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Copper tolerances of *Adenophlebia auriculata* (Eaton) 1884 (Insecta: Ephemeroptera) and *Burnupia stenochorias* Cawston 1932 (Gastropoda: Ancylidae) in indoor artificial streams

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Abstract

Copper tolerances of two potential African model species for toxicity testing, the mayfly *Adenophlebia auriculata* (Eaton) 1884 (exposed to 10 different concentrations in the range of 0.02–65 mg l^{-1}) and the limpet *Burnupia stenochorias* Cawston 1932 (exposed to eight different concentrations in the range of 0.02–3 mg l^{-1}) were determined in continuous flow-through indoor artificial streams. The following parameters were monitored daily: Cu, pH, temperature, oxygen, survival and location of each individual in the channels. Behavioural effects after 96 h exposure on the mayflies were measured with impedance conversion technique. *B. stenochorias* (LC_{50}: 0.36 mg l^{-1} (24 h), 0.10 mg l^{-1} (48 h), 0.07 mg l^{-1} (72 h)) was more sensitive to copper than *A. auriculata* (LC_{50}: 1.78 mg l^{-1} (24 h), 0.79 mg l^{-1} (48 h), 0.18 mg l^{-1} (96 h)). *A. auriculata* occurred most frequently under the stones, however moved up to the top of the stones just before death at $\geq$ 0.5 mg Cu l^{-1}. With increasing exposure time the mayflies significantly preferred the stones close to the outflow ($P = 0.016$). *B. stenochorias* reacted to Cu-exposure by moving out of the water ($P = 0.009$) and towards the inflow or outflow of the channels at $\geq$ 0.06 mg l^{-1}. After 96 h of exposure, Cu-exposed mayflies ventilated more than the controls ($P < 0.05$). A dose-dependent sequence of different behaviours was seen with increasing Cu-concentrations: increased abdomen undulations ($\geq$ 0.046 mg l^{-1}), increased locomotion (escape) at $\geq$ 0.231 mg l^{-1} and increased ventilation ($\geq$ 0.277 mg l^{-1}) combined with increased variance in the data sets. In both species the Cu body burdens increased proportionally with copper exposure.

Keywords: Copper; Tolerance; Artificial streams; *Adenophlebia auriculata*; *Burnupia stenochorias*

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1. Introduction

In South Africa, aquatic toxicology is a rather new research field. The development of water and sediment quality criteria is based on toxicity bioassays with standard and indigenous test species. The aim of the present paper is to reveal short-term lethal and sublethal toxicity of Cu for two new potential African test species, the mayfly *Adenophlebia auriculata* (Eaton) 1884 and the gastropod *Burnupia stenochorias* Cawston 1932.

Short-term toxicity tests using survival and sublethal responses of macroinvertebrates are an important component of the routine battery of tests to evaluate toxicity of chemicals, support registration of pesticide products for outdoor application, monitor effluents, establish water quality criteria and provide aquatic safety assessments for chemicals (Bitton et al., 1995). Benthic macroinvertebrates are particularly suited as model species for such tests due to the relative sedentary habits of some taxa (e.g. molluscs) and their wide range of sensitivities to contaminants (e.g. mayflies) (Pontasch and Cairns, 1991; Kifney and Clements, 1994).

Copper (Cu) is an essential micronutrient for most living organisms, being incorporated in at least 30 different enzymes mediating metabolic processes and oxygen transport (e.g. haemocyanin) (Flemming and Trevors, 1989). Copper has been considered as a priority pollutant by the USEPA (Suedel et al., 1996). Its wide use as an algicide, aquatic herbicide, fungicide and molluscicide reflects selective toxicity at high concentrations. There are numerous short-term toxicity studies which reveal a wide range of tolerances to Cu amongst aquatic invertebrates: LC_{50}: 5–86 $\mu g\ l^{-1}$ for *Daphnia* sp. (Crustacea), 17–98 $\mu g\ l^{-1}$ for *Chironomus tentans* (Diptera), 10–890 $\mu g\ l^{-1}$ for *Tubifex* sp. (Oligochaeta) (Flemming and Trevors, 1989), 44 $\mu g\ l^{-1}$ for *Villosa iris* (Unionidae), 83 $\mu g\ l^{-1}$ for *Anodonta grandis* (Unionidae) (Jacobson et al., 1993), 30–64 000 $\mu g\ l^{-1}$ for caddisflies, 37 $\mu g\ l^{-1}$ for *Gammarus pulex* (Crustacea) (Taylor et al., 1991), 1.7 mg l^{-1} for *Dugesia tigrina* (Turbellaria) (See, 1976) and 0.075 to 1.1 mg l^{-1} for different species of freshwater fish (Atchison et al., 1987).

