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Effects of precipitations of iron hydroxides on *Leptophlebia marginata* (L.) (Insecta: Ephemeroptera) in the field

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With 6 figures and 3 tables in the text

Abstract: For 3 months, nymphs of *L. marginata* (Ephemeroptera) were exposed in a clearwater stream (Wispe) with permanent Fe_2O_3 precipitations on the streambed and in a brownwater stream (Mullra-stream) with $Fe_x(OH)_y$-humus precipitations.

The body burden of Fe of mayflies exposed in the Wispe were significantly higher than those in the Mullra-stream. Fe-precipitations on the body surface of the mayflies accounted for 80 % of the Fe-body loads in the Wispe, whereas only 40 % of the body loads in Mullra-stream were adsorbed on the body surface of the mayflies. However, survival of the mayflies was not affected by the different kinds of Fe-precipitations on the mayflies' body and gills. The internal uptake of Fe by the mayflies was higher in Mullra-stream than in Wispe.

Frequent molting did not allow for an incrustation of more than 50 % of the body. Incrustation occurred randomly as no body part was preferred. The chemical composition of the precipitations on the mayflies differed. Fe^{3+} was found in the precipitates on mayflies in the Wispe, while Fe^{3+} and Fe^{2+} were found on the nymphs in Mullra-stream.

Within the body, Fe^{2+} was found in eye- and cuticle-pigments and in the eggs of the last instar.

Introduction

Effects of pollution with iron have been investigated in connection with coal mining at low pH. Studies about the effects of acid mine drainage on aquatic life revealed an impoverishment of the macrobenthic community of several streams. Already at concentrations of ≥ 1.2 mg Fe l^{-1} the species diversity decreased (Walter 1966). At concentrations as low as 0.3 mg Fe^{2+} l^{-1} the number of taxa in a benthic community decreased from 67 to 53 (Rasmussen

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& Lindegaard 1988). In pH-neutral streams, Fe-hydroxide precipitations have been observed to exert negative effects on species richness (Greenfield & Ireland 1978, McKnight & Feder 1984, Rasmussen & Lindegaard 1988). Several explanations for the decrease in the number of benthic species have been put forward: 1) Fe-hydroxide precipitations on the sediment may inhibit the growth of periphyton and lead to a decrease in the number of grazers (McKnight & Feder 1984, Rasmussen & Lindegaard 1988). 2) The precipitations may cause respiratory stress and gill damage (Larson & Olsen 1950, Sykora et al. 1972). 3) The spawning grounds for fish may be destroyed by Fe-precipitations (Sykora et al. 1972). 4) Fe-precipitations on the gill and gut membranes of mayflies may reduce oxygen consumption, food consumption and mobility of the animals (Gerhardt 1992).

The aim of this field experiment was to test how precipitations of iron compounds affect the survival and the uptake of Fe by the benthic particle feeding mayfly *Leptophlebia marginata* in a brownwater and a clearwater stream.

Material and methods

Characterisation of the study sites

The Mullra-stream in South Sweden is a brownwater stream unaffected by anthropogenic waste (Fig. 1). It flows through mixed forest vegetation on gravel sediment (Gerhardt 1990). The bedrock is iron-rich gneiss. The Wispe is a clearwater forest stream in the Hils Mountains in the German province Niedersachsen (Fig. 1). The bedrock is sandstone with iron inclosures. Both are submontaneous silicate streams with a circumneutral pH during mean water discharge situations, but in Mullra-stream the pH can drop to pH 5 during floods. Fe_{tot}-concentrations and other chemical parameters in the water column are similar in both streams (Table 1). The concentrations of FPOM (fine particulate organic matter) in water and sediment of Mullra-stream is up to 10times higher than that in the Wispe stream (Table 2). Precipitations of Fe-compounds have been observed on the sediment as well as on invertebrates in both streams, mainly during low water levels. The precipitations were, however, of different color and probably also of different chemical composition. One part of the Wispe is covered by thick, firm, yellow-brown Fe_2O_3-precipitations (ochre), since Fe- and CO_2-rich groundwater

Table 1. Chemical characterisation of the study sites.

