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Effects of subacute doses of iron (Fe) on *Leptophlebia marginata* (Insecta: Ephemeroptera)

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SUMMARY

1. The objective of this paper was to reveal the toxicity of Fe^{3+} and Fe^{2+} at pH 4.5 and 7 on larvae of the mayfly *Leptophlebia marginata*, by examining survival, motility, gill ventilation, moulting and feeding in experiments.
2. Fe^{2+} was the dominant metal species at pH 4.5, and Fe^{3+} at pH 7. Precipitation of Fe occurred only at pH 4.5, where Fe-precipitations were observed on the thorax and the gills of the larvae.
3. Both feeding activity and motility of the animals decreased at pH 4.5 and 10, 20 or 50 mg l^{-1} Fe_{tot}. After a short period of normal feeding, the animals stopped feeding for approximately 2 weeks and did not start to feed again until the end of the experiment. They were constipated. Survival was >95% in all treatments, except at pH 4.5 and 50 mg Fe_{tot}. In this group, about 20% of the animals died after having been constipated for 2 weeks.

Introduction

During acidification of surface waters the solubility of several metals increases, resulting in acute or chronic toxic effects on the aquatic invertebrate fauna. Depending on the pH and redox potential of the water, Fe appears as the dissolved ions Fe^{2+} and Fe^{3+} or as precipitates of both species. Shifts in between pH 4.5 and 5.0 cause changes in the redox state of the Fe, which then changes its solubility (McKnight & Bencala, 1990). There are only a few studies that deal with the toxicity of Fe, mostly LC_{50}-tests or simple field observations (reviewed in Gerhardt, 1990), and the results seem to be contradictory. For example, the LC_{50} (62 h) of *Asellus aquaticus* is 3 mg Fe^{2+} l^{-1} according to Walter (1966) but 200–400 mg Fe^{2+} l^{-1} according to Maltby, Snart & Calow (1987). In field surveys, Fe seems to be more toxic at neutral pH than at low pH, probably due to precipitation of Fe on to the gills and body as well as on to the sediment, preventing algal growth which in turn leads to decreased availability of food for grazers (McKnight & Feder, 1984).

The purpose of this study was to test three hypotheses; (i) that speciation of Fe depends on pH, (ii) that Fe is more toxic at a low pH than at a neutral pH, and (iii) that the toxicity of Fe is caused mainly by precipitation onto the body surface of the animals.

Experiments were performed on larvae of the common and acid-tolerant (Økland & Økland, 1986) mayfly, *Leptophlebia marginata* collected from a small stream in the northern part of the southern Swedish province Scania. The stream is humus-rich, usually of neutral pH, and passes through an area of gneiss bedrock with pine and deciduous forest on the banks. The Fe_{tot} concentration of the water varied between 1.0 and 3.5 mg Fe_{tot} l^{-1} according to discharge. At low water levels, Fe–humus precipitates can be found on the sediment and on some benthic invertebrate species. The animals try to remove this coating of iron precipitate using their mouth parts (field observation).

Materials and Methods

Animals were collected in November 1990 and kept in individual cubicles of 60 cm^3 within net-bottomed PVC frames (500-μm mesh size), which were submerged in PVC boxes containing 8 l of stream water. Earlier experiments have shown that this type of design is appropriate for this particular test species (A. Gerhardt, unpublished data). The animals were

acclimated to a pH of 4.5 by a stepwise decrease in pH (0.5 units per day from an initial pH of 7). pH was then kept at a constant level by daily adjustments with 0.1 M H_2SO_4 and 0.1 M NaOH. The temperature was 11 (SD 2)°C and the animals had a 12 h light: 12 dark light regime. Fe concentrations in the water were measured daily with flame AAS and photometer (Fe_{tot}, Fe^{2+} at 522 nm). Al_{tot} was measured with inductive coupled plasma emission spectrometry (ICPES). Oxygen concentrations were measured with an electrode, as the traditional Winkler method did not work properly because of the oxidizing effects of iron compounds. The stream water was changed every week to avoid both fungal growth and precipitation of Fe. The animals received food on a weekly basis in the form of fine detritus. This was obtained by filtering detritus, from sandy sediments from the stream, on to Whatman GF/C glassfibre filters using a vacuum pump.

