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# Effects of Subacute Doses of Cadmium on pH-stressed *Leptophlebia marginata* (L.) and *Baetis rhodani* Pictet (Insecta: Ephemeroptera)

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*ABSTRACT*

*Cd uptake, emergence, survival and locomotory activity at two pH levels were studied in a 27-day experiment with* Leptophlebia marginata *and in a 19-day experiment with* Baetis rhodani. *Model ecosystems containing recirculating stream water, patches of sediment and leaves, simulated a natural stream. During the experiments, concentrations of Al, Cd, Fe and Zn increased in the water at pH 5, while conductivity decreased. Cd was taken up by both species at a rate which indicated linear uptake kinetics. No steady state of Cd in the animals was observed. The larvae of* B. rhodani *contained significantly more Cd than the adults (*$p < 0{\cdot}001$*). Uptake of Cd by* Baetis rhodani *was higher at pH 7 than at pH 5 (*$p < 0{\cdot}05$*). The survival of* L. marginata *was not influenced by pH and Cd stress, but emergence was significantly reduced (*$p < 0{\cdot}05$*). The survival and the emergence of* B. rhodani *were reduced by low pH and also by Cd addition (*$p < 0{\cdot}01$*). At pH 7, Cd had no significant adverse effect on survival. Locomotory activity of* Baetis rhodani *was reduced by low pH and additionally by Cd stress.*

## INTRODUCTION

Knowledge of the combined effects of acidification and heavy metal toxicity on invertebrates is limited (Campbell & Stokes, 1985), although the effects of pH and Al on invertebrates are well known (Raddum & Fjellheim, 1984;



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Herrmann & Andersson, 1986; Ormerod *et al.*, 1987; McCahon *et al.*, 1989). Most toxicity studies have been performed with fish or *Daphnia* sp. The majority of them involve single $LC_{50}$ or short time mortality tests and results vary widely because of different test species and experimental designs (e.g. Rehwoldt *et al.*, 1973; Thorp & Lake, 1974; Ravera, 1984; Williams *et al.*, 1984).

Although the importance of sublethal effects of metals has been known for several years, little work has been done in which hatching, moulting, emergence, reproduction or behaviour have been examined (e.g. See, 1976; Best & Morita, 1983; Colborn, 1987).

The aim of the present study was to investigate the effect of subacute doses of Cd at two pH levels on uptake, emergence, locomotory activity and survival of mayflies.

## MATERIALS AND METHODS

### Animals

Mayflies are generally pH-sensitive and accumulate more Cd than other aquatic insects (Burrows & Whitton, 1983). *Leptophlebia marginata* (L.) is moderately abundant in lakes and slower parts of running waters. It is univoltine with a synchroneous emergence (April–June). *L. marginata* will not occur in streams with a pH below 4·5 (Økland & Økland, 1986). *Baetis rhodani* Pictet is common in rapid parts of rivers and small streams with a wide tolerance of water quality ($\beta$-oligosaprob to $\alpha$-mesosaprob) (Mueller-Liebenau, 1969). *Baetis rhodani* is bivoltine (Bengtsson, 1973) and pH sensitive. It will not survive in streams with a pH minimum below pH 5 (Harmanen, 1980). However, it tolerates chronic pH values of pH 5·2 (McCahon *et al.*, 1989). Both species feed on fine particulate organic matter (FPOM); *B. rhodani* also on microalgae (Mueller-Liebenau, 1969).

### Experimental design

To simulate a natural stream, continuous flow systems were constructed using circular glass aquaria. Each system contained stream water, which had been collected from a small stream in South Sweden in spring 1989 and was led from a 10-litre tank and circulated through three replicate aquaria at a flow rate of *ca.* 20 cm/s. The animals were collected from the same stream. The aquaria were additionally aerated and contained patches of sand, stones and leaves. The mean water temperature was 12°C (sd = 1°C) in the experiment with *L. marginata* and 9·6°C (sd = 0·6°C) in the experiment with *B. rhodani*. The daily light regime was a 16 h day. Since the sediment and stream water contained detritus and algae in sufficient quantities, no additional food was given. Stream water in the tanks was changed after 2 weeks to suppress fungal growth in the system. The pH in the systems was adjusted to the nominal values of pH 5 and 7, which remained stable, as can be seen below.