Stream	pH* min./max.	Ca (mg/l)	Cond. µS/cm	Hardness d°H	Fe_{tot}* (mg/l)	FPOM* (mg/l)
Mullra-stream	6.0–7.4	<30	100–235	1–2	1.64	12.9
Wispe-above Fe	6.0–7.5	14–26	145–207	3–4	0.34	n.d.
Wispe-below Fe	6.0–7.1	12–22	127–184	2–4	1.46	1.5

* Measured during the experiment; FPOM = 0.45 µm < x < 1 mm.

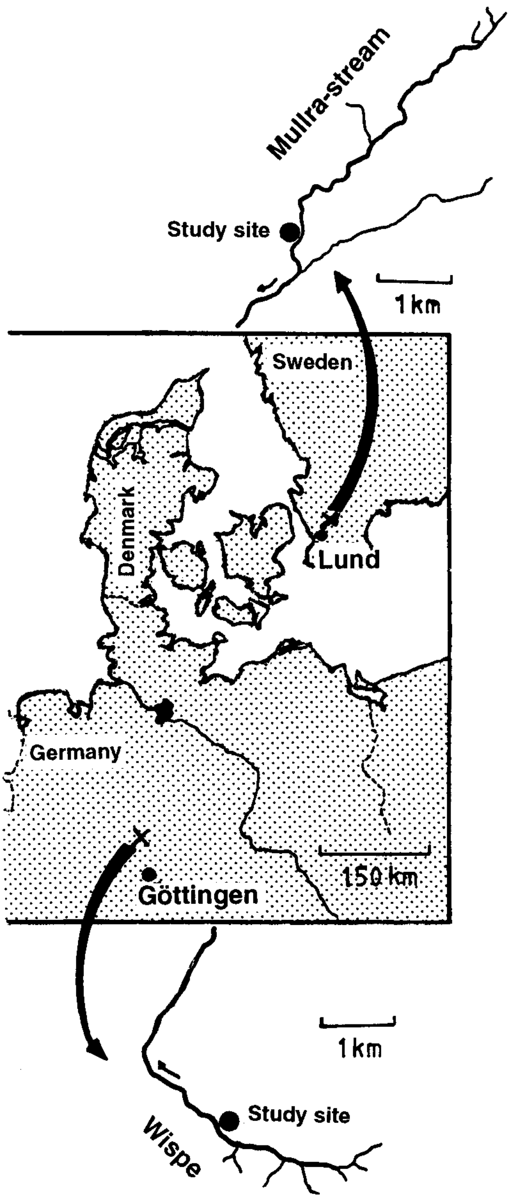

Fig. 1. The study sites in Sweden and Germany.

reaches the stream from a well. In the Mullra-stream, however, positively charged Fe-hydroxides form complexes with negatively charged humic material. These dark brown, slimy aggregates precipitate during low water level, but are in suspension during medium and high discharge. Upstreams the influx of Fe-rich groundwater, the number of macrobenthic species in the Wispe was 75, downstreams the species diversity decreased to 41 macroinvertebrate species. Mayflies were especially affected, with only *Baetis rhodani* and *Ecdyonurus venosus* being found (WESTERMANN 1990).

Cultures of microorganisms

Aliquots of 1 ml from the sediment and water samples of both streams were suspended in 0.9 % NaCl solution. Sediment solutions were vortexed for 0.5 min and sonicated for 1.5 min in order to release microorganisms from sediment particles. A dilution series from that solution in 0.9 % NaCl was made and incubated on two different media at 15 °C for about 3 weeks. Medium 1 was prepared by adding 4.9 g l^{-1} $FeSO_4$ to Wolfs-Medium at pH 4.5 to select for chemolithotrophic iron-oxidizing bacteria. Medium 2 was prepared by adding 0.005 g l^{-1} $FeSO_4$ to Nutrition Broth Agar at pH 6.9, to estimate the total number of microorganisms (GERHARDT et al. 1981).