Groups of sixty individuals of *L. marginata* were exposed to 0, 10, 20 or 50 mg $Fe_{tot}\,l^{-1}$ as $FeSO_4$ at pH 4.5, and pH 7, for about 30 days. Twice a week the following biological parameters were recorded: survival, feeding activity, gill ventilation, moulting and motility (measured as escape movements after a mechanical stimulus). After the acclimation phase to the experimental pH values, the water contained 0.30 mg $Al_{tot}\,l^{-1}$ (SD 0.02, $n=8$) in all treatments, which is higher than the concentration found in the stream (0.15 mg $Al_{tot}\,l^{-1}$; SD 0.05, $n=3$). The Fe concentration of the detritus, given as food, was 62.5 mg $Fe_{tot}\,kg^{-1}$ (SD 1.7, $n=6$), which agrees with values found in other river systems (Grahn & Håkansson 1987).

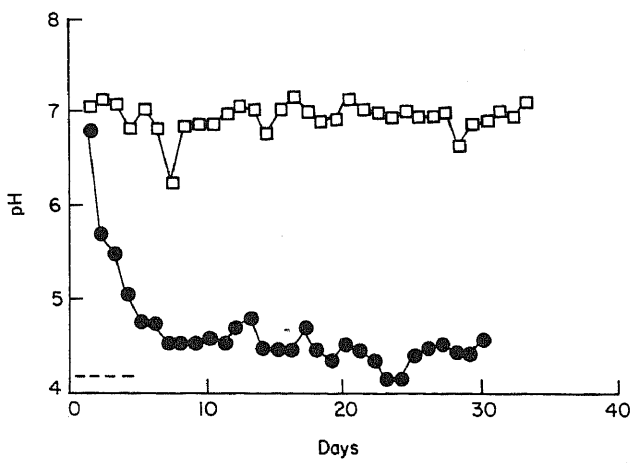

Fig. 1 pH values during the experiments. The values are means ($n=4$). □, pH 7 treatments; •, pH 4.5 treatments; ——, acclimation phase.

Once a week the Fe concentrations in a number of randomly selected animals were measured with flame AAS after drying (80°C, 2 days) and destroying the samples in 100% HNO_3 (80°C, 4 h). At the end of the experiments, three animals of each treatment were analysed histochemically to determine the localization of Fe compounds in the body. Longitudinal microtome dissections of the animals were stained with K_3 ($Fe(CN)_6$) to reveal Fe^{2+} and K_4 ($Fe(CN)_6$) to reveal Fe^{3+} (Moewis, 1978).

Results

Chemical changes in the experiments

Fig. 1 shows the pH values during the experiments. At pH 4.5 the maximal deviation from the nominal value was 0.3 pH units, and 0.7 pH units at pH 7. The deviations occurred mostly after the weekly change of stream water, when both Fe and pH levels needed to be adjusted.

Fig. 2 shows the concentration of Fe_{tot} and Fe^{2+} during 1 week between two changes of water. At pH 7, Fe^{2+} decreased immediately after $FeSO_4$ addition, which apparently gave a decrease in pH due to changes in the redox status of the water. At pH 7 Fe_{tot} was almost constant and Fe^{2+} was always below 1 mg l^{-1}. At pH 4.5, Fe_{tot} decreased after 5 days as a result of precipitation processes. The dominant species was Fe^{2+}.

The oxygen saturation level was between 60 and 70% in all treatments due to the large contact area between the water and air in the boxes, ensuring free diffusion of oxygen to compensate for removal of oxygen caused by the oxidation of iron compounds.

Survival motility and feeding behaviour of the larvae

Survival was greater then 90% in all treatments, with no significant differences between the different treatments. At the end of the experiments, however, the mortality in the pH 4.5/50 mg Fe-treatment increased abruptly to more than 20% (Fig. 3).

At pH 7, no significant differences between the control and the Fe treatments were observed in motility of the larvae, measured as locomotion after

