addition of dissolved humic material in the water clearly reduced the toxicity of aluminium and iron. The humus preparations used in the present experiments may have a different binding affinity to metals compared to the humic sub-

Table 2. The mortalities of one-summer-old grayling after being exposed to different concentrations of aluminium and iron in humic or humus-free water. No fish died in any of the control groups.

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>pH</th>
<th>Temp. °C</th>
<th>Al μg l⁻¹</th>
<th>Fe mg l⁻¹</th>
<th>Humus mg l⁻¹</th>
<th>Mortality %</th>
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<td>3 d</td>
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Fig. 5. The percentage of affected (dead or non-swimming) newly-hatched fry in different pHs and aluminium concentrations (μg l⁻¹); grayling after a seven-day exposure at 12 °C and brown trout from the Luutajoki stock after a 32-day exposure at 5 °C.
stances in natural waters (Kukkonen 1991). The protecting effect of humus was also seen in the exposures of grayling yolk-sac fry to natural waters, one of which (i.e., with high humic material content) was not toxic in spite of a high aluminium and iron concentration. Similarly, of acidic brook waters containing moderate amounts of aluminium and iron, those with a higher humus content were less toxic to rainbow trout than were low-humic waters (Hulsman et al. 1983). Humus has previously been shown to reduce the acute toxicity of aluminium (Baker and Schofield 1980), while the growth of juvenile rainbow trout in an exposure to aluminium was better in water with dissolved humic material than in humus-free water (Gundersen et al. 1994). According to Roy and Campbell (1997), natural organic matter may play an independent protective role in acidic aluminium-containing water in addition to decreasing aluminium toxicity by complexation. In the present study, dissolved humic material alone was beneficial to the yolk-sac fry of grayling in iron-poor acidic water which did not contain any aluminium or iron.

Humic material did not entirely ameliorate the toxicity of aluminium and iron. Even at pH 6 with dissolved humic material and a colour corresponding to the colour of natural waters, the metals had toxic effects on grayling yolk-sac fry as well as on the oxygen consumption of one-summer-old grayling. The sodium content of yolk-sac fry was lower and swimming activity also decreased in humic water. Some of the fry even died when exposed to a tributary water from the River Isojoki containing high concentrations of aluminium and iron but also dissolved humic material. These natural waters were not very acidic, with the lowest pH being just below six. The most toxic of the tested river waters had the highest aluminium concentrations. The aluminium tolerance of fish is known to depend on the ionic strength of water, in particular the calcium concentration, in addi-

**Fig. 6.** Gill lamellae of one-summer-old grayling exposed for three days at 10 °C to iron and aluminium (Fe 2 mg l⁻¹, Al 250 μg l⁻¹) at pH 6 (A) without humus and (B) with 15 mg l⁻¹ of dissolved humic material. (C) Gills of the control group, pH 6.3. Adhered gill lamellae are indicated with an arrow.

Water acidity was one of the most essential environmental factors which affected the density of the brown trout population in the tributaries of Isojoki (Jutila et al. 1998). However, in addition to physical properties no other water quality parameters but pH and conductivity were available for that study. In a study where a number of water quality characteristics were measured, the catch per unit effort for brown trout was highest in acidified lakes with the highest calcium concentration and acid neutralizing capacity in inflowing secondary streams (Hesthagen and Jonsson 1998). Reader et al. (1989) also suggest that aluminium cannot be regarded as the only metal affecting the decline of fish populations in acidic waters. Natural waters may also contain other deleterious metals, such as manganese, which disturbs the ion uptake of brown trout yolk-sac fry even at pH 6.5 (Tuunainen et al. 1991). This may be explained by the great number of mucous cells in the skin of newly-hatched fry because the mucus forms com-

Fig. 7. Plasma chloride concentration (mean ± SE) of one-summer-old grayling exposed for three days at 10 °C to iron or/and aluminium (Fe 2 mg l⁻¹, Al 250 µg l⁻¹) at pHs 5 and 6 without humus or with dissolved humic material (15 mg l⁻¹). Number of fish measured in brackets. A significant difference (p < 0.05) between the groups is indicated by dissimilar letters above the columns. pH 6.3 = control.