Experimental setup

The benthic mayfly *Leptophlebia marginata* was chosen for the field tests, because it is frequent both in Swedish brown-water streams with elevated Fe-concentrations and in submontaneous streams in Europe. The *Leptophlebia-Paraleptophlebia* species complex is distributed throughout the holarctic region (PETERS 1988).

Nymphs of *Leptophlebia marginata* were collected from the Mullra-stream (M) in February 1992 and exposed in the Mullra-stream as well as in the Wispe (W) both upstreams and downstreams the Fe-rich groundwater influx. Groups of 20 nymphs were placed in net cages (20 × 10 × 80 cm^3) made of a wood frame with nylon nets (mesh size: 1 mm) on all sides (Fig. 2). The cages contained local sediment (gravel, sand) and fine detritus as food for *L. marginata.* The sediment had been dried (24 h, 80 °C) in order to remove all living animals or eggs. The cages were dug 5 cm into the stream sediment, but were sufficiently high that, even during high water levels one third of the cage would still be above the water level. During extremly low water situations the animals had access to sediment pore water. The construction assured that the animals got sufficient water and oxygen even if the net was clogged with Fe-precipita-

Table 2. Composition of microorganisms in the Wispe and Mullra-stream.

Wispe			Mullra-stream		
FPOM (ppm)	Medium 1 (CFU)	Medium 2 (CFU)	FPOM (ppm)	Medium 1 (CFU)	Medium 2 (CFU)
Sediment					
90	420	1000	143	10 000	1 000 000
Water					
1.5	30	680	12.9	10	200

Medium 1 for chemolithoautotrophic bacteria.
Medium 2 for all aerob bacteria.
CFU = Colony forming units per ml.

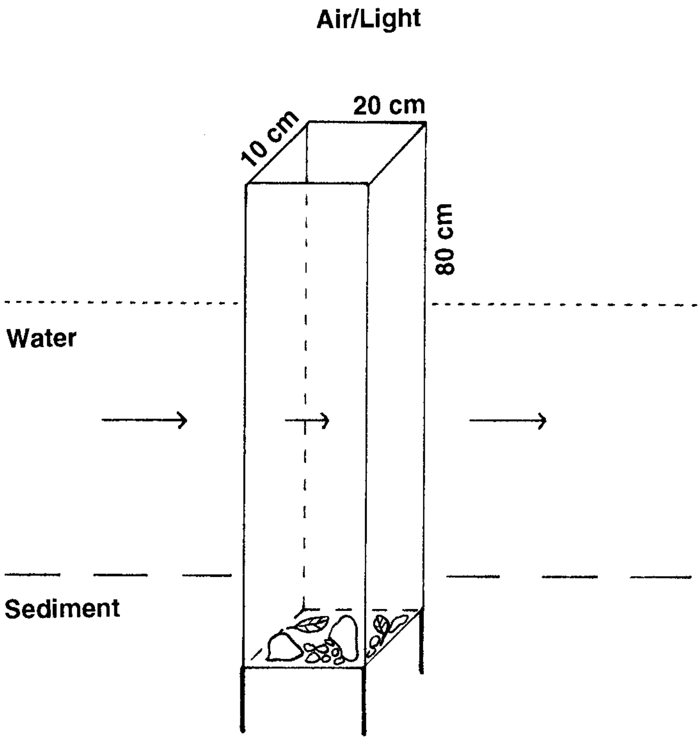

Fig. 2. The cages used for the field exposures, built of a wood frame with nylon nets (1 mm mesh size) on all sides.

tes. Tests of the cages during 6 weeks in the Mullra-stream showed that no macroinvertebrates (e.g. predators) from the stream penetrated into the cages. For the field experiment, six cages with 20 mayflies each were placed in the Mullra-stream and exposed for 8 and 12 weeks. Two cages were collected 8 and 12 weeks after exposure. In the Wispe, six cages with 20 mayflies each were placed upstreams and additionally six cages were placed downstreams the Fe-influx. After 8 and 12 weeks of exposure 2 cages from both sites were collected.

