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Monitoring Behavioural Responses to Metals in *Gammarus pulex* (L.) (Crustacea) with Impedance Conversion

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Abstract

An impedance conversion technique was used to study the behaviour of *Gammarus pulex* (L.) exposed to acutely toxic concentrations of Pb (0.01, 0.05, 0.1 and 0.5 mg Pb l^{-1}) and to field concentrations of Cu ($\leq$ 0.05 mg Cu l^{-1}). Initial stress responses were studied during short-term exposure (1 h) and sublethal toxic effects were monitored during 7 (Pb) and 35 days (Cu), respectively.
Exposure to Pb caused 30 % mortality and resulted in a bioconcentration factor (BCF) of 2700 at 0.5 mg Pb l^{-1} after 168 h. Exposure to Cu polluted stream water caused no mortality within 35 days and uptake was low (BCF 5.8).
Gammarus pulex reacted with initial stress responses to metal exposure within 30 min. (Cu) or 1 h (Pb). The reactions consisted of increased ventilation and decreased locomotion.
Sublethal concentrations of Pb and Cu caused toxic effects on the behaviour of G. *pulex* after several days of exposure, consisting of increased ventilation and decreased locomotion.
Impedance conversion is an appropriate method for detecting stress responses to metals and can be used in "early warning" biomonitoring systems as well as for acute and chronic behavioural toxicity testing.

Key words: Biomonitoring; exposition; *Gammarus pulex;* crustacea; metals: Cu, Pb; impedance-conversion technique; Pb $(NO_3)_2$

1 Introduction

Behaviour is the final outcome of a sequence of neurophysiological events including stimulation of sensory and motor neurons, muscular contractions and release of chemical messages (LAGADIC et al. 1994). According to the **Stimulus-Integration-Response model (SIR)** any overt behavioural response may be seen as a result of integrating external abiotic and biotic stimuli (SCHERER 1992). Behavioural responses integrate many cellular processes vital to an organism's survival and reproduction, thus reflecting both biochemical and ecological consequences of toxic impact (JANSSEN et al. 1994). Changes in behaviour appear to be among the most sensitive indicators of environmental alterations (WARNER 1967; ATCHISON et al. 1987; BEITINGER 1990).

Behavioural responses to pollutants can generally be of two different characters:

1) A trial to maintain homeostasis in the body by compensatory responses due to "loading stress", which increases the cost of maintenance,
2) an overt effect at toxic concentrations above the regulative capacity, caused by "limiting" stress.

These two categories of environmental stress have been defined in terms of oxygen transport and aerobic metabolism (WILSON et al. 1994), but might be used in a broader sense. This makes behavioural parameters to come into focus in aquatic toxicity testing (toxic effects) and biomonitoring of water pollution (compensatory initial stress responses).

Biomonitoring has several advantages over chemical monitoring in the continuous survey of pollutants in streams. Biomonitoring results are more relevant than chemical monitoring because

(1) it takes the whole mixture of toxicants into account, including synergistic or antagonistic effects,
(2) it puts the organism in focus instead of giving concentration levels, which do not say anything about toxic effects at the organism-, population- or ecosystem level and

(3) it allows for temporal integration of the effects of different pollutant exposures throughout the life cycle of an organism.

Chemical monitoring may be relevant only where there is a defined source and the concentration dependency of the effect of the chemicals at that source is known. Biomonitoring methods allow measurement of initial stress reactions to pollution peaks as well as physiological effects of pollutants on organisms after chronic exposure.

With the technical development during the last decade it has become possible to record behavioural patterns on an automated continuous real-time basis and on-line biomonitoring systems have been developed using physiological or behavioural responses of fish, *Daphnia* sp. and mussels as parameters (GERHARDT et al. 1994; GRUBER & DIAMOND 1988). The choice of test organism is crucial for the sensitivity and reliability of the system. Many of the existing test systems monitor acute toxicity, however none works reliably when sublethal pollution levels have to be detected (GRUBER & DIAMOND 1988).

A new test system, based on an impedance conversion technique has been developed and is capable of simultaneously recording different kinds of behaviour of a great number of aquatic species (GERHARDT et al. 1994). *Gammarus pulex* was found to be an appropriate test organism in this system, being geographically widely distributed, easily cultured and displaying different kinds of behaviour which are sensitive to pollutants.

In this paper the behavioural responses of *Gammarus pulex* to acutely toxic concentrations of Pb and sublethal concentrations of Cu were investigated. Initial early stress responses during short term exposure as well as toxic effects of the metals due to chronic exposure were measured. Pb was chosen because it represents one of the most widespread and toxic metals (BERGBÄCK et al. 1992; GOYER 1993), whereas Cu represents a widely used, essential micronutrient, being incorporated into haemocyanin, which is responsible for oxygen binding and transport in crustaceans.

2 Material and Methods

2.1 Test Organism

Gammarus pulex (L.) was chosen as an experimental animal because it is frequent in running waters, where it is an important link in the aquatic foodweb decomposing detritus and serving as prey for both invertebrate and vertebrate predators (GARMENDIA TOLOSA & AXELSSON 1993). *Gammarus pulex* is sensitive to a wide range of toxicants (WILLIAMS et al. 1984) and has been recommended for use in toxicity tests both in the laboratory and in the field (PASCOE et al. 1994). Several new toxicity tests using G. *pulex* as a test organism are under development with endpoints such as feeding activity (TAYLOR et al. 1993), precopula separation (PASCOE et al. 1994) and scope for growth (MALTBY et al. 1990). These underline the increasing importance of G. *pulex* in aquatic toxicology.

2.2 Sampling Sites

The organisms were sampled from Skäralidbäcken, an unpolluted first order stream in the province of Scania in Southern Sweden (→ *Fig. 1*). This stream runs through deciduous forest areas and the substrate contains gravel. The streambed is not anthropogenically affected and there are meanders with pool sites and detritus leaf packs, which are the habitats preferred by G. *pulex*. Metal levels are around the natural background values and the water is of circumneutral pH, which make the stream an excellent control site (Cu: 2.0 $\mu g l^{-1}$; Pb: 1.0 $\mu g\ l^{-1}$; Fe: 0.84 mg l^{-1}).

The water for the experiments was collected from Ståstorpsbäcken, a small sandy stream in an agricultural area in the province Scania (→ *Fig. 1*). The stream receives effluents from a sewage treatment plant as well as from an electronics factory. Regular chemical analyses of the water show elevated metal levels, especially of Cu (→ *Table 1*). The Cu concentrations in the stream vary from day to day and are greater than ten to hundred times the Cu levels reported for natural waters in Sweden (0.8 to 2.5 μg Cu l^{-1}; BINGMAN 1987). The pH values are around 7.5 and the conductivity is about 89 mS/m. The faunistic analysis reveals only a few invertebrate species of high densities, e.g. Chironomidae and Oligochaeta (WALKER 1991).

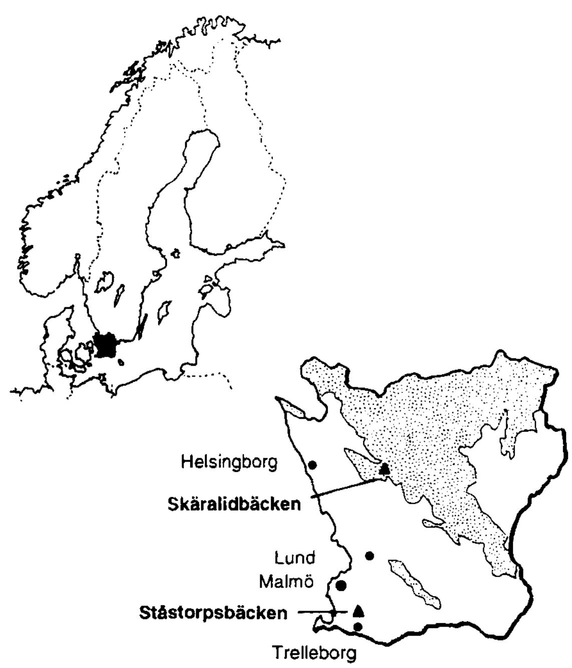

Fig. 1: Location of the sampling sites in the province Scania (South Sweden)

Table 1: Cu concentrations in Ståstorpsbäcken during the experimental period

Date	Cu µg l⁻¹	Date	Cu µg l⁻¹	Date	Cu µg l⁻¹	Date	Cu µg l⁻¹
02.05.94	360.0	09.05.94	11.0	16.05.94	45.0	23.05.94	n.d.
03.05.94	21.0	10.05.94	366.0	17.05.94	14.0	24.05.94	5.3
04.05.94	13.0	11.05.94	19.0	18.05.94	8.0	25.05.94	8.1
05.05.94	17.0	12.05.94	n.d.	19.05.94	5.9	26.05.94	8.3
06.05.94	12.0	13.05.94	248.0	20.05.94	2.4	27.05.94	4.7
07.05.94	n.d.	14.05.94	n.d.	21.05.94	n.d.	28.05.94	n.d.
08.05.94	n.d.	15.05.94	n.d.	22.05.94	n.d.	29.05.94	n.d.
30.05.94	8.9	06.06.94	20.0	13.06.94	35.0		
31.05.94	3.8	07.06.94	n.d	14.06.94	n.d.		
01.06.94	38.0	08.06.94	15.0	15.06.94	13.0		
02.06.94	25.0	09.06.94	34.0	16.06.94	n.d.		
03.06.94	51.0	10.06.94	27.0	17.06.94	n.d.		
04.06.94	n.d.	11.06.94	n.d.	18.06.94	n.d.		
05.06.94	n.d.	12.06.94	n.d.	19.06.94	14.0		

n.d.: not determined

3 Experimental Design

3.1 Acute Exposure to Pb

Gammarus pulex from the control site was acclimated in the laboratory for four days and then exposed in two replicate groups of 12 specimens each to the following concentrations of Pb, added as $Pb(NO_3)_2$ at pH 7: 0.01, 0.05, 0.1 and 0.5 mg Pb l^{-1}. Concentrations up to 0.01 mg Pb l^{-1} have been reported for some field sites (JØRGENSEN et al. 1991). Two replicate groups of 12 animals each were kept in water from the control site and served as controls. During the test period of 7 days, no food was added in order to minimise the loss of Pb by binding to organic detritus. The experiments were performed in aquaria (12 x 12 x 4 cm^3) in a static-renewal test design at 10 ± 1 °C with daily renewal of water with the appropriate metal concentration and a 12h photoperiod. The actual metal concentrations were always close to the nominal concentrations (0.01 ± 0.001; 0.06 ± 0.002; 0.11 ± 0.003 and 0.53 ± 0.01 mg Pb l^{-1}). Survival of the animals was recorded daily. After 1, 24, 72 and 168 h, five randomly selected animals from each replicate and Pb-treatment were analysed for behavioural changes due to Pb exposure and then returned to their aquaria. The organisms were put in the test chamber filled with water from their respective aquaria and acclimated to the chamber for five minutes before start of the behaviour recordings.

3.2 Biomonitoring of Cu Contaminated Stream Water

The biomonitoring experiments were carried out each week as soon as the polluted water was collected from Ståstorpsbäcken, in order to avoid metal losses due to precipitation. The Cu concentrations of the water were determined immediately with furnace-AAS. After 5 min. acclimation to the electrode chamber filled with the test water, the behavioural pattern of at least four organisms was monitored in three subsequent recordings of 280 s for each animal. Simultaneously, four specimens of *G. pulex* kept in unpolluted water were monitored as controls. A total of 64 animals was studied.

3.2.1 Chronic Exposure to Cu Polluted Stream Water

Gammarus pulex was collected at the control site and acclimated to the laboratory conditions for 4 days. The invertebrates were held in the same test system as described for the Pb experiment. Two replicate groups of 12 organisms each were exposed to metal-polluted stream water from Ståstorpsbäcken, whereas two more groups of 12 animals each were kept in unpolluted water from the control site. The exposure lasted for 35 days, water and food (elder leaves) being renewed each week. Each week one day after renewal of the water, at least 8 animals (4 exposed and 4 controls) were randomly collected for analyses of their behavioural patterns in three subsequent recordings of 280 sec. each. The animals were acclimated to the test chamber for five minutes and measured in the water which they have been exposed to. A total of 72 animals was measured during the 5 weeks of exposure. Survival of the invertebrates was recorded each week. Metal concentrations were determined in the water each week and in the animals at the end of the experiments.

3.3 Behaviour Studies

The behavioural patterns of the invertebrates were recorded using an impedance conversion method (GERHARDT et al. 1994). The organisms were placed individually in the test chamber filled with 2 ml of unpolluted stream water (control measurements) or polluted water (exposure measurements). The test chamber served as a static water system and was submerged in cooling water at 10 ± 1 °C. Two electrodes placed on the side walls of the chamber were connected to an alternating current of 0.3 mA. The movements of the organism caused changes in the current, which were measured by another pair of electrodes, placed in between the current-generating pair of electrodes. The signals were recorded on-line via a MacAdios peripherial unit and processed on a Macintosh LC III computer using programs written in the graphical programming language LabView (National Instruments).

The software contained a data collection program for two channels and a data analysis program. The data collection program collected data (voltage signals from the impedance converter) at a frequency of 50 Hz while plotting on the computer screen in real time. The signals could be described by amplitudes and frequencies, specific for different types of behaviour. The data analysis program mainly used the frequencies of different types of behaviour because the absolute values for amplitudes changed according to the position of the animal in the test chamber. A Discrete Fourier Transformation was performed for short time periods (6.4 sec., 128 data points) over the whole sampling period of 280 sec. A histogram for 12 frequencies was calculated and plotted as percentages of the total time. Moreover, the dominant frequency was calculated. Besides the histogram, the original signal and a multiplot graph for 12 different frequencies in different colours were given (→ *Fig. 2*). Times spent on different types of behaviour were calculated manually when scrolling through the behaviour plot.

3.3.1 Classification of Types of Behaviour

In *G. pulex* the following types of behaviour could be clearly distinguished with the impedance converter (GERHARDT et al. 1994).

1) Locomotion consisted of swimming, which meant stretching the body generating a signal of 1 – 2 V, followed by smaller leg movements of about 500 mV. Locomotion signals were of low frequency (0.8 to 1.2 Hz).

2) Gill ventilation consisted of high mono-frequent rhythmic movements with the pleopods, generating a regular signal of 2 to 7 Hz and 200 mV.

3) Inactivity was defined for immobile animals and small movements at 20 mV, which was the noise level of the impedance converter.

3.4 Metal Uptake

After the acute and chronic exposure experiments, the animals were dried (48h, 80 °C), weighed individually and digested in two steps with concentrated $HN0_3$ and H_20_2 at 80 °C before metal analyses with flame or furnace atomic absorption spectrophotometry. Metal concentrations in the unfiltered water samples were determined with furnace AAS.

4 Statistical Analyses

4.1 Behaviour Data

In the Pb experiments, there were several exposure concentrations and several dates on which behaviour was recorded, therefore a two factor analysis of variance ANOVA (concentration levels, dates) was used to evaluate effects of Pb concentration and exposure time on the animals. Analysis of covariance (ANCOVA) was used for the evaluation of ventilation data, as there might be a covariation between time spent on ventilation, number of ventilation phases and ventilation frequencies.

All behavioural data from the chronic Cu exposures were analysed as follows. The mean values of the three subsequent records for times spent on different types of behaviour (locomotion, ventilation, inactivity, number of ventilation phases, ventilation frequency) were calculated and these dependent variables were compared between the two locations (control or Cu-exposed) in a one factor ANOVA.

In the analysis of the biomonitoring experiments, an additional repeated measurement ANOVA was performed for the three subsequent behaviour records to define significant differences in the behaviour within the first half hour of exposure to Cu.

4.2 Metal Uptake

Metal concentrations of the invertebrates were evaluated using a one factor ANOVA (five concentration levels) for the Pb experiment and Student's t-test for the Cu experiment (exposed versus control) according to procedures described in SOKAL & ROHLF (1987).

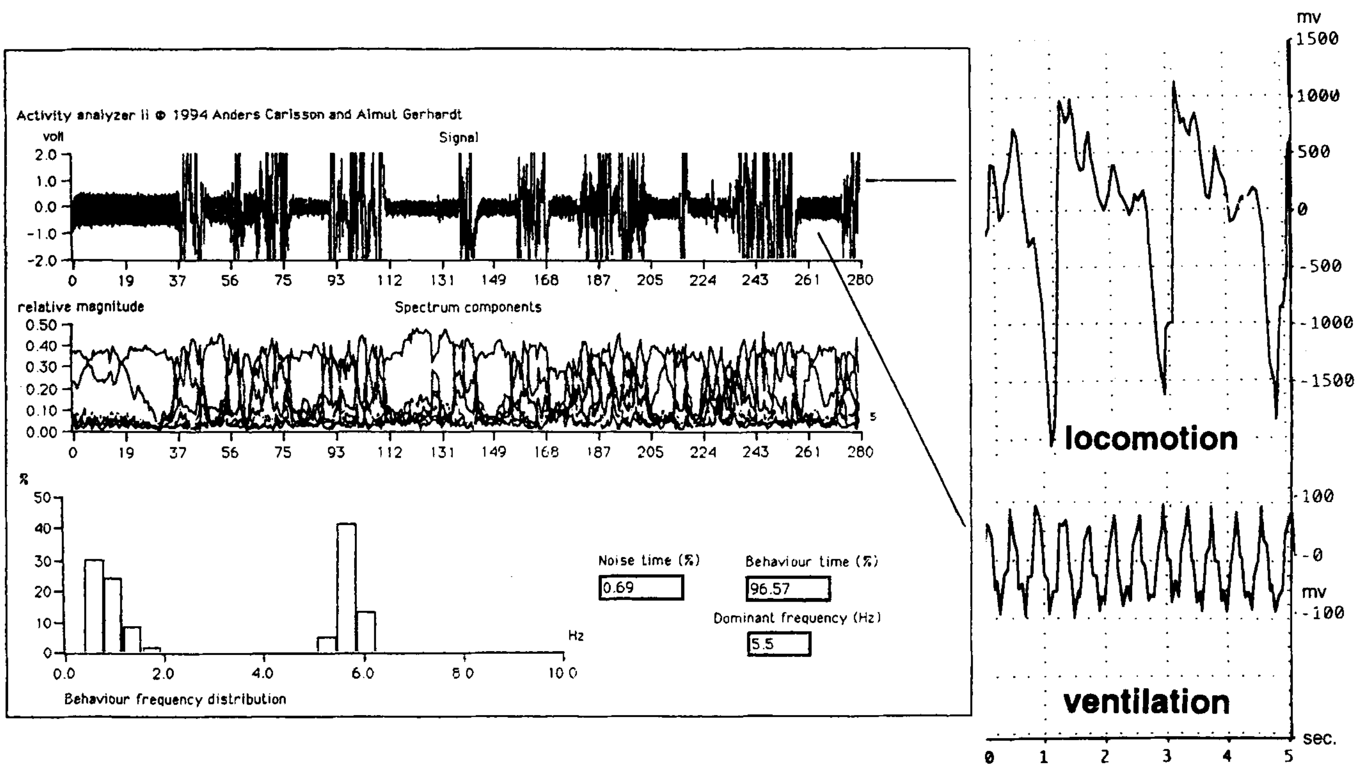

Fig. 2: Example of a behavioural record of *Gammarus pulex* with two typical behaviours, ventilation and locomotion

5 Results

5.1 Behaviour

5.1.1 Acute Exposure to Pb

The natural behaviour of *G. pulex* as observed in the controls was characterised by alternation between locomotion (swimming), resting (inactivity) and gill ventilation phases (→ *Fig. 2*).

The time used for ventilation was significantly related to the exposure concentrations ($p = 0.0001$; F = 6.4) and exposure time ($p = 0.0001$; F = 10.9). After only 1 h of exposure, the time spent on ventilation increased at 0.01 mg Pb l^{-1} (→ *Fig. 3 a*). After 168 h, there was a pronounced increase in ventilation time at 0.5 mg Pb l^{-1}.

Ventilation frequency was very variable but independent of Pb exposure. The time spent on resting (inactivity) was significantly dependent on Pb- exposure ($p = 0.0001$; F = 5.3) and exposure time ($p = 0.0001$; F = 10.6). After 24 h, resting increased at concentrations of $\geqq 0.05$ mg Pb l^{-1} (→ *Fig. 3 b*).

5.1.2 Biomonitoring of Cu Contaminated Stream Water

Gammarus pulex exposed to Cu polluted water spent significantly less time on locomotion than the controls in unpolluted water ($p = 0.04$; F = 8.8; *Fig. 4 a*). This difference increased during the three subsequent recordings (T1: $p = 0.06$, T2: $p = 0.02$, T3: $p = 0.007$). *G. pulex* exposed to Cu polluted water spent significantly more time on ventilation than the controls ($p = 0.012$; F = 6.7; *Fig. 4 b*). While the differences were not significant during the first recording period, they were for T2: $p = 0.005$, and T3: $p = 0.012$. The increased time spent on ventilation was correlated to a higher number of ventilation phases in organisms exposed to polluted water than in those of the controls ($p = 0.019$). However, no significant difference in ventilation frequencies was found between Cu exposed and control animals. Ventilation frequencies showed a wide range (3.0 to 7.5 Hz) and seemed to be more dependent on biological differences such as size and sex than on short-term exposure to Cu.

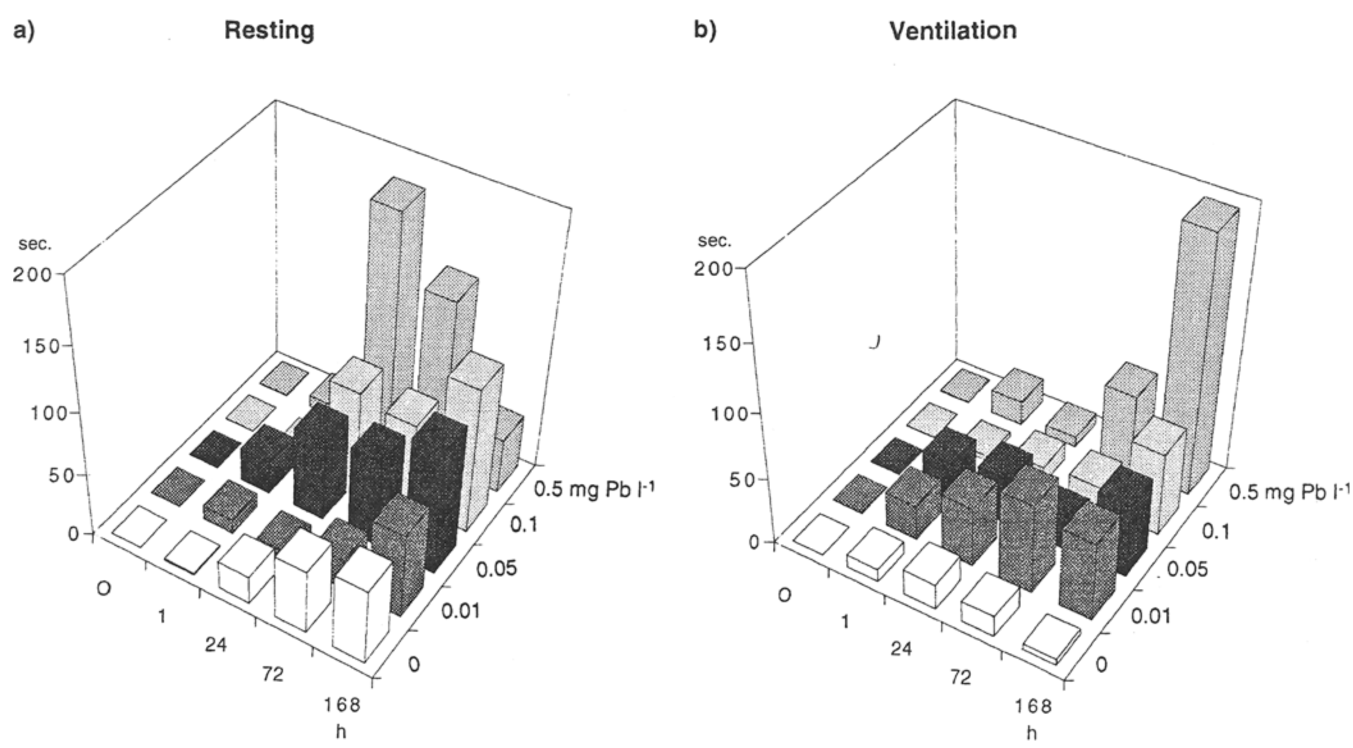

Fig. 3: Effects of Pb on (a) ventilation and (b) resting of *Gammarus pulex* during 168 h of exposure

5.1.2.1 Chronic Exposure to Cu Polluted Stream Water

Gammarus pulex exposed to Cu polluted water spent less time on locomotion than those kept in unpolluted water ($p = 0.005$, F = 8.5). Both in polluted, as well as in unpolluted water, the amphipods decreased their locomotory activity with increasing exposure time ($p = 0.0001$; F = 15.i; *Fig. 5 a*). *Gammarus pulex* exposed to Cu polluted water spent more time on ventilation than those kept in unpolluted water ($p = 0.014$, F = 6.4. *Fig. 5 b*). Ventilation increased after 8 days of exposure to Cu contaminated water. The number of ventilation phases co-varied with time spent on ventilation (p = 0.001; F = 11.9) and increased with exposure time in both groups ($p = 0.0001$; F = 16.4). No differences in ventilation frequency were found between Cu exposed and control animals.

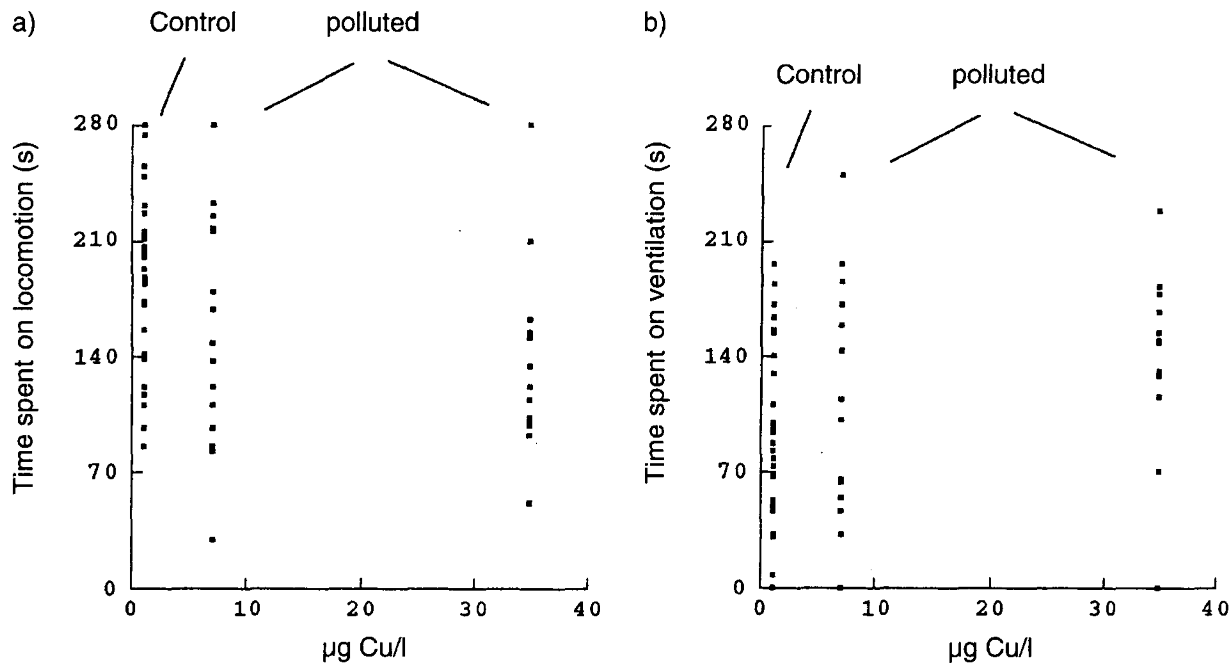

Fig. 4: Early stress responses to Cu polluted stream water of *Gammarus pulex* exposed for 30 min.: (a) locomotion, (b) ventilation

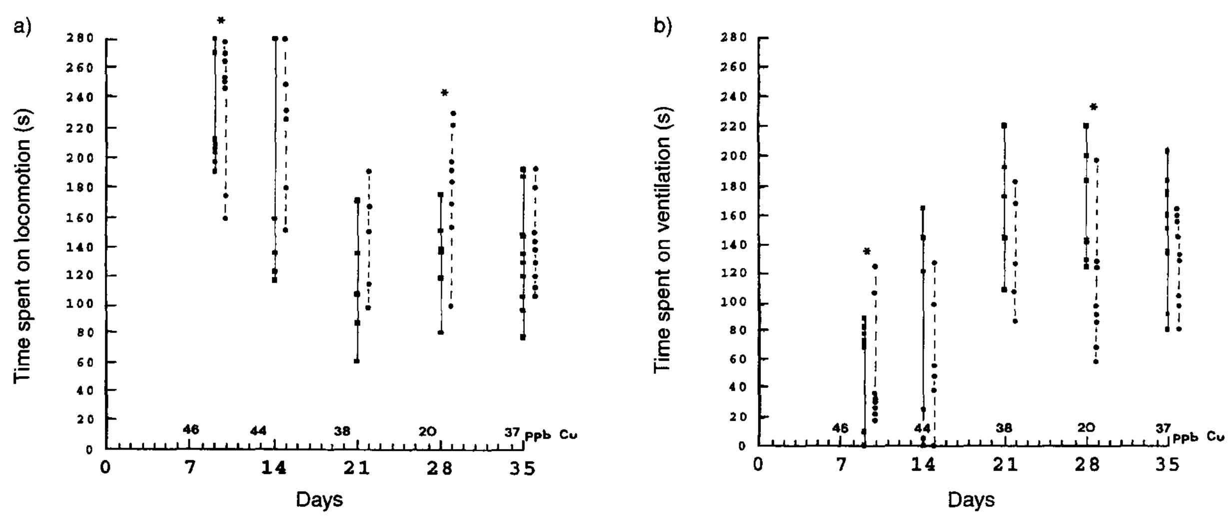

Fig. 5: Effects of Cu polluted stream water on (a) locomotion and (b) ventilation of *Gammarus pulex* during chronic exposure.
polluted water —— control water - - - - - -
* = sign. diff. ($p < 0.05$) between the controls and the exposed animals

6 Survival

6.1 Acute Exposure to Pb

All individuals exposed to Pb concentrations ≤ 0.05 mg Pb l^{-1} survived the exposure period of 168 h. At 0.1 mg Pb l^{-1}, 4 % of the animals died after 120 h. Significant decreases in survival occurred only at the highest exposure concentration (0.5 mg Pb l^{-1}), where 10 % died after 120 h and 30 % after 168 h of exposure.

6.2 Exposure to Cu Contaminated Stream Water

Handling of *G. pulex* in the test chambers and exposure to Cu contaminated stream water containing up to 50 μg Cu l^{-1} for 35 days did not cause any mortality.

6.3 Metal Body Burdens

The Pb body concentrations of *G. pulex* increased in proportion to the exposure concentrations (p = 0.0001) (→ *Table 2*). Pb concentrations in the controls were below the detection limits (10 μg Pb l^{-1}).

Table 2: Pb-body burdens (mg kg^{-1}) of *Gammarus pulex* exposed to different Pb concentrations during 168 h

0.001 mg l^{-1}	0.01 mg l^{-1}	0.05 mg l^{-1}	0.1 mg l^{-1}	0.5 mg l^{-1}
0.01 ± 0.01	7.91 ± 4.50	147.29 ± 77.31	339.24 + 125.72	1270.45 ± 647.26

The values are means (n = 10) and standard deviations (1 sd)

After 35 days of exposure to field concentrations up to 50 μg Cu l^{-1}, *G. pulex* contained about twice as much Cu as those kept in unpolluted water (Cu: 0.66 ± 0.3 mg kg^{-1}, controls: 0.37 ± 0.15 mg kg^{-1}, n = 17; p = 0.001, t-test).

7 Discussion

7.1 Acute Exposure to Pb

7.1.1 Ventilation

Gammarus pulex reacted to Pb exposure as follows: 1) an initial early warning stress response consisting of increased ventilation at ≥ 0.01 mg Pb l^{-1} after only 1 h, and 2) a sublethal physiological effect consisting of increased ventilation at 0.5 mg Pb l^{-1} after 168 h of exposure.

The increase in ventilation after only 1 h of exposure indicated an early stress-response of *G. pulex* at concentrations as low as 0.01 mg Pb l^{-1}, a concentration reported for polluted rivers (JØRGENSEN et al. 1991). This reaction might be used as an early warning parameter in a continuous on-line biomonitoring system.

At 0.5 mg Pb l^{-1} where the total Pb burden of the organisms was about three times higher than that at sublethal concentrations a drastic increase in ventilation time could be seen, indicating a toxic effect of Pb on the organisms after several days of exposure. The lethal concentrations of lead for *G. pulex* are similar to LC_{50} values of *Daphnia magna* (LC_{50}(48h) 0.45 mg Pb l^{-1}) and *Gammarus pseudolimnaeus* (LC_{50}(96h) 0.12 mg Pb l^{-1} (GARMENDIA TOLOSA & AXELSSON 1993). Thus, increased ventilation may also be used as a parameter for toxic effects of lead in bioassays.

The toxic mechanism of Pb at the gills may involve a blocking of the Ca^{2+} channels in the gill membrane by Pb^{2+}, leading to disruptions in ion regulation, for which the organisms may try to compensate for by increased ventilation. Increased ventilation, however, involves increased energy consumption, which will lead to even more ventilation in order to get enough oxygen. This increased energy consumption may lead to a loss of fitness, if not compensated for by a larger energy intake.

Ventilation frequency was highly variable and unaffected by Pb exposure. Other factors such as size, sex and stage in the moult cycle might be responsible for the high variation of this well regulated parameter, thus hiding a possible effect of Pb on ventilation frequency.

7.1.2 Locomotory Activity

The time the organisms spent resting (inactive) increased after 24 h at ≥ 0.05 mg Pb l^{-1} and no significant mortality was observed. Decreased locomotory activity can be interpreted as a sublethal effect of Pb.

Decreased activity might affect several essential biological processes, such as feeding, competition, upstream-migration, mating or seeking shelter. Inhibition of locomotion may reduce avoidance behaviour, geotaxis and phototaxis (LAGADIC et al. 1994). Reduced activity due to metal exposure has also been documented for *Baetis niger* (≥ 0.5 mg Pb l^{-1} after 72 h exposure) and *Leptophlebia marginata* (≥ 0.5 mg Cd l^{-1} after 120 h exposure) (GERHARDT, unpubl.). At 0.1 mg Pb l^{-1}, zebra fish (*Brachydanio rerio*) decreased their reaction time and increased their handling time of prey (*Daphnia spp.*) after exposure for 14 days (NYMAN 1981). Pb caused also persistent dysfunctions in the exploratory behaviour and motor skills in rats (LUTHMAN et al. 1992).

The mechanism behind the reduction of locomotory activity due to Pb exposure could be an effect of Pb on neuronal functions, e.g. inhibition of Ca^{2+} transport in synapses leading to changes in the affinities of neurotransmitters as observed for bivalves (SALANKI 1992). Competition of divalent metal ions with calcium at the Ca^{2+}-channels seems to be a widely accepted mechanism of toxicity of divalent metal ions at membranes (MARKICH & JEFFREE 1994).

7.2 Biomonitoring of Cu Contaminated Stream Water

Within 30 min. of exposure to concentrations up to 50 μg Cu l^{-1}, *G. pulex* showed increased ventilation and less locomotion. Increased ventilation may be a mechanism to counteract disturbances in ion balances at the gills, which has been reported for fish gills at 12 – 50 μg Cu l^{-1} during 2 – 3 h of exposure (PLAYLE et al. 1993).

The fast response time agrees well with other findings. *Gammarus pulex* showed reactions to permethrin at ≥ 500 μg l^{-1} (MUIRHEAD-THOMSON 1978) within 30 min. The crustacean *Brachionus calyciflorus* reacted within 5 min. to 250 μg Cu l^{-1} by a 50 % decrease in swimming activity (JANSSEN et al. 1994). As the actual costs for locomotion account for a large part of the animals total metabolism, swimming behaviour is a sensitive indicator of environmental stress caused by changes in pH, water quality, temperature, food availability and ammonium (JANSSEN et al. 1994).

G. pulex reacted rapidly to Pb contamination of ≥ 0.01 mg Pb l^{-1} in the present study by increased ventilation. *G. pu-*

lex has been shown to react rapidly to organic chemicals such as phenolic compounds (BORLAKAGLU & KICKUTH 1990) and pollution from oil industry (AUNAAS et al. 1991; UDALOVA et al. 1990). Moreover, *G. pulex* reacted within 1 h to an industrial effluent, containing metals and organic chemicals with decreased activity and decreased ventilation (GERHARDT unpubl.).

Thus, *G. pulex* seems to be a sensitive indicator organism for different kinds of pollutants and its behaviour (ventilation, locomotion) appropriate biomarkers for pollution stress originating from short-term variation in metal concentrations.

7.2.1 Chronic Exposure to Cu Polluted Stream Water

Gammarus pulex survived 35 days of exposure to $\leq$ 50 μg Cu l^{-1}. Cu accumulation was low with a bioconcentration factor (BCF) of 5.8 compared to the uptake of Pb during 168 h (BCF 2700 at 0.5 mg Pb l^{-1}). This was expected because Cu is an essential element, a component of enzymes (up to 26 μg g^{-1} in Crustacea) and haemocyanin (57 μg g^{-1}) (WHITE & RAINBOW 1985) and essential elements are characterised by a regulated uptake. However, in *Echinogammarus pirloti* no evidence for Cu regulation was found (RAINBOW & WHITE 1989).

The behavioural changes observed in *G. pulex* exposed to sublethal Cu concentrations indicate sublethal effects, expressed as decreased locomotion and increased ventilation. Cu, like Pb, affects the permeability of neuronal membranes in bivalves (SALANKI 1992) and depresses acetylcholine activity in the synapses of *Chironomus decorus* (KOSALWAT & KNIGHT 1987). Thus, decreased locomotion may be explained by decreased muscular function. Increased ventilation due to Cu indicates a higher energy consumption, maybe as a consequence of detoxification processes.

Sublethal effects of Cu on activity have earlier been reported for fish at $\geq$ 10 μg l^{-1} (BEITINGER & MCCAULEY 1990) and in bivalves at 5 μg Cu l^{-1} (SALANKI 1992). The lowest observed effect concentration (LOEC) for *G. pulex* showing altered feeding behaviour has been estimated to 39 μg l^{-1} (SANDHEINRICH & ATCHISON 1989). Behavioural responses to Cu in *G. pulex* have been found at concentrations below 50 μg l^{-1} in this study, corresponding to other behavioural results for *G. pulex*, such as effects on precopula separation at 23 μg l^{-1} (PASCOE et al. 1994), impaired recruitment at $\geq$ 15 μg l^{-1} (MAUND et al. 1992), impaired feeding behaviour at 8 μg l^{-1} after 96 h (TAYLOR et al. 1993) and delayed growth of *G. pseudolimnaeus* at $\geq$ 8 μg l^{-1} (ARTHUR & LEONARD 1970). Sublethal behavioural effects (avoidance) due to Cu have been observed for *Anodonta grandis* at 33 μg l^{-1} after 24 h of exposure (JACOBSON et al. 1993). At $\geq$ 1.6 mg Cu kg^{-1} larval growth of chironomids was reduced, emergence delayed and increased frequencies of morphological deformities of the epipharynx were found (KOSALWAT & KNIGHT 1987). Behavioural effects have been observed at concentrations not much lower than the Cu-LC_{50}(96 h) values for *G. pulex* reported in the literature (37 μg l^{-1}, TAYLOR et al. 1993; PASCOE et al. 1994), 90 μg l^{-1} for *Gammarus* sp. (LC_{50}96 h) (REHWOLDT et al. 1973). In comparison to that *Daphnia pulex* had a LC_{50} (48 h) of 21 μg Cu l^{-1} while reproduction was impaired at concentrations as low as 3 μg Cu l^{-1} (ROUX et al. 1993). ENSERINK et al. (1991) reported a 21d-LC_{50} of 69 μg Cu l^{-1} for *Daphnia magna* in hard water.

8 Conclusions

This study proved *G. pulex* to be a sensitive bioindicator to metal stress. Changes in ventilation and locomotion can be used as non-destructive biomarkers for acute and chronic toxicity testing as well as "early stress responses" in a continuous biomonitoring-system as part of ecological risk assessment.

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