The animals were kept in triplicate under the following different conditions:

| *pH regime* | *Cd exposure* | *Symbol* |
|---|---|---|
| *L. marginata* | | |
| pH 6·9 (sd = 0·16) | no Cd addition | (control 1) |
| pH 5·2 (sd = 0·12) | 0·2 mg litre$^{-1}$ Cd | (pH 5/Cd high) |
| *B. rhodani* | | |
| pH 7·2 (sd = 0·13) | no Cd addition | (control 2·1) |
| pH 7·2 (sd = 0·10) | 0·02 mg litre$^{-1}$ Cd | (pH 7/Cd low) |
| pH 5·2 (sd = 0·13) | 0·02 mg litre$^{-1}$ Cd | (pH 5/Cd low) |
| pH 5·2 (sd = 0·10) | no Cd addition | (control 2·2) |

**Parameters**

Cd was chosen because of its toxicity to aquatic organisms, its widespread occurrence and its tendency to leach from soils under acidic conditions (Monitor, 1987). The mayfly *L. marginata* was exposed to a high metal concentration (0·2 mg litre$^{-1}$) because of its general tolerance to pollutants, e.g. Cd, where an $LC_{50}$ (120 h) of 7 mg litre$^{-1}$ (pH 7) and 9·0 mg litre$^{-1}$ (pH 5) was found (A. Gerhardt, unpublished). *B. rhodani* was exposed to a lower metal concentration (0·02 mg litre$^{-1}$) because toxicity data reported in the literature showed it to be less tolerant $LC_{50}$ (96 h):0·7 mg litre$^{-1}$ (Williams *et al.*, 1985), $LC_{50}$ (120 h) at pH 5:1·8 mg litre$^{-1}$ and at pH 7:1·5 mg litre$^{-1}$ (A. Gerhardt, unpublished).

Cd was determined in living larvae in pooled samples from three aquaria at the beginning and the end of the experiments. The level of Cd was also determined in larvae that died during the exposure.

Cadmium, added to the water tanks as $3CdSO_4 . 8H_2O$, was measured every second or third day and adjusted to the nominal concentration three times in the experiment with *B. rhodani* and once in the experiment with *L. marginata*. It was determined by flame AAS (detection limit 1 $\mu$g litre$^{-1}$).

Al and Zn were also determined because of their tendency to leach during acidification. Fe was investigated because there were elevated concentrations in the stream (1·4 to 3·6 mg litre$^{-1}$) compared to the general

**TABLE 1**
Data on Water Chemistry and Concentrations of Total Metals in the Unfiltered Water and Animals before the Experiments

| | *pH* | *Temp.* (°C) | *Cond.* (µS) | *Hardn.* (mmol litre$^{-1}$) | *FPOM* (mg litre$^{-1}$) | *Fe* (mg litre$^{-1}$) | *Al* (mg litre$^{-1}$) | *Zn* (mg litre$^{-1}$) | *Cd* (mg litre$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| water | 6·71 | 6·0 | 235 | 0·5 | 14 | 1·48 | 0·2 | nd | nd |
| *L. marginata* (*n* = 9) | | | | | | 21·30 | 0·8 | 0·2 | 0·002 |
| water | 7·06 | 12 | 100 | 0·4 | 5·3 | 3·69 | 0·1 | nd | nd |
| *B. rhodani* (*n* = 14) | | | | | | 4·57 | 0·2 | 0·6 | 0·008 |

nd = not detected with ICPES (detection limits: Zn: 4 µg litre$^{-1}$, Cd: 1 µg litre$^{-1}$) or AAS (detection limit: Cd: 1 µg litre$^{-1}$).
Metal concentrations in the animals are given in mg g$^{-1}$ DW (dry weight).

background of 0·8 mg litre$^{-1}$ (Monitor, 1987), because of its tendency to precipitate onto the sediment in the brook, and its capacity to adsorb other metals (Allard *et al.*, 1987).

pH was determined twice a day. If deviations from the expected mean exceeded 0·1 pH units, adjustments were made with 0·1M $H_2SO_4$ or 0·1M NaOH. The use of buffers was avoided because this would have affected ionic strength and metal speciation.