Treatment of the mayflies after exposure

Live nymphs from the cages were checked for Fe-precipitations on the head, thorax, abdomen and gills using 40× magnification. The extent of the incrustation was divided into 3 classes: 0, <50 %, >50 % (Fig. 3).

Three nymphs from each cage at each time were prepared for histochemical analysis of the localization of Fe^{2+} and Fe^{3+} in the body (Moewis 1978). The nymphs were dehydrated in an ethanol gradient and in xylol before embedding in paraffin. Transversal microtome sections (8 µm) through the animals were cut and mounted on slides, which were stained with $K_4[Fe(CN)_6]$ for Fe^{2+} and $K_3[Fe(CN)_6]$ for Fe^{3+} (Gerhardt 1992).

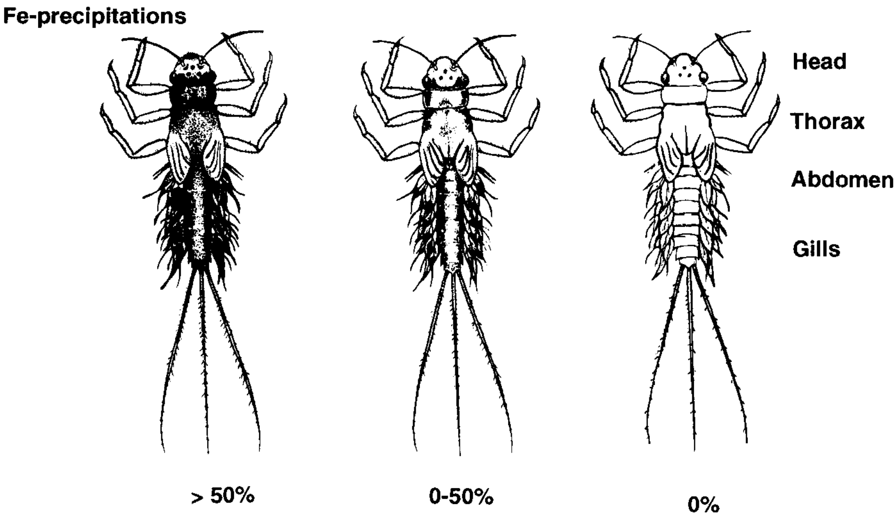

Fig. 3. Classification criteria for the extent of Fe-incrustation of the body of *L. marginata.*

About 5 animals from each cage were acid washed (0.1 M H_2SO_4 for about 10 min) to remove the $Fe(OH)_x$-precipitations from the body surface. These and five other nymphs were prepared for metal analysis with flame AAS (GERHARDT 1990, 1992). Fe_{tot} concentrations in the water samples were determined after acidification of the water to pH 2, which was done to preserve the sample and digest remaining particulate matter.

Statistical analysis

The parameters whole-body loads and internal uptake of Fe by the mayflies were analysed by one-factor ANOVA followed by pairwise comparisons with Tukey HSD tests. The groups were compared according their exposure place and time (Wispe above and below the Fe-influx and Mullra-stream). The proportion of Fe-precipitation on the body of the mayflies in relation to the exposure locations were compared by the use of Log-Likelihood Ratio tests (ZAR 1984).

Results

Comparison of microorganisms in the streams

The sediment of the Mullra-stream was much richer in bacteria, also in chemolithotrophic bacteria than that of the Wispe stream (Table 2). This indicates that in the Mullra-stream iron-oxidizing bacteria, belonging to the group of chemolithotrophic bacteria, may play an important role in Fe-speciation and bioavailability. The water, of both streams, however, contained similar amounts of bacteria.

Table 3. Statistical analysis (one-factor ANOVA and pairwise comparisons with Tukey HSD tests).

Anova	df	F	p		df	F	p
	Fe-load				internal Fe-uptake		
8 weeks							
Locations	2	11.16	0.001		2	11.8	0.001
Error	22				21		
12 weeks							
Locations	2	21.43	0.001		2	3.01	0.08
Error	15				17		
Tukey HSD		1	2	3	1	2	3
8 weeks							
	1	1			1		
	2	0.11	1		0.03	1	
	3	0.02	0.001	1	0.001	0.06	1
12 weeks							
	1	1					
	2	0.001	1				
	3	0.45	0.001	1			

1: Mullra-stream; 2: Wispe above Fe-influx; 3: Wispe below Fe-influx.

