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Technical Note

Behavioural and feeding responses of *Echinogammarus meridionalis* (Crustacea, Amphipoda) to acid mine drainage

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Abstract

As a result of mining activities the exposure of metal sulphides to oxidation takes place with consequent release of acid mine drainage (AMD). Biomonitoring instruments have been proven to have the best deterrent effect upon polluters. A new approach in online biomonitoring, with the Multispecies Freshwater Biomonitor™ (MFB), was developed combining behavioural and feeding responses of the Portuguese indigenous benthic shredder, *Echinogammarus meridionalis* (Pinkster, 1973) (Crustacea, Amphipoda). These endpoints, along with mortality, were measured and analyzed for a gradient of sublethal doses of AMD. Original river water was used as the control and three doses of treatments were attained by adding increasing volumes of AMD to the control. The increase in AMD concentration and concomitant decrease in pH and increase in the concentration of most metals was followed by an overall increase of the mortality, decrease of locomotion/feeding activity and inhibition of the feeding rate. Mortality was observed in the two highest concentrations of AMD. Significant decrease in average locomotion/feeding activity took place in the second treatment. Although an inhibition of feeding was observed along the gradient of AMD concentration only in the highest concentration the feeding rate was significantly reduced.

Keywords: Online biomonitor; Behavioural endpoints; Feeding inhibition; *Echinogammarus meridionalis*; Acid mine drainage

1. Introduction

Biomonitoring instruments have been proven to have the best deterrent effect upon polluters (Gerhardt, 2000a) addressing the need to determine the effects of pollutants on benthic invertebrates. The study of biological responses allows the assessment of toxicity of environmental pollutants taking bioavailability into account (Hoekkstra et al., 1994). These responses can be measured with online biomonitoring systems (Gerhardt et al., 1998) having the advantage of giving information of the exposure of toxic compounds on a continuous real-time basis (Gerhardt, 2001). Indigenous organisms are better biomonitors than standard test species since they play an ecological role in the aquatic ecosystems where they occur, and have been proven to be better indicators for anthropogenic stressors (Pontasch and Cairns, 1991), hence providing more environmentally realistic results (Gerhardt, 2000a).

Biomonitoring should rely on sublethal endpoints rather than mortality alone. Behaviour is a sublethal parameter readily altered by stress (Beitinger, 1990) and its changes may have ecological consequences, *e.g.* avoidance behaviour or decreased activity may influence population levels by affecting migration or susceptibility to predation (Gerhardt, 1995b). Behavioural endpoints are more sensitive than mortality tests (Gerhardt, 1995a), reflecting different levels of susceptibly of organisms to environmental stimuli, and are therefore appropriate indicators for biomonitoring purposes (Gerhardt et al., 1994; Ulfstrand, 1996). Once a determined behaviour can be quantified, it has the potential to be used as a biomarker in the assessment of stress (Beitinger, 1990). Feeding inhibition can be used as a general

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stress response to a wide range of contaminants and has been applied with a variety of organisms (Naylor et al., 1989; McWilliam and Baird, 2002). Feeding responses can be applied with ecological relevant species for the assessment of water quality linking physiological organism-level responses to population or community-level effects (Maltby et al., 2000). Behavioural studies of feeding habits or mobility patterns help to clarify differences in the response of organisms to anthropogenic stressors (Ulfstrand, 1996).

Abandoned mines can be responsible for several impacts in their surrounding environment due to the chemical nature of their effluents. Mining activities often focus on ores rich in heavy metals and expose metal sulphides to oxidation, with consequent release of acid mine drainage (AMD) (Boult et al., 1994).

In the research work presented in this paper a new approach for online biomonitoring, using the Multispecies Freshwater Biomonitor™ (MFB), was developed combining behavioural and feeding responses of *Echinogammarus meridionalis*. The purpose of this study is to test the hypothesis that an observable and quantifiable behavioural response and an inhibition in the feeding rate of *E. meridionalis* is elicited, outside the normal range of variability, when exposed to a gradient of AMD concentration.

2. Methodology

2.1. Test species

The amphipod *E. meridionalis* is found in slow running streams with gravel substrate that may be slightly polluted with domestic effluents. It is a benthic macroinvertebrate feeding primarily on detritus such as decaying leaves. In common with other freshwater amphipods it plays an important role in lotic systems, namely in the detritus processing and as an important food item for several species of fish. The specimens were collected in river Lena (N 38°35′28.3″, W 8°40′30.2″), near Porto-de-Mós (district of Leiria, Portugal), four d before the beginning of the experiment so that they could acclimate to the laboratory conditions: temperature of 20 ± 2 °C; cycles of 16 h of light, 8 h of dark (0800–2359 light/0000–0759 dark). During acclimation time they were fed *ad libitum* with dried alder leaves.

Along with the organisms, river water (pH 7.6) was also brought to the laboratory to be used as the *E. meridionalis*' aquaria medium before the experiment began and to be used in the test trays during the experiment.

2.2. Experimental design

The AMD was collected from the abandoned mine of S. Domingos, southeast of Portugal (N 37°39′56″, W 7°28′46″), near Beja, with pH 2.4. The ore-body of the mine consists of cupric pyrite (Oliveira, 1997) and therefore its high acidic drainage is enriched in dissolved metals that are abundant in the sulphide minerals exposed to weathering by mining activity (*e.g.*, Fe, Mn, Cd, and Pb).

It was decided to use AMD dilutions in order to obtain a realistic chemical approach since in nature populations are exposed to mixtures of chemical contaminants (Logan and Wilson, 1995). Four test trays were labelled according to the following pH gradient: A – pH 7.6 (control tray), B – pH 7.0, C – pH 6.4 and D – pH 5.8. The lowest pH level (tray D) was selected since, in spite of the fact that there were no published data for *E. meridionalis* pH lethal concentration (LC) values, it has been reported that exposure to pH 4.5–6.0 results in rapid mortality for several Gammaridae species (Havas, 1981; Økland and Økland, 1986; Hargeby and Petersen, 1988).

The pH of each tray was attained adding AMD to the water from the river Lena with a difference of *ca.* 0.6 pH units between trays. The trays had a volume of 4 l of river water, AMD was added while the pH was measured with a pH meter, maintaining the total volume at 4 l by draining excess liquid into a connected bucket where the medium (*ca.* 5 l) was aerated (Fig. 1). The water from this bucket was returned to the original tray via a Watson–Marlow peristaltic pump (90 rpm). The 24 tubes from the pump were divided by the 4 trays which gave a number of six tubes per tray with a total pumping rate of 132.6 ml min^{-1}. The exposure design therefore consisted of a semistatic system where the medium pH was adjusted every two d by addition of AMD.

The following physical parameters were recorded daily: pH, oxygen concentration (%), conductivity (μS cm^{-1}) and temperature (°C). At the end of the experiment, mortality was registered.

Three samples of the AMD from S. Domingos mine and daily samples of the medium in the trays were taken for chemical analysis. The samples were analysed by both "inductively coupled plasma atomic emission spectroscopy" (ICP-AES) and "inductively coupled plasma mass