FPOM (<1 mm) in the water was gravimetrically estimated after filtration (Whatmann GFC:0·45 µm pore size), drying (24 h, 80°C) and ashing (4 h, 550°C).

Table 1 gives some chemical parameters of the stream water and total metal concentrations in the water and the animals. This indicates elevated levels of Al and especially Fe, which was precipitated on the thorax of *L. marginata*.

Cd uptake, emergence and survival were calculated daily, locomotory activity was measured twice a day as the numbers of passages of an animal over a marked line in the aquarium per hour. Locomotory activity was defined as any sort of movement of the animals, including downstream drift, upstream movement and food searching behaviour.

## Statistical analysis

All data represent measurements along a time scale. A test of autocorrelation (Durbin Watson test, *D*-test) revealed a significant positive autocorrelation for all time dependent measurements ($D < 0·8$). The Mann Whitney *U*-test was used to reveal significant differences between the different treatment groups.

# RESULTS

## Changes in chemical parameters

Because of seasonal changes in waterflow and chemistry of the stream water, the water used in the two experiments was not exactly of the same quality (Table 1). During the experiments, conductivity decreased with the exception of the pH 7/Cd low treatment in the experiment with *B. rhodani*, Fe, Zn and Al increased at pH 5 (Tables 1 and 2) independently of Cd addition.

Cadmium concentrations in the water were easily held constant in the experiment with *L. marginata*, where the dosing was high, while they varied in the experiment with *B. rhodani* because of low Cd addition (pH 5:Cd mean = 19·8 $\mu$g litre$^{-1}$ standard deviation (sd) = 5·2 $\mu$g litre$^{-1}$; pH 7:Cd mean = 7·7 $\mu$g litre$^{-1}$; sd = 3·6 $\mu$g litre$^{-1}$) (Figs 1 and 2). At pH 5, Cd concentration in the water became twice that at pH 7, in spite of the addition of the same concentration, perhaps because of increased precipitation on the aquaria walls at high pH.

## Cd uptake

Uptake in this context is defined as uptake into and adsorption onto the organism. *L. marginata* showed a low initial Cd concentration (0·002 mg g$^{-1}$ dw) in comparison with the Cd addition (0·2 mg litre$^{-1}$). The uptake kinetics following a linear model (Fig. 3). No steady state was observed during the experiment. After 19 days of Cd exposure, the bioconcentration

**TABLE 2**
Conductivity and Total Metal Concentrations of the Water at the End of the Experiments

| | *Cond.* (*$\mu$S*) | *Fe* (*mg litre$^{-1}$*) | *Al* (*mg litre$^{-1}$*) | *Zn* (*mg litre$^{-1}$*) | *Cd* (*mg litre$^{-1}$*) |
|---|---|---|---|---|---|
| *L. marginata* experiment | | | | | |
| control 1 | 84 | 5·10 | 0·13 | nd | nd |
| pH 5/Cd high | 189 | 3·60 | 0·24 | 0·17 | 0·22 |
| *B. rhodani* experiment | | | | | |
| control 2·1 | 100 | 3·80 | 0·15 | nd | nd |
| pH 5/Cd low | 126 | 3·93 | 0·31 | 0·24 | 0·050 |
| pH 7/Cd low | 42 | 5·60 | 0·03 | nd | 0·023 |
| control 2·2 | 100 | 5·20 | 0·14 | nd | nd |

nd = not detected (detection limit of ICPES: Zn: 4 $\mu$g litre$^{-1}$, Cd: 1 $\mu$g litre$^{-1}$).

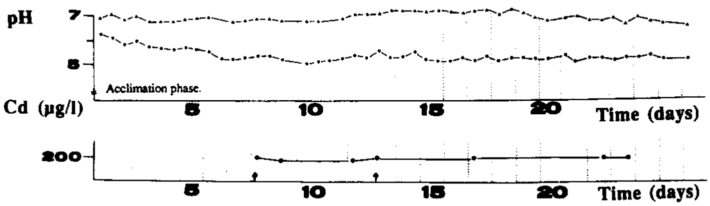


**Fig. 1.** pH values and concentration of total Cd (µg litre$^{-1}$) in the water during the experiment with *L. marginata* in control 1 (pH 7, no Cd addition) (▲) and pH 5/Cd high treatment (●). Each treatment group consisted of three replicates. Adjustments of Cd to the nominal concentration of 200 µg litre$^{-1}$ (↑).