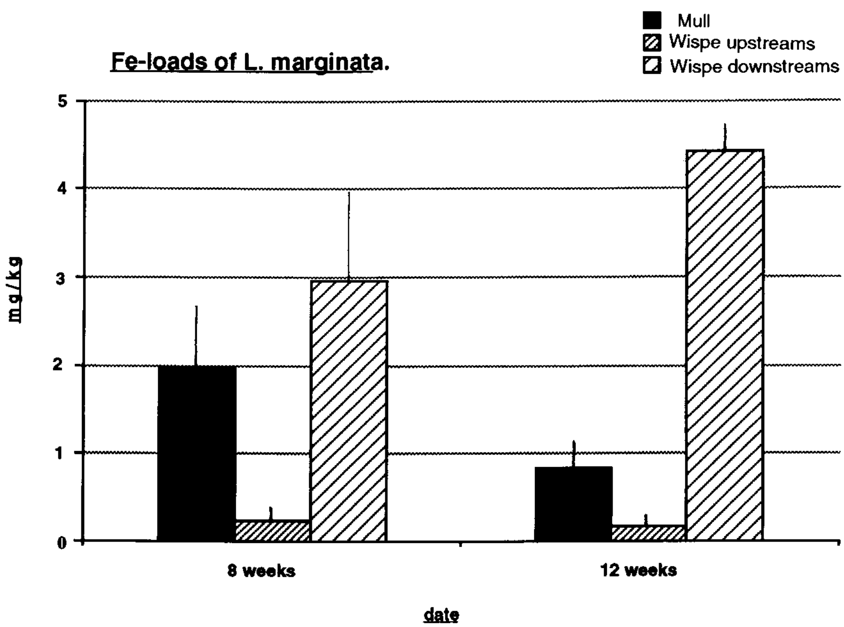

Fig. 4. Whole-body Fe-loads of *L. marginata* exposed in the Mullra-stream and Wispe.

Survival

Nymphs of *L. marginata* survived all exposure situations in all cages to 100%.

Fe-body loads

Significant differences in Fe-loads were found between the different exposure places after 8 and 12 weeks of exposure (Fig. 4) ($p < 0.001$, ANOVA). After 8 weeks of exposure, mayflies kept in the Wispe below the Fe-influx contained significantly more Fe than those kept above ($p < 0.001$, Tukey HSD) and those in Mullra-stream ($p = 0.02$, Tukey HSD). Moreover, mayflies kept in Mullra-stream contained more Fe than those kept in the Wispe above the Fe-influx ($p < 0.025$, Tukey HSD).

Fe-uptake

Mayflies exposed in the Mullra-stream showed a significantly higher internal Fe-uptake than those in the Wispe downstreams the Fe-influx after 8 weeks of exposure ($p < 0.001$, ANOVA). After 12 weeks of exposure, the differences were no longer significant ($p = 0.08$, ANOVA).

Fe-precipitations

The difference between the whole-body loads of Fe and the internal Fe-uptake revealed the amount of Fe which was externally adsorbed on the body of the mayflies. Thick crusts of Fe-precipitates on the mayflies' body surface accounted for a great part of Fe body load. These precipitates were more pronounced in the Wispe ($\bar{x}_{(r=2,\ n=5)}$: 80% of Fe-load) than in Mullra-stream ($\bar{x}_{(r=2,\ n=5)}$: 40% of Fe-load) after 8 weeks of exposure. However, after 12 weeks, mayflies in Mullra-stream molted just before sampling and no Fe-precipitations were found at all (Fig. 5).

A detailed analysis of the extent of the Fe-crusts on different body parts of the nymphs revealed that no body part was preferred. The precipitations were located randomly on all body parts ($p > 0.05$, Log-Likelihood Ratio tests). In general, the body parts were covered up to 25–50% by Fe-precipitations (Fig. 5).