Sublethal effects of Cu include the valve closure of juvenile Unionidae at 20 $\mu g\ l^{-1}$ and shortening of activity phases at 100 $\mu g\ l^{-1}$ (Jacobson et al., 1993), reduced filtration rate of mussels at 94 $\mu g\ l^{-1}$ (Blaxter and Ten Hallers Tjabbes, 1992), fewer movements and reproductive success in *Lymnaea truncatula* at ≥ 1 mg l^{-1} (Rondelaud, 1988), changes in macroinvertebrate species composition and abundance at ≥ 6 $\mu g\ l^{-1}$ (Clements et al., 1989, 1990) and reduced emergence of *Clistoronia magnifica* (Trichoptera) (Nebeker et al., 1984).

The variety in tolerances for survival and other toxicity endpoints indicates that it is necessary to perform several toxicity bioassays with different parameters, such as survival, behaviour and growth for each species to be considered as indigenous test species.

2. Materials and methods

2.1. Test species

Compared to standard toxicity test species such as *Daphnia magna*, toxicity assessment with indigenous species has the advantage of increased ecological relevance. The disadvantage is, however, the results are restricted to the area of distribution of the test species. Apart from geographical distribution, abundance, availability and ecological importance are key factors for the choice of indigenous toxicity test species (APHA, 1992). *Adenophlebia auriculata* (Ephemeroptera: Leptophlebiidae) has been chosen because it is easily naturally available throughout the year (O'Keeffe and Palmer, 1995), comparatively large (up to 3 cm length), and easy to feed and rear in the laboratory (Haigh and Davies-Coleman, 1996). The species is abundant in 'deadwater' areas of many headwater streams such as behind big stones and pools in the Eastern Cape Province, South Africa (Palmer et al., 1994). *A. auriculata* is classified as an omnivorous brusher collector (Palmer et al., 1993). It survived well between 15 and 25°C in still water and in recirculating systems with different food sources such as detritus, commercial fish food, leaves, and algae (O'Keeffe and Palmer, 1995). The taxonomy is rather easy as

South African Leptophlebiidae comprise three genera, of which *Adenophlebia* contains two species (Mc Cafferty, 1990).

Burnupia stenochorias (Gastropoda: Ancylidae) is sedentary and easy to breed in the laboratory on different sources of artificial food. A starving period of 4 days can be survived without negative effects (Davies-Coleman, personal communication). The species is found on stones in the current in many streams of the Eastern Cape Province (O'Keeffe and Palmer, 1995). The Ancylidae are distributed worldwide, comprising seven genera (Hubendick, 1964), three of which occur in Africa and one of them, *Burnupia* being restricted to Africa, however little is known about their ecology and ecotoxicology (Brown, 1980). The ancylid freshwater limpets have lost nearly all traces of pulmonary cavity and instead have developed well-vascularized, extensive neomorphic gills, resulting in cutaneous respiration (Russel-Hunter, 1978). The limpets seem to have an especially high oxygen demand, as they are confined almost entirely to small, stony streams and the wave-washed shores of lakes (Brown, 1980).

2.2. Sampling sites

A. auriculata was collected from the Berg/Palmiet River system, ~12 km south west of Grahamstown (33°22′ S, 26°28′ E) (Eastern Cape) using hand nets in June 1996. The river is unpolluted (Table 1), has a stony bed and flows through natural Valley Bush vegetation. *B. stenochorias* was collected from the Blaaukrantz River, ~13 km outside Grahamstown, (33°20′26″ S, 26°40′45″ E), partially shaded by trees on grassed banks. The limpets' abundance was higher than in the Berg/Palmiet River system. The bed consisted of silt and boulders and current speeds ranged from 0.06–0.42 cm s^{-1}. The stream receives domestic effluents from the townships and from the municipal waste water purification plant of Grahamstown (Table 1).

2.3. Flow-through systems

Frutiger (1984) has stated that stream-dwelling organisms were physiologically dependent on current and that their behaviour might be seriously affected by being kept in lentic conditions. Experimental systems with current maintain the largest species diversity and numbers of organisms (Pontasch and Cairns, 1989). All toxicity tests were therefore performed in a flow-through system. Dechlorinated tapwater was pumped from 25-l aerated reservoirs into white PVC channels (1.5 m length) at 10 ml/min with peristaltic pumps. The water passed from each channel via an overflow pipe, covered with nylon netting (500 μm) and was collected in 100-l containers for treatment and safe disposal. Four hand-sized stones from the Berg/Palmiet River system and two Whatman GF/C filterpapers covered with fine detritus according to Gerhardt (1992a) were added in each channel as substrate and food for the mayflies. The substrate and food for the limpets were two filter papers and a plastic sheet marked with a grid (2.54 cm spacing). The plastic sheet allowed for fast and easy determination of the survival of the animals without touching them as dead limpets fell off when it was lifted out of the water.