Fig. 8. Oxygen consumption (mean ± SE) of one-summer-old grayling at pHs 5 and 6 exposed for three days to iron (2 mg l⁻¹) or/and aluminium (250 µg l⁻¹) without humus or with dissolved humic material (15 mg l⁻¹). Number of fish measured in brackets. A significant difference (p < 0.05) between the groups is indicated by dissimilar letters above the columns. pH 6.3 = control.
plexes with aluminium and thus protects the fish against aluminium (Hughes 1985). In newly-hatched fry without well-developed gills the gas exchange (Rombough 1988) and probably the ion exchange as well (Alderdice 1988, Li et al. 1995) occur mainly through the skin. In brown trout fry, the number of skin mucous cells is highest immediately after hatching, approximately twice as high as in older fry (Blackstock and Pickering 1982). The yolk-sac phase of the brown trout lasted several weeks at 5 °C while that of grayling at 12 °C took only about one week. Although the test with brown trout yolk-sac fry continued considerably longer (32 d) than with grayling fry (7 d), the yolk of the grayling was completely absorbed by the end of the test period whereas brown trout fry still had yolk left. However, newly-hatched fry are proposed to be more sensitive than older fry if the exposure continues for the entire yolk-sac phase (Tuunainen et al. 1987). When the acute toxicity of many inorganic substances (e.g. metals) to the yolk-sac fry of grayling, rainbow trout and coho salmon were compared, grayling was the most sensitive species, but when older fry were compared there were hardly any differences in sensitivity between the species (Buhl and Hamilton 1991). Brown trout parr were also more tolerant to aluminium than yolk-sac fry (Weatherley et al. 1990).

As discharges from the forestry treatment areas can sometimes be short-term peaks, it is important for the survival of the fish that their gills recover from possible damage caused by metals. The gill epithelium is able to recover in a few days, and after the recovery, the metal tolerance of the gills improves (McDonald and Wood 1993). In the test with one-summer-old grayling, recovery took place at 13 °C but no longer at 3 °C. Though there were no mortalities at 3 °C, the low temperature in itself did not protect the fish against the toxicity of the metals. The low mortality in the cold water was most probably due to a slowed metabolism and reduced oxygen demand alone. However, the damage to the gill epithelium under cold conditions possibly prevented the recovery of ionoregulation. The inability of the gill epithelium to recover from the metal exposure in cold water can be fatal to fish in the spring, for example, when waters are still cold. The harmfulness of the melt waters to brown trout has been observed in caging experiments in a river (Andersson and Nyberg 1984). Furthermore, the oxidation of the toxic ferrous iron to a ferric form is slowed down at low temperatures (Decker and Menendez 1974, Amelung 1982, Roekens and van Griegen 1983).

Melt waters or autumn rains can increase the harmfulness of changes in water quality caused by forestry land use. They can increase the acidity and/or the metal concentrations of the waters, and changes in water pH change the form in which iron and aluminium occur. Dissolved humic material forms complexes with metal ions (Petersen et al. 1987, Kukkonen 1991) thereby either increasing or decreasing the bioavailability of metal ions (Kullberg et al. 1993). In natural conditions, fish are acclimatized to low metal concentrations and will thus better tolerate the short-term increases in metal concentrations (Mount et al. 1990), but this is not the case with newly-hatched fry.

Fig. 9. Plasma chloride concentration (mean ± SE) of one-summer-old grayling exposed to iron and aluminium with or without dissolved humic material for six days at pH 5.5 and after a one-week recovery. Experiments were performed at 3 °C and at 13 °C. AlFe = iron (1 mg l⁻¹) and aluminium (100 μg l⁻¹), AlFeH = iron (1 mg l⁻¹) and aluminium (100 μg l⁻¹) plus dissolved humic material (15 mg l⁻¹). Number of fish measured in brackets. A significant difference (p < 0.05) between the groups is indicated by dissimilar letters above the columns.
Conclusions

Aluminium and iron, in concentrations found in the natural environment, may be toxic both to brown trout and grayling, and the toxicity of these metals is augmented with increasing acidity. Even in slightly acidic water, the increase in the concentrations of these metals will make the water more harmful to fish. Dissolved humic substances reduce the toxicity of iron and/or aluminium but do not entirely prevent it. Waters in forest drainage and peat production areas can have toxic effects on fish particularly during spring-melt and autumn rains, because in cold water fish cannot recover quickly from the gill damage caused by the metals. The experiments with yolk-sac fry showed that some natural waters in the areas influenced by forestry treatments and peat production are harmful to the physiology of the fish and some waters can even be acutely lethal.

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