Histochemical analysis

Microtome sections showed that *L. marginata* placed in the Wispe contained less particles in the gut than those kept in the Mullra-stream. While the gut contents of the nymphs in the Mullra-stream stained for Fe^{2+} and Fe^{3+}, that of

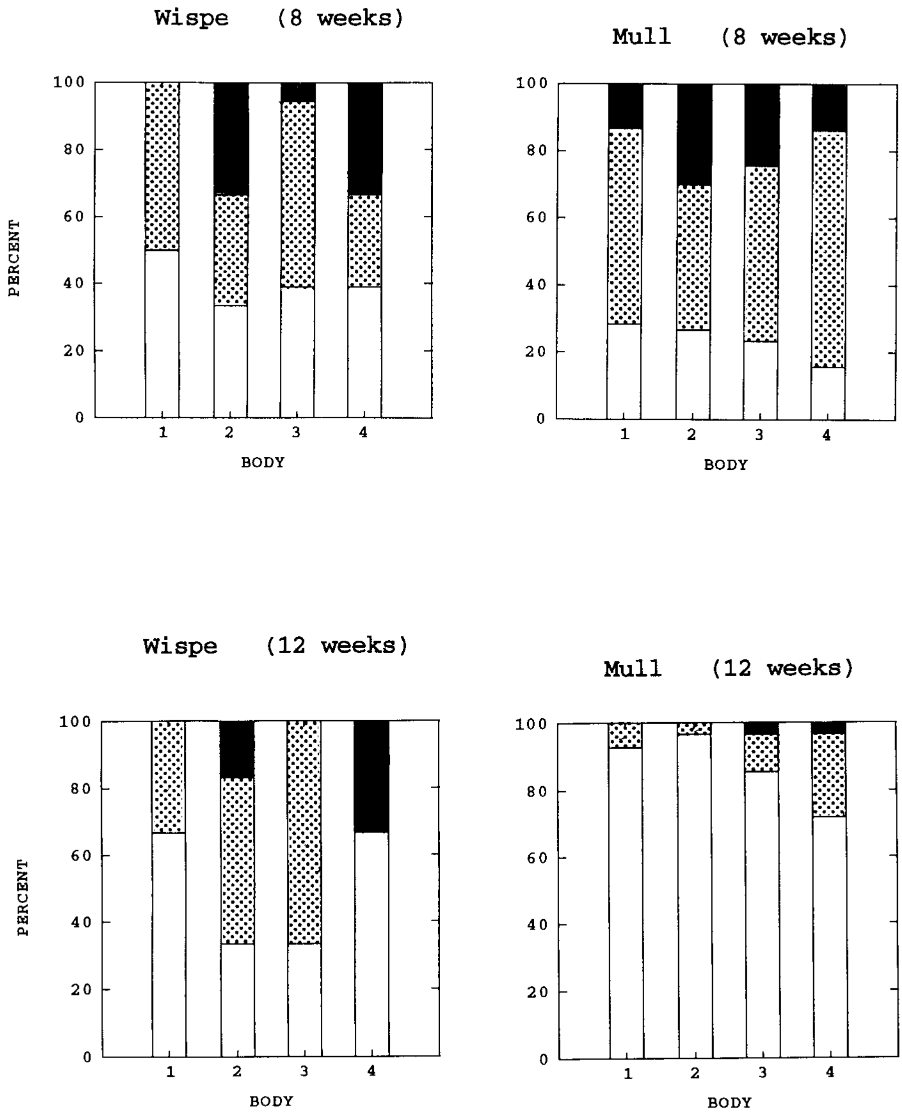

Fig. 5. Fe-crusts on different body parts of *L. marginata* from different sites of exposure.

animals in the Wispe contained less organic matter which stained only for Fe^{3+}. A thick crust of Fe^{3+} was found on the body surface of mayflies exposed in the Wispe while mayflies kept in the Mullra-stream had a crust of Fe^{3+} and Fe^{2+} on their body. Internally, only Fe^{2+} was found. Fe^{2+} was located in the eyes, tracheae and just before emergence also in the ovaries (Fig. 6).