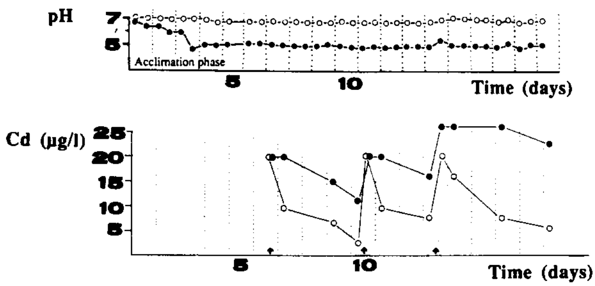


**Fig. 2.** pH values in the treatments pH 5 (●, △) and pH 7 (○, ▲) and concentrations of total Cd (µg litre$^{-1}$) in the water during the experiment with *B. rhodani*. The following treatments, each consisting of three replicates, were used: control 2·1. (pH 5) (△), pH 5/Cd low (●), pH 7/Cd low (○), control 2·2. (pH 7) (▲). Adjustments of Cd to the nominal concentration of 20 µg litre$^{-1}$ (↑).

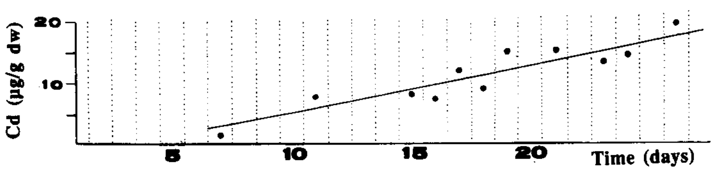


**Fig. 3.** Cd uptake by larvae of *L. marginata* (µg g$^{-1}$ dw) at pH 5 during the experiment. Cd concentration was measured in pooled samples of the three replicates.

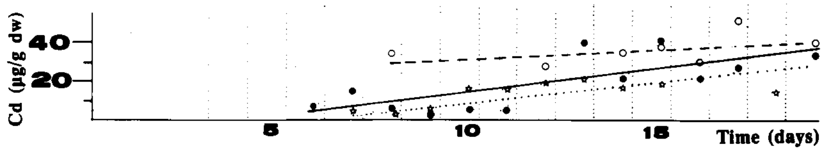


**Fig. 4.** Cd uptake by larvae of *B. rhodani* (µg g$^{-1}$ dw) at pH 5 (●) and pH 7 (○) during the experiment. Cd uptake by adults of *B. rhodani* at pH 7 (☆). At pH 5, no further animals emerged after 4 days after Cd addition. Cd concentration was measured in pooled samples of the three replicates in each treatment group.

factor (BCF) was 8·6 (pH 5) and 7·3 (pH 7) ($n = 9$). The BCF was calculated as $(Ca - Ck).\ Cw^{-1}$, where $Ck$ and $Ca$ are the concentrations in the animals at the beginning and at the end of the experiment, respectively. $Cw$ denotes the concentration of Cd in water (Taylor, 1983).

*B. rhodani* showed a higher initial concentration of Cd 0·008 mg g$^{-1}$ dw (dw = dry weight, $n = 14$) in comparison with the following Cd exposure (0·02 mg litre$^{-1}$). At pH 7, larvae contained significantly more Cd than adults ($p < 0{\cdot}001$, $U$-test) (Fig. 4) and Cd uptake was twice as high as at pH 5 ($p < 0{\cdot}01$, $U$-test). The BCF of *B. rhodani* larvae after 13 days of Cd exposure was 1·3 at pH 5 and 7·1 at pH 7.

## Emergence

More larvae of *L. marginata* emerged in the controls than in the pH 5/Cd high treatment ($p < 0{\cdot}05$, $U$-test) (Fig. 5). Emergence of *B. rhodani* was significantly lower at pH 5 than at pH 7 ($p < 0{\cdot}01$, $U$-test) irrespective of Cd addition (Fig. 6).