Table 1
Water quality parameters of the sampling sites and of the tapwater used for the experiments

Parameter ($mg\ l^{-1}$)	Water body		
	Blaaukrantz	Palmiet/Berg	Tapwater
pH	7.8	6.7	7.6
NH_4-N	0.76	0.03	0.04
NO_3/NO_2-N	0.8	0.01	0.1
PO_4-P	0.49	0.008	0.43
SO_4	56	37	28
Na	282	23	54
K	6.7	0.9	3.9
Ca	66	1	14
Mg	50	3	10
F	0.4	0.1	0.1
Cl	426	16	95
EC (mS/m)	188.3	16.3	47.9

2.4. Short-term toxicity tests

The animals were distributed randomly between the channels and evenly over the area of each channel. They were all of similar dry weight (*A.*

auriculata: 0.55 (S.D. 0.1) mg; *B. stenochorias*: 1.39 (S.D. 0.76) mg). After 4 days of acclimation to the dechlorinated tap water (Table 1) and the new environment (16 ± 1°C, 12 h: 12 h daily photoperiod), 20 *A. auriculata* and separately 25 *B. stenochorias* in each channel were exposed to different nominal Cu-levels for 4 days (Table 2). Based on experience from the experiment with the mayflies and the greater sensitivity of molluscs to Cu, maximal Cu-concentrations of 3 mg l^{-1} were used for *B. stenochorias*.

The concentrations of copper for each channel were prepared daily by dilutions from a freshly prepared stock solution (100 mg Cu^{2+} l^{-1}) at pH 4 and mixed with 25 l tapwater of circumneutral pH. The following chemical parameters were measured daily: Cu_{tot} at inflow and outflow of each channel, pH, oxygen and temperature.

Survival was recorded daily. The filling of the gut was recorded daily on a semiquantitative basis ($< 50\%$, $> 50\%$) (Gerhardt, 1992a). Different parameters of behaviour were recorded daily after the acclimation period. *B. stenochorias*: position of each individual in the grid system painted on the plastic sheet, at the inflow, at the outflow, on or under the plastic sheet; *A. auriculata*: location on, under the stones, on food-plates or in the channel, longitudinal distribution within the channel, i.e. on which of the four stones.

After 4 days of exposure, the behaviour of nine mayflies from each Cu-treatment with at least nine survivors was measured with a quadrupole impedance conversion technique (Gerhardt et al., 1994). Placed in a water-filled chamber, an organism functions as resistance in a circuit of alternating current generated by a pair of electrodes on the opposite chamber walls. Movements of the animal change the impedance between a second non-current carrying pair of electrodes and thus generate specific electrical signals for different behaviours (Gerhardt, 1995, 1996; Gerhardt and Janssens de Bisthoven, 1995; Gerhardt et al., 1998). Each organism was placed individually in a test chamber ($2 \times 1 \times 1$ cm^3) filled with water of its treatment. After 10 min of acclimation a recording of 120 s was performed with a sampling frequency of 50 Hz using Super Scope Software (GW Instruments). Behaviours such as ventilation, abdomen-undulations, resting and swimming/locomotion were quantified.

2.5. Element analyses

Element analyses for the chemical characterisation of the sampling sites and the water used for the experiments were done with ICP-AES for Na, K, Ca, Mg, F, Cl; Cu_{tot}-analyses of the water and individual animals were performed with flame and graphite furnace AAS. After drying (48 h at 80°C) and weighing of the individual animals (limpets with shell) they were prepared by wet digestion in 30% HNO_3 (1 night) and 30% H_2O_2 (1 night) in an aluminium blockheater at 80°C. The Cu-analyses of MA-A-3/TM shrimp homogenate which served as reference material reached 90% of the certified values.

2.6. Statistical analyses

Stephan and Rogers (1985) recommended a regression instead of ANOVA design for toxicity data as regression analysis can accommodate unexpected inversions in the data, it gives clear trends and it does not require treating experimental units as replicates if in fact they are not, e.g. the actual toxicant concentrations in several experimental units are seldom exactly the same. Survival data were used to calculate LC_{50} values after probit transformation from a linear regression model (Weber, 1986). As the probit analysis is restricted to cumulative binomial data, no attempt was made to calculate EC_{50} values from the quantitative behavioural data. Regression analysis was also used to describe the dependency between Cu-levels in the water and Cu body loads.

Concentration-related differences in different behaviours (e.g. location of animals in the channels) over time were evaluated with non-parametric repeated measurement analysis (Friedman test) followed by non-parametric post-hoc tests (Siegel and Castellan, 1988). Concentration-related differences in behaviour after 96 h exposure to Cu (locomotion, ventilation, number of activity and ventilation phases, ventilation frequency) were analysed by using Kruskal Wallis one-way

Table 2
Exposure conditions for *A. auriculata* and *B. stenochorias* for Cu_{tot} (mg l^{-1})