Fig. 6. Transversal section through the abdomen of *L. marginata* from Wispe. – a) Localization of Fe in the gut and ovaries. b) Localization of Fe in the eyes. c) Localization of Fe in the tracheae.

Discussion

Fe is an essential element which is needed for accessory pigments in the eyes as well as for melanin production and cuticle formation in insects (LOCKE &

NICHOL 1992). Thus, slightly elevated concentrations of Fe in the water may stimulate these processes instead of being toxic, as Fe can be regulated by insects (LOCKE & NICHOL 1992). Fe-body loads of the mayflies and the proportion of Fe adsorbed on the body surface were higher in the Wispe than in Mullra-stream. However, internal uptake of Fe by the mayflies seemed to be higher in the Mullra-stream than in the Wispe. These results may be related to the occurrence of different Fe-species in the streams such as permanent Fe_2O_3 (ochre) precipitations in the Wispe and occasional $Fe_x(OH)_y$ coprecipitations with humic matter in the Mullra-stream. The low internal uptake of Fe may be explained by difficulties in grazing on a firm ochre layer that covered the sediment in the Wispe. In the Mullra-stream, however, coprecipitations of iron together with humic substances may have allowed for Fe-uptake together with organic particles, which are the main food source for *L. marginata*. Thus, Fe-compounds in the Mullra-stream might be more bioavailable than those in the Wispe. As the Fe-precipitations were only of occasional nature in the Mullra-stream, Fe-adsorption on the body surface of *L. marginata* was proportionally smaller compared to that in the Wispe. Fe^{3+} and Fe^{2+} form strong complexes with organic bases, e.g. tannic acid (WETZEL 1983, WELLS & MAYER 1991). This explains, why these Fe-species were found in the crusts on the mayflies from Mullra-stream, whereas only Fe^{3+} was found in the crusts on the mayflies exposed in the Wispe. As the amount of organic matter in the water and sediment of the Wispe was low, coprecipitation processes may be less important for Fe speciation than in the Mullra-stream. Fe-speciation in the Wispe may be mainly controlled by chemical oxidation processes, whereas microbial processes may contribute to Fe-speciation in Mullra-stream, due to the higher content of microorganisms in the sediment.

In both streams, Fe-precipitation occurred randomly onto the different body parts of *L. marginata*. Molting may be a mechanism to get rid of the Fe-crusts on the body, as newly molted mayflies showed no precipitations on their bodies. According to KJELLBERG (1972) the closely related species *L. vespertina* molted usually every 10 to 14 days. Such short intermolt phases may be the reason that *L. marginata* nymphs were not covered more than 50 % with Fe-precipitations, which did not seem to affect survival of the nymphs in the cages.

In conclusion, neither Fe-concentrations of up to 1.64 mg $Fe_{tot} l^{-1}$ in the water nor Fe-precipitations of different chemical forms on the sediment and invertebrates did affect *L. marginata* during an exposure of 3 months. *L. marginata* is one of the few fine particle feeding mayfly species, which may be less affected by Fe-incrustations on the sediment than grazers such as *Epeorus sylvicola, Rhitrogena iridina, Baetis* sp., which did not occur in the Wispe downstreams the iron rich groundwater influx. Grazers were also reduced by Fe-precipitations due to the absence of epiphyton in a Danish stream (RAS-

MUSSEN & LINDEGAARD 1988). Detritus-feeders, especially shredders (Plecoptera) seem to be less affected by Fe-precipitations (SCULLION & EDWARDS 1980), probably because they rely on an allochthonous food source. Also in the Wispe, the number of plecopteran species was only slightly reduced from 24 species upstreams to 20 species downstreams the Fe-influx. Indirect effects of Fe-incrustation such as prevention of epilithic algae, clogging of the sediment and impairment of exchange processes between the hyporheic interstitial and the water column may be more important for the occurrence of an invertebrate species in an iron-rich stream than direct toxic effects of Fe-ions and Fe-hydroxides on the animals' body.

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