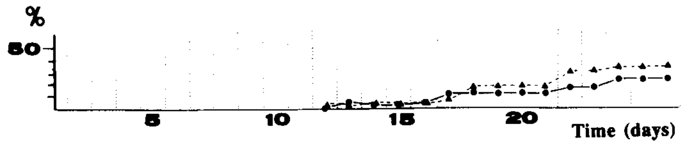


**Fig. 5.** Percentage of emergence of *L. marginata* in the control (▲) and pH 5/Cd high treatment (●) as a summation diagram. The values represent means of three replicates.

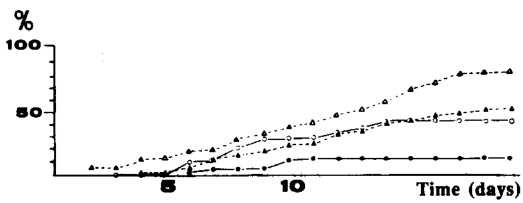


**Fig. 6.** Percentage of emergence/moulting of *B. rhodani* in control 2.1. (△) (moulting into the last instar), control 2.2. (▲), pH 7/Cd low (○) and pH 5/Cd low (●) treatment as summation diagram. The values represent means of three replicates.

## Survival

pH and Cd-stressed animals of *L. marginata* survived better than the controls ($p < 0{\cdot}05$, $U$-test) (Fig. 7).

During the acclimation phase, survival of *Baetis rhodani* varied between the two groups held at low pH. Because of acid stress, the survival of the treated animals at low pH was lower than in the controls ($p < 0{\cdot}05$, $U$-test). After Cd addition, survival in the pH 5/Cd low treatment was significantly

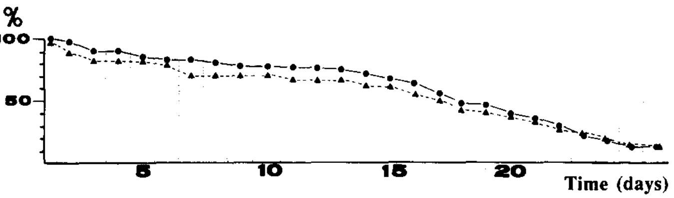


**Fig. 7.** Percentage of survival of untreated (▲) and pH 5/Cd high (●) stressed *L. marginata* during the experiment. The values represent means of three replicates.

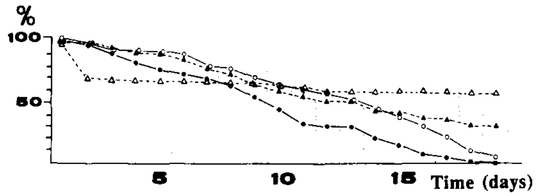


**Fig. 8.** Percentage of survival of *B. rhodani* in the different treatments: control 2.1. (△), control 2.2. (▲), pH 5/Cd low (●), pH 7/Cd low (○). The values represent means of 3 replicates.

lower than that in the pH 7/Cd low treatment ($p < 0{\cdot}05$, *U*-test). Moreover, survival in the control groups at both pH values differed significantly from each other ($p < 0{\cdot}01$, *U*-test). However, if one compares survival of animals held at low pH, with and without Cd addition, one notices a generally expressed significant difference ($p < 0{\cdot}005$, *U*-test) (Fig. 8).

## Locomotory activity

After an initial phase of high activity in both species because of handling stress and pH decrease, locomotory activity of *Baetis rhodani* was reduced by

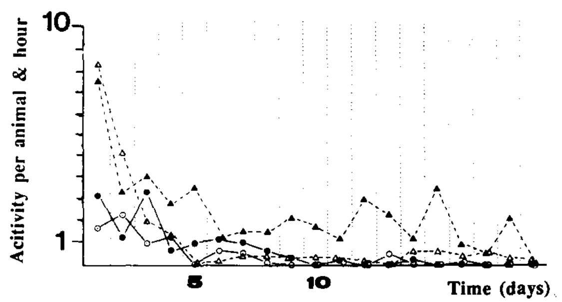


**Fig. 9.** Locomotory activity (all sorts of movement) of *B. rhodani* during the experiment in the different treatments: control 2.1. (△), control 2.2. (▲), pH 5/Cd low (●), pH 7/Cd low (○). The values represent daily means of two observations and are means of three replicate aquaria. Activity is given as number of passages of animals per hour and animal.

low pH and by Cd stress ($p < 0.01$, *U*-test). Only animals held at pH 7 without Cd addition showed a pattern of phases with elevated locomotory activity until the end of the experiment (Fig. 9). The locomotory activity of *L. marginata* was not affected by low pH and Cd.