CH	*A. auriculata*		*B. stenochorias*	
	Mean	S.D.	Mean	S.D.
1a	0.017	(0.0002)	0.029	(0.015)
1b	0.017	(0.0002)	0.025	(0.011)
2a	0.050	(0.02)	0.030	(0.0008)
2b	0.041	(0.02)	0.030	(0.001)
3a	0.070	(0.02)	0.060	(0.006)
3b	0.068	(0.02)	0.059	(0.006)
4a	0.240	(0.07)	0.099	(0.007)
4b	0.210	(0.08)	0.098	(0.007)
5a	0.296	(0.015)	0.518	(0.038)
5b	0.258	(0.019)	0.496	(0.034)
6a	0.644	(0.007)	0.747	(0.031)
6b	0.613	(0.05)	0.787	(0.065)
7a	2.940	(0.69)	2.000	(0.35)
7b	2.030	(0.12)	1.500	(0.14)
8a	5.410	(1.76)	3.000	(0.20)
8b	3.626	(0.07)	2.700	(0.50)
9a	22.030			
9b	21.635			
10a	67.259			
10b	65.712			

CH, artificial stream channel, (a) at inflow, (b) at outflow. The values represent means of four measurements and standard deviations (S.D.).

analysis of variance as there were not enough data left for a reliable regression analysis.

3. Results

3.1. Chemical parameters

The pH values during both experiments were between 6.5 and 7.4 with a slight increase over time irrespective of Cu-treatment. Oxygen saturation of the water was $\geq 70\%$ and no significant difference between the Cu-treatments was found. Precipitation of copper salts occurred at concentration levels ≥ 20 mg l^{-1} within 24 h and there was no gradient in Cu-concentrations along the channels (Table 2).

3.2. Survival

A significant dose-dependent decrease in survival with exposure time was observed for both species (Figs. 1 and 2). The limpets were more sensitive to Cu than the mayflies (Table 3).

Table 3
Cu-LC_{50} values (mg l^{-1}) for *A. auriculata* and *B. stenochorias*

Parameter	*Adenophlebia auriculata*	*Burnupia stenochorias*
LC_{50} (95% CI)		
24 h	0.66 (0.16–17.7)	0.36 (0.29–1.1)
48 h	0.50 (0.25–1.12)	0.10 (0.06–5.4)
72 h		0.07 (0.06–6)
96 h	0.18 (0.05–0.25)	

3.3. Cu body burdens

A concentration-dependent increase in Cu body loads was found after 24, 48 and 120 h (Fig. 3). Regressions were significant after 24 h ($P = 0.001$, $R^2 = 0.9$ for both species) and after 48 h ($P = 0.034$, $R^2 = 0.6$ for *A. auriculata*).

3.4. Distribution in the channels

Irrespective of treatment, between 60 and 80% of the mayflies were found sitting under the stones, indicating a negative phototactic behaviour during the day. During the course of the experiment, there was a significant trend of *A. auriculata* to prefer the stones close to the outlet of the channel ($P = 0.06$ after 48 h, $P = 0.016$ after 96 h) (Table 4). There was a slight trend of the mayflies to move onto the stones (increase by 20% after 3 days of exposure) before they died at Cu-concentrations ≥ 0.5 mg l^{-1}. Irrespective of Cu-treatment, more limpets moved under the plastic sheet with exposure time ($P = 0.019$, df = 4) (Table 5). There was a dose-dependent increase of limpets moving to the water/air line of the channels, starting in the higher concentration levels already after 24 h and subsequently increasing in the lower Cu-treatments ($P = 0.009$, df = 4) (Table 5). Once they reached these places, the number of immobile limpets increased linearly from 20% at 0.02 mg Cu l^{-1} to 50% at 0.1 mg Cu l^{-1}.

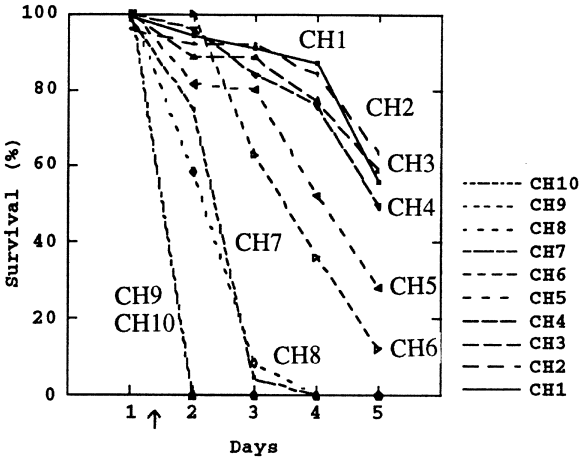

Fig. 1. Survival of the mayfly *Adenophlebia auriculata* exposed to different concentrations of copper as described in Table 2. The beginning of Cu-exposure is marked with an arrow.

3.5. Behavioural effects

The mayflies showed different types of behaviour such as swimming/locomotion, sitting/resting, ventilation and abdomen-undulations. With increasing Cu-concentrations more organisms either ventilated or swam ('escape', 'avoidance') during the whole recording period, which resulted in increased variance of the data compared to the control animals, which showed all types of behaviour (including resting and abdomen-undulations) more evenly distributed over the whole recording period (Fig. 4). Cu-exposed mayflies ventilated significantly more than control animals ($P = 0.05$, df = 5). However, there was no significant difference in ventilation frequency between the Cu-treatments; the mayflies ventilated within a range of 3.2 (S.D. 0.8) to 4.7 (S.D. 0.5) Hz. With increasing Cu-concentrations the following sequence of behavioural effects could be seen: increased abdomen undulations (≥ 0.046 mg l^{-1}), increased escape (swimming) at ≥ 0.23 mg l^{-1} followed by increased ventilation (≥ 0.27 mg l^{-1}) (Fig. 5).

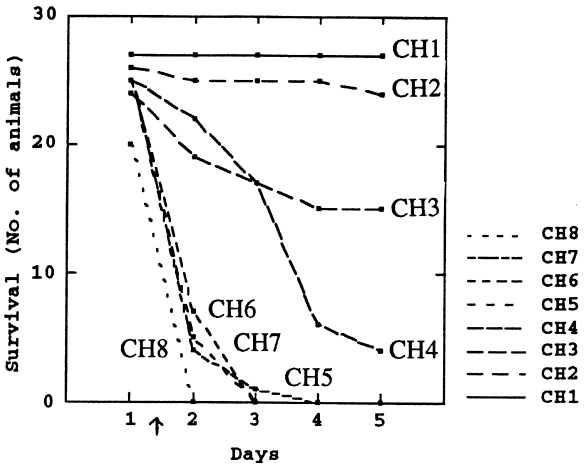

Fig. 2. Survival of the limpet *Burnupia stenochorias* exposed to different concentrations of copper as described in Table 2. The beginning of Cu-exposure is marked with an arrow.

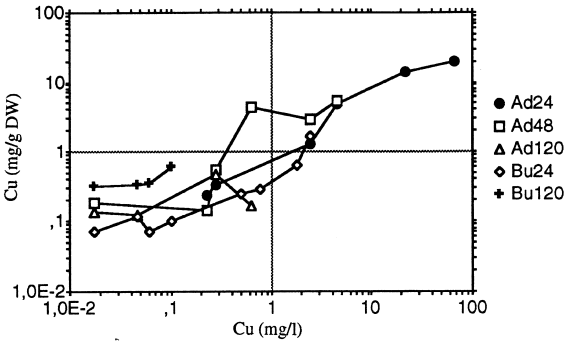

Fig. 3. Cu_{tot}-body burdens in *Adenophlebia auriculata* (Ad) and *Burnupia stenochorias* (Bu) after 24, 48 and 120 h of exposure in relation to actual Cu_{tot}-concentrations in the water.

4. Discussion

4.1. Water chemistry

The Cu-concentrations in the streams are in the range of freshwater background concentrations (Naimo, 1995). The pH of the tapwater used for the experiments was between 6.5 and 7.5. According to speciation models, copper exists mostly as $Cu(OH)_2$-solid and $Cu(CO_3)_2$ at pH values above 6 (Stumm and Morgan, 1981). The copper stock solution was prepared at pH 4 to keep the Cu(II) ions in solution. After dilution with tapwater of circumneutral pH to the different concentrations of the treatments, however, precipitation of $Cu(OH)_2$, CuO and $Cu_2(OH)_2(CO_3)$ (green) occurred in the reser-

Table 4
Distribution of *A. auriculata* (%) on each of the four stones in the channels

Parameter		Mean Cu_{tot} conc. (mg l^{-1})									
		0.017	0.046	0.07	0.231	0.277	0.629	2.486	4.482	21.8	66.48
Day	Stone										
0	1	24	60	19	64	52	20	42	50	21	61
	2	36	24	27	8	11	8	0	42	17	19
	3	24	8	27	20	22	60	50	33	33	4
	4	16	4	27	0	15	0	8	12	25	11
1	1	13	29	54	64	0	56	26	17	35	21
	2	43	17	25	12	4	12	9	21	0	8
	3	22	8	12	8	12	12	17	0	26	17
	4	13	38	0	12	58	20	39	58	13	21
2	1	14	26	39	54	32	24	33	36		
	2	59	9	30	4	4	4	5	7		
	3	4	17	22	17	32	8	22	7		
	4	9	44	9	17	23	56	33	36		
3	1	5	22	17	47	35	25	0			
	2	42	26	22	0	6	10	0			
	3	16	4	17	0	12	15	0			
	4	32	48	44	24	41	50	100			
4	1	23	9	25	37	0	0				
	2	6	10	20	0	7	22				
	3	47	38	10	21	14	33				
	4	24	38	30	37	79	44				

Day 0: after 48 h acclimation; day 1–4: under Cu exposure; stones 1, 2, 3 and 4: stones placed in the channel longitudinally from inflow to outflow.

voirs, the tubes of the pumps and the channels within 24 h in the treatments of ≥ 20 mg Cu_{tot} l^{-1}. The tapwater contained a number of cations (Na, K, Ca, Mg) as potential ligands for precipitates.

4.2. Survival

In both experiments, survival decreased in a dose-dependent way with increasing Cu-concentrations. *Adenophlebia auriculata* was more tolerant to Cu than *Burnupia stenochorias*. The LC_{50} values for the limpet (0.36 mg l^{-1} after 24 h, 0.07 mg l^{-1} after 72 h) are in the range of those found for other molluscs, e.g. LC_{50}-24 h: 83 μg l^{-1} for *Anodonta grandis*, 44 μg l^{-1} for *Villosa iris*, both Unionidae (Jacobson et al., 1993). For *Corbicula fluminea* LC_{50}-24 h of 0.59 μg l^{-1} and LC_{50}-96 h of 0.04 μg l^{-1} were found (Rodgers et al., 1980). $CuCl_2$ is used as a molluscicide (Rondelaud, 1988) and mussels have been found to be more sensitive to Cu than to other metals such as Hg, Cd, Pb and Zn (Salanki, 1992).

The LC_{50}s for *A. auriculata* (48 h: 0.50 mg l^{-1}, 96 h: 0.18 mg l^{-1}) were higher than for e.g. *Gammarus pulex* (LC_{50}-48 h: 0.047 mg l^{-1}) and daphnid crustaceans (LC_{50}-48 h: 0.019 mg l^{-1}, Bitton et al., 1995), but lower than that for *Chironomus riparius* (LC_{50}-48 h: 1.2 mg l^{-1}, Taylor et al., 1991), similar to those reported for different fish species (LC_{50} s: 0.1–1.1 mg l^{-1}, Atchison et al., 1987), *Chironomus tentans* (LC_{50}-96 h: 0.63 mg l^{-1}) or *Hyalella azteca* (LC_{50}-96 h: 0.65 mg l^{-1}) (Suedel et al., 1996). Leptophlebiid mayflies have earlier been reported to be metal tolerant, e.g. Cd-LC_{50}-96 h for *Leptophlebia marginata* were

Table 5
Distribution of *B. stenochorias* (%) in the channels

Parameter Day	Mean Cu_{tot} conc. (mg l^{-1})							
	0.027	0.03	0.06	1.00	0.50	0.70	2.00	3.00
% Limpets under the plastic sheet with coordinate system								
0	19	24	38	6	35	22	28	25
1	17	18	63	15	45	31	33	44
2	37	20	53	17	83	30		
3	53	25	77	76				
4	67	41	50	32				
% Limpets at the water/air interface on the channel walls								
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	17	10	11
2	10	50	53	67	17	40		
3	47	63	100	62				
4	46	94	100	67				

Day 0: after 48 h acclimation; day 1–4: under Cu-exposure.

≥ 4.4 mg l^{-1} depending on pH and test design (Gerhardt, 1992b), Pb-LC_{50}-96 h were ≥ 1.1 mg l^{-1} and Fe-LC_{50}-96 h were 89.5–106.3 mg l^{-1} depending on pH (Gerhardt, 1994). The leptophlebiid *Atalophlebia australis* showed an LC_{50} for Cd of 0.84 mg l^{-1} (Thorp and Lake, 1974). *Adenophlebia auriculata* tolerated ≥ 31.6 mg Zn l^{-1} (LC_{50}-96 h) depending on data-analysis method (Probit, Spearman Kärber) and test design (size of aquaria and aeration) (Everitt, personal communication). Leptophlebiid mayflies seem to be less sensitive to metal toxicity than crustaceans and molluscs; their tolerance is similar to that of chironomids and some caddisflies (Gerhardt, 1992a, 1994).

4.3. Cu body loads

Both species accumulated copper to the same extent and proportionally with increasing exposure concentration. The Cu body loads of the limpets were similar to those mentioned in the literature such as *Asellus meridianus* (200–800 μg g^{-1} dry weight after 14 days to 0.57 mg l^{-1}: Brown, 1977), *Asellus aquaticus* (250 μg g^{-1} dry weight after 50 days to 0.25 mg l^{-1}: van Hattum et al., 1993), *Chironomus decorus* (450 μg g^{-1} after 24 h to 0.2–0.8 mg Cu l^{-1}: Kosalwat and Knight, 1987), *Chironomus riparius* (44.5 μg g^{-1} after 28 days: Janssens de Bisthoven et al., unpublished) and *Gammarus pulex* (0.66 μg g^{-1} after 35 days to 0.05 mg l^{-1}: Gerhardt, 1995). The bioconcentration factor of *A. auriculata* and *B. stenochorias* was in the range of 1000 to 10 000, similar to those reported for *Daphnia magna* (Winner and Gauss, 1986) and freshwater plankton (Jørgensen et al., 1991). Indications of a threshold value for Cu regulation have been given for *Anodonta cygnea* (Salanki and Balogh, 1989) and *Palaemon elegans* of about 120 μg l^{-1} (Rainbow and White, 1989).

4.4. Distribution in the channels

Both test species changed their distribution in the channels during exposure to copper. *A. auriculata* tended to crawl on top of stones instead of the negative phototactic and cryptic behaviour of sitting under stones. A loss of positive phototactic behaviour was found for calanoid copepods exposed to pCu: 13 (Stearns and Sharp, 1994). A slowing down of the crawling speed in *Dugesia tigrina* as a sign of decreased negative phototactic behaviour, was found at ≥ 0.5 mg Cu l^{-1} (See, 1976). After 4 days of exposure, the mayflies preferred stones close to the outlet instead of being evenly distributed on the four stones in the channel. *B. stenochorias* moved to the channel

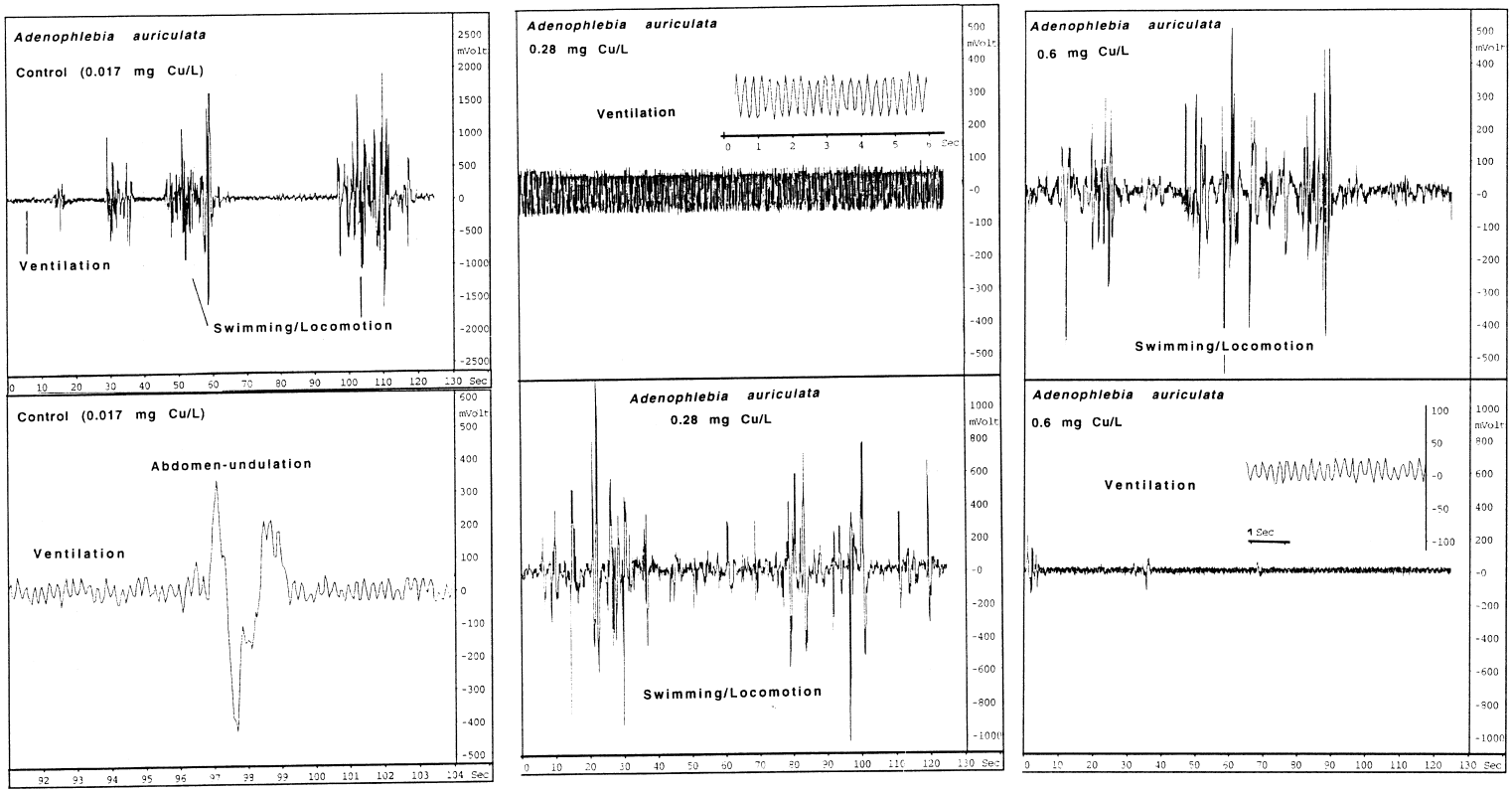

Fig. 4. Examples of typical behavioural records (120 s) of *Adenophlebia auriculata* exposed to different Cu_{tot}-concentrations (0.017 mg l^{-1}, 0.28 mg l^{-1} and 0.6 mg l^{-1}) and signal magnifications for abdomen-undulation and ventilation.

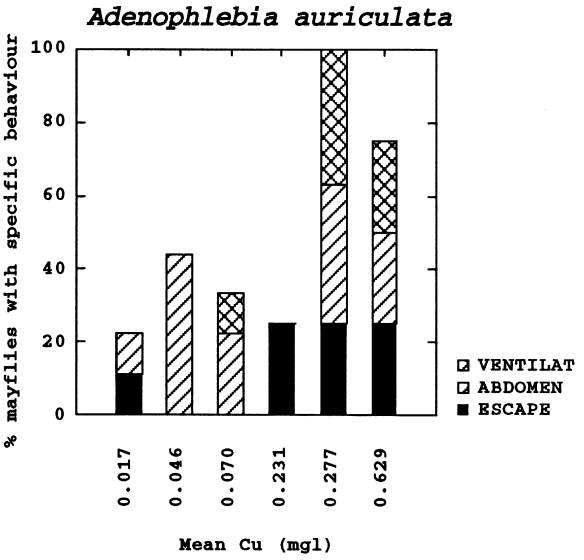

Fig. 5. Changes in the behavioural patterns (locomotion/swimming/escape, abdomen-undulation, ventilation) of the mayfly *Adenophlebia auriculata* after 96 h exposure to different Cu_{tot}-concentrations in the water.

edges and accumulated significantly more at the water/air line of the channels treated with Cu (≥ 0.03 mg l^{-1}) compared to the control. With increasing exposure time the limpets moved under the plastic sheet, which was placed in the channel. All these migrations might be seen as avoidance or escape behaviour. Similar sensitive avoidance reactions in molluscs e.g. the valve closure of mussels (Blaxter and Ten Hallers Tjabbes, 1992; Salanki, 1992; Jacobson et al., 1993), indicate that Cu can be sensed by chemoreceptors (Morton, 1971). Changes in migrations due to Cu were found at ≥ 0.03 mg l^{-1} for *B. stenochorias* and for *A. auriculata* at ≥ 0.3 mg l^{-1}. The values are in the range of those cited in the literature, e.g. valve closure-EC_{50}-24 h for Unionidae: 27–33 μg l^{-1} (Jacobson et al., 1993), LOEC for valve closure in *D. polymorpha* 0.03 mg l^{-1} (Sloof et al., 1983), swimming-EC_{50}-3 h for *Brachionus calyciflorus*: 15 μg l^{-1} (Janssen et al., 1994), filtration rate-EC_{50} for mussels: 94 μg l^{-1} (Blaxter and Ten Hallers Tjabbes, 1992), feeding rate-EC_{50}-6 h for *Ceriodaphnia dubia*: 14.5 μg l^{-1} (Bitton et al., 1995), avoidance-LOEC for fish: 0.1–6.4 μg l^{-1} (Atchison et al., 1987), ingestion-NOEC for *Brachionus calyciflorus*: 50 μg l^{-1} (Juchelka and Snell, 1994), pumping rate-LOEC for *Anodonta cygnea*: 5 μg l^{-1} (Salanki, 1992) and life-cycle parameter-NOEC for *Clistoronia magnifica*: 8.3 μg l^{-1} (Nebeker et al., 1984). A plating industry effluent containing Cr, Ni and Fe produced an avoidance reaction in rainbow trout at $1{:}300 < x < 1{:}75$ LC_{50} (Hadjinicolaou and Spraggs, 1989).

4.5. Behavioural effects after 96 h of exposure

Besides the migrations of both species in the channels, different toxic effects on the behaviour of the mayfly *A. auriculata* were found after 96 h of exposure to Cu, such as increased abdomen undulations at ≥ 0.046 mg l^{-1}, increased locomotion and at ≥ 0.231 mg l^{-1}, increased ventilation at ≥ 0.277 mg l^{-1} and variances in behaviour. Increased ventilation was also found in *Gammarus pulex* exposed to 50 μg Cu l^{-1} and to ≥ 0.01 mg Pb l^{-1} (Gerhardt, 1995). Increased ventilation might be a trial to get rid of Cu-ions bound to the gill membranes, thus blocking the Ca-binding sites (Winner and Gauss, 1986). Hyperactivity was found for estuarine copepods exposed to sublethal pCu after 24 h (Sullivan et al., 1983). With increasing Cu-concentrations, more mayflies either swam or ventilated during the whole recording period, resulting in an increasing variance of the behaviour. Similar results have been found for the chironomid *Glyptotendipes pallens* exposed to Cd for 96 h (Heinis et al., 1990). This kind of 'bimodal' or 'dichotomous' behavioural response, described by Scherer (1992) impedes statistical analyses based on normal distributions and analyses of means as often no significant results can be found with these traditional methods. There is a need to develop methods to deal with interindividual variability in ecotoxicology (Depledge, 1990).

5. Conclusions

Both species showed sublethal behavioural and lethal responses to copper within 96 h of exposure at concentration levels which are 10–100 times higher than the background levels in the

field. The limpet was more sensitive to Cu than the mayfly, the bioconcentration factor and the LC_{50} value for the mayfly fell in the range of those reported for standard toxicity test species such as daphnids and chironomids. Thus, the two species proved to be appropriate indigenous toxicity test species for the evaluation of freshwater ecosystems where they occur and where the standard organisms are lacking. However, more toxicity tests with these new species and simultaneously with standard organisms have to be performed with different contaminants to further support this conclusion.

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