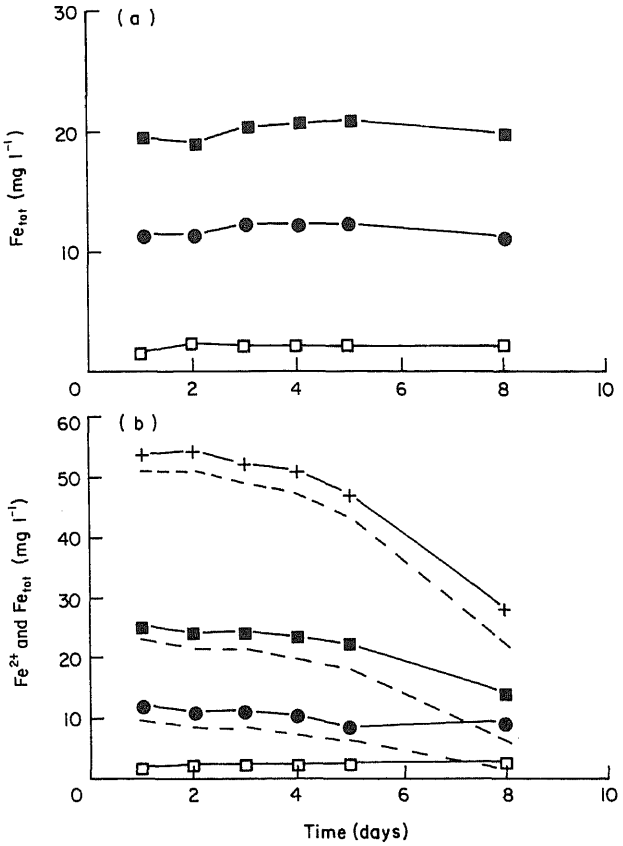

Fig. 2 Fe_{tot} and Fe^{2+} values in the water between 2-weekly changes of experimental water. (a) pH 7, (b) pH 4.5. □, controls (no Fe addition); •, 10 mg $Fe_{tot}l^{-1}$; ■, 20 mg $Fe_{tot}l^{-1}$; +, 50 mg $Fe_{tot}l^{-1}$; ------ Fe^{2+}.

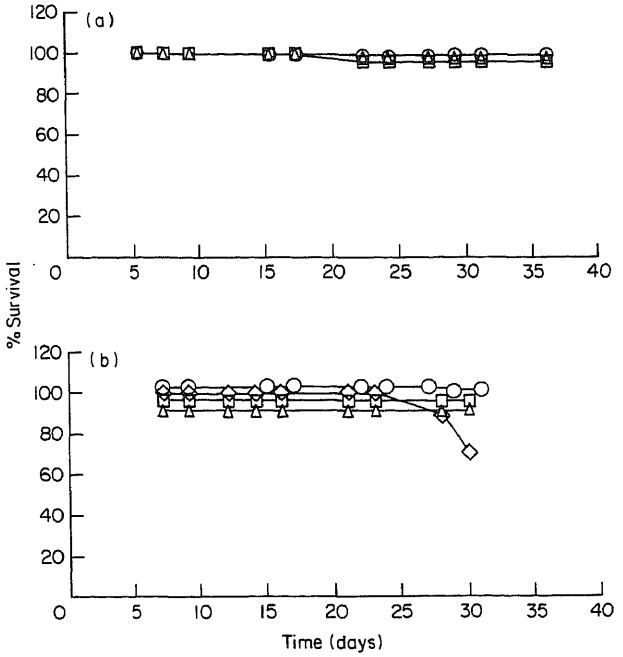

Fig. 3 Survival of *Leptophlebia marginata* in the Fe treatments at (a) pH 7 and (b) 4.5. o, controls; □, 10 mg $Fe_{tot}l^{-1}$; △, 20 mg $Fe_{tot}l^{-1}$; ◇, 50 mg $Fe_{tot}l^{-1}$.

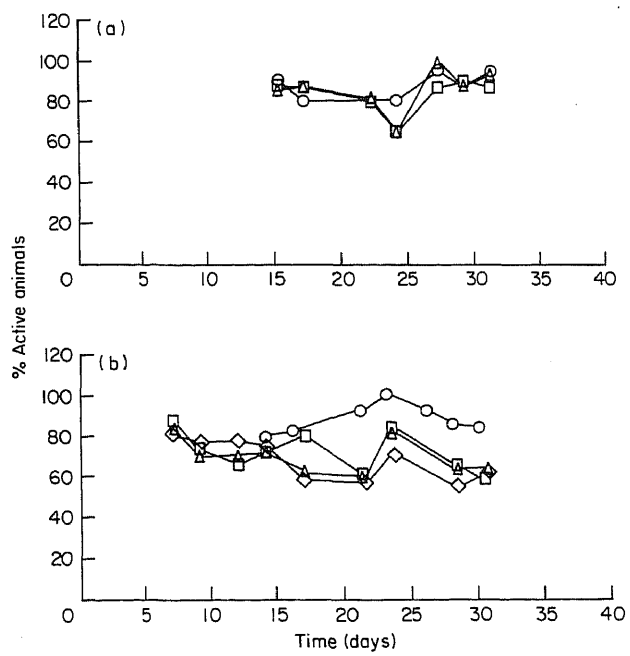

Fig. 4 Activity of *Leptophlebia marginata* in the Fe treatments at (a) pH 7 and (b) 4.5. Activity was measured as escape movement (yes or no) after a mechanical stimulus. Symbols as in Fig. 3.

a mechanical stimulus. At pH 4.5, however, the motility of the animals in the control group (no Fe added) differed significantly ($P < 0.025$, *U*-test) from that in the Fe treatments, in which the decrease in motility was proportional to the Fe concentration in the water (Fig. 4).

At pH 7, more than 90% of the animals continued to eat the provided food during the course of the experiment, irrespective of Fe exposure (Fig. 5). At pH 4.5, the percentage of animals eating the detritus decreased throughout the experiment, inversely proportional to the time and concentration of Fe exposure. The difference between the pH 4.5/Fe treatments and the control group was significant ($P < 0.01$, *U*-test). While the animals in both the control and all pH/Fe treatments contained food throughout the whole gut, the pH 4.5/Fe-treated animals were constipated. In the latter, the midgut was bloated and filled with food so that neither

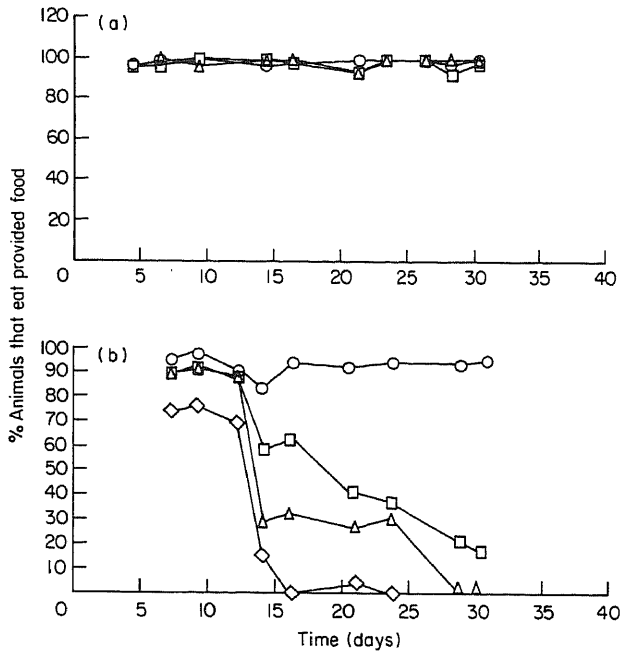

Fig. 5 Feeding activity of *L. marginata* in relation to Fe exposure at (a) pH 7 and (b) 4.5. Feeding activity was measured as percentage of animals that scraped the provided food (detritus) from the filter paper. Symbols as in Fig. 3.

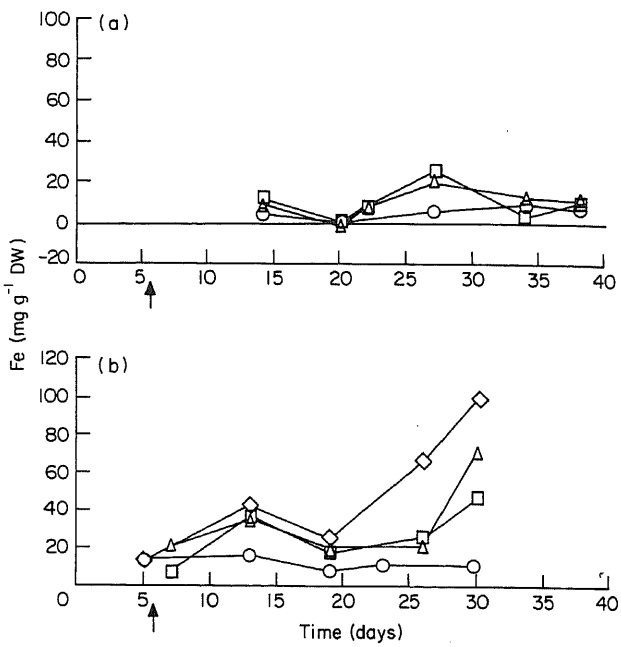

Fig. 6 Total body Fe in *L. marginata* at (a) pH 7 and (b) 4.5. Symbols as in Fig. 3. (↑) start of Fe addition. Symbols as in Fig. 3.

transport nor intake of new food was able to take place. After 2 weeks of starvation with a filled gut, the animals in the highest Fe treatment began to die. This period of starvation affected motility, which decreased simultaneously (Figs 4 & 5).

Uptake of iron

At pH 7, no significant net uptake of Fe into or on to the animals was observed (Fig. 6). At pH 4.5, however, the Fe content of the animals increased, especially in the 50 mg Fe treatment. At the same time, precipitation of Fe compounds on to the animals was observed, suggesting that surface adsorption mainly accounts for the 'uptake' of iron. This was confirmed by the histochemical analysis, which revealed Fe-compounds in the gut at both pH levels (pH 7 and 4.5) and on the body surface and gills at pH 4.5, but not in other tissues.

Discussion

The results of these experiments appear to be new as no other studies about the effects of Fe on behaviour and food digestion in invertebrates have been found in the literature. There are some LC_{50} tests, revealing a high tolerance of invertebrates (Anderson, 1944; Walter, 1966) and fish to Fe (Weigelt, 1900; Dyk, 1942). This is confirmed by this study, where the animals survived at concentrations up to 50 mg Fe_{tot} l^{-1} for 30 days at pH 7. Some mortality studies using pulverized fuel ash on marine invertebrates revealed highly variable toxicity (Jenner, Bowmer & Kema, 1990). Field observations emphasize the importance of Fe precipitates for the structure and function of the ecosystem (e.g. changes in species composition at Fe^{2+} <1 mg l^{-1}: Greenfield & Ireland, 1978; Rasmussen & Lindegaard, 1988). Scullion & Edwards (1980) and McKnight & Feder (1984) found more species in acid coal-waste water than in pH-neutral Fe-contaminated water. Keller *et al.* (1984) measured Fe stress in Crustacea and found that at a concentration of more than Fe 2 mg l^{-1}, *Gammarus minus* increased its oxygen consumption.

Fe^{2+} seems to be the most toxic metal species according to the present results. This is supported by a study where taconite tailings in acidified water of pH 5 proved to exert acute toxic effects upon rainbow trout (Oxberry, Douderhoff & Anderson, 1978). The authors assumed that the effects were

caused by the dissolved Fe content in the water.

The medical literature contains some ideas about the mechanisms of Fe toxicity. Intracellular Fe^{2+} may increase oxidative stress by inducing the formation of oxygen radicals, which cause damage to DNA and membranes (Stevens & Kalkwarf, 1990). Besides these carcinogenic effects, Fe overload can cause ulceration of the gastrointestinal mucosa and liver cirrhosis in mammals if it is present in sufficient concentrations for enough time in the parenchymal cells (Bothwell *et al.*, 1979; Friberg, Nordberg & Vouk, 1979). Segner & Storch (1985) found disruptions in the liver cell membranes of fish, possibly due to Fe stress. Although the gut membranes of vertebrates and invertebrates may be very different, the toxic effects of Fe^{2+} taken up from the acidified water in the current experiments may have occurred directly at the membrane as histochemical analysis with the light microscope showed no significant uptake of Fe into other organs. Fe^{2+} may mechanically destroy the membrane by ulceration or formation of precipitates on to the membrane, thus preventing absorption of food from the gut. In man, Fe uptake is regulated by transferrin and much less than 10% (Stevens & Kalkwarf, 1990) (3–6% according to Ganong, 1979) of the ingested Fe is absorbed. Transferrin has also been found in invertebrates (Finch, 1986) and seems to take up Fe^{2+} more rapidly than the vertebrate carrier molecules (Webb *et al.*, 1986). On the other hand, Fe^{2+} may have affected the muscular system of the experimental larvae, because peristalsis of the gut and larval motility decreased in the Fe-treated animals at pH 4.5. Finally, dehydration of the food, probably caused by changes in the permeability of the gut membrane, could have hardened the food so that transport through the gut became more difficult.

Feeding inhibition in fish has been observed as a reaction to other toxicants, including fenitrothion, Cu and Zn (Beitinger, 1990), but the fish started feeding again after 15 days, while in these experiments with mayflies, the animals started to die due to starvation and constipation.

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