## DISCUSSION

### Exposure conditions

The seasonal changes in the quality of the water used for the experiments (e.g. FPOM content) may have caused different bioavailability of Cd to the organisms and therefore, may have influenced Cd uptake.

The elevated Fe concentrations in the water are typical for the stream and animals living there may be habituated to this. At pH 7, the Fe levels decreased during the experiment, perhaps because of precipitation processes. This may also have reduced the bioavailability of Cd by means of coprecipitation. Iron hydroxide precipitation on organisms may have deleterious effects on invertebrates (McKnight & Feder, 1984). Fe precipitation on invertebrates was observed in the stream where the animals were collected. However, no visual precipitations occurred during the experiments, probably because the exposure time was too short. McCahon *et al.* (1989) observed elevated Fe concentrations in a stream after artificial acidification, but this had no additional effect on mortality of *B. rhodani* after 48 h.

During the experiments, the Al concentrations were below 0·32 mg litre$^{-1}$, even at pH 5. Similar values were observed in a stream with a mean pH of 5·2 (McCahon *et al.*, 1989), where both of the species used in my experiments were found. Weatherley and Thomas (1989) observed, after acidifying a stream, that at pH values between 5·5 and 5·0 Al concentrations were *ca.* 0·2 mg litre$^{-1}$, while at lower pH (5·0 to 4·5) Al increased drastically to $>$0·4 mg litre$^{-1}$, which indicates that the mobility and increasing toxicity of Al may affect the animals only below pH 5. However, such pH levels were not used in the presented experiments. Ormerod *et al.* (1987) observed increasing drift of *B. rhodani* in an acidified stream at Al concentrations of 0·4 to 1·3 mg litre$^{-1}$ at pH 5. Such concentrations of Al neither occurred in the experiments, nor were measured in field. It may be concluded that the effects of Fe and Al on the animals can be neglected because the values are below the levels of adverse effects and the animals are habituated to the metal levels used in the experiments. Moreover, the increase in Cd, because of Cd addition, was 10- to 100-fold, which may account for all adverse effects observed in the experiments.

During the experiments, conductivity decreased, indicating a loss of free ions probably as a result of adsorption and precipitation processes. However, at pH 5 the conductivity increased in the experiment with *B. rhodani* and this may have been a result of a remobilisation of metal ions from the sediment (Allard *et al.*, 1987; Broberg & Lindgren, 1987). This may indicate that the substrate can serve as ion sink as well as ion source.

## Cd uptake

The different bioconcentration factors of Cd at pH 5 in the two species may be due to species dependent accumulation rates and tolerances (Petersen, 1986), as well as Cd concentration in the water. Moreover, the surface/volume ratio may affect metal concentrations of the species caused by surface adsorption (e.g. *L. marginata*: Fe). However, the number of moultings, which may differ between the two species may counteract this effect.

Cd uptake in both species is characterized by a linear regression model. This observation is supported by results of v. Hattum *et al.* (1989), where *Asellus aquaticus* accumulated Cd according to a linear model during the first 20 days. No steady state was reached after 19 days (*Leptophlebia marginata*) or 13 days (*Baetis rhodani*) in my experiments, nor in those of v. Hattum *et al.* (1989) after 30 days (*Asellus aquaticus*). Cd uptake by *B. rhodani* at pH 5 was slow (BCF: 1·3) and delayed (*ca.* 5 days), while at pH 7 elevated Cd concentrations could be measured immediately and the BCF was 7·1. This indicates the importance of surface adsorption of Cd onto the animals, which may be faster than uptake into the organism. Cd concentrations in the animals varied remarkably because of the great size variation among the individuals (4–10 mm). Whilst the concentration of Cd in the water became twice as high at pH 5 as at pH 7, the Cd uptake was higher at pH 7 than at pH 5 (Fig. 4), which also indicates fast precipitation processes at pH 7. No significant effects of pH (pH range: 5·9 to 7·6) on uptake of aqueous Cd were found by v. Hattum *et al.* (1989), when studying isopods. The elevated Cd concentrations in animals at pH 7 can be explained as follows:

1. At pH 7, precipitation and adsorption of the metal onto the exoskeleton (presumably together with Fe hydroxide) may be the dominant uptake mode. This is confirmed by the fact that larvae contained more Cd than adults, and the faster initial uptake of Cd at pH 7 than at pH 5. Emergence and moulting may be mechanisms to decrease the metal content in the animal. Similar observations were made by Krantzberg & Stokes (1988) and Colborn (1987).
2. Because of precipitation processes, Cd may accumulate in the sediment

to a greater extent at pH 7 than at pH 5, which will increase exposure to animals feeding on sediment particles. Studies of the importance of metal uptake from soluble and dietary sources are scarce for freshwater invertebrates. pH-dependent variations in the two uptake modes have been observed by Lewis and McIntosh (1986).

3. Although direct uptake from the water may be more important at low pH, because of increased concentrations of $Cd^{2+}$ ions in the water and the great amount of water passing respiration organs, metal ions probably compete with $H^+$ ions at the binding sites, which may counteract metal uptake from the water (Broberg & Lindgren, 1987). An ameliorating effect of $H^+$ ions (pH 4·5) for toxicity of Al (mortality) was also observed for *B. rhodani* (Herrmann, 1986).

## Emergence and survival

*L. marginata* seems to be a Cd- and pH-tolerant species since its emergence was only slightly decreased under chemical stress. The species tolerated pH 4·8 (0% mortality after 25 days) and 40% of the animals survived at pH 4 for 25 days (Herrmann, 1986). The animals survived even better under chemical stress than in the controls, perhaps because of decreased fungal growth at the low pH. However, survival in the controls also decreased, which indicates adverse effects of other stress factors, such as insufficient food supply. Willoughby (1988) investigated survival of *B. rhodani* on different food items and without food. Unfed animals died within 2 weeks while animals fed on a specific algae survived over 60 days. In spite of the increased mortality of unfed animals, differences due to pH were found, however, indicating reduced survival at pH $< 5{\cdot}0$, confirming the results of the present study with *B. rhodani*. Emergence and survival of *B. rhodani* were mainly influenced by low pH, and additionally by Cd, since significant differences were found between the pH 5/Cd low treatment group and the pH 5 control, but not between the pH 7/Cd low treatment group and the pH 7 control. Økland and Økland (1986) reported that 50% of the animals held in circular aquaria survived a pH of 4·5 for only 4·5 days. McCahon *et al.* (1989) observed that mortality at pH 4·9 was 14% after 48 h, while that at pH 4·7 and 0·4 mg litre$^{-1}$ Al it was 20%, which may indicate that pH alone accounts more for mortality than Al. Willoughby (1988) suggested moulting to be a sensitive stage for pH stress. The same may be true for emergence (Bell, 1971). During moulting and emergence the animals have an increased ion exchange through the integument until the end of the sclerotisation process. Thus, loss of $Na^+$ and a detrimental impact of increased $H^+$ concentrations can take place all over the body, which may cause severe damage to *Baetis rhodani* in these sensitive phases.

## Activity

Activity proved to be an appropriate parameter for subacute chemical stress. During the first days of pH stress, *B. rhodani* showed high activity, perhaps in order to try to get away from stress. But as stress (pH and Cd) became chronic, the animals' activity declined. Raddum and Fjellheim (1984) observed that the drift of *B. rhodani* in artificial running water trays increased during the first 3 days of acid stress. Ormerod *et al.* (1987) observed the same after acidification and addition of Al to a stream. Drift was elevated, however, only in the Al zone and not by low pH alone. During chronic stress (acid, Al), drift and activity were reduced in fish (Muniz & Leivestad, 1980) and zooplankton (Havas, 1985). Reduced activity may affect feeding behaviour and the animals will die of starvation, in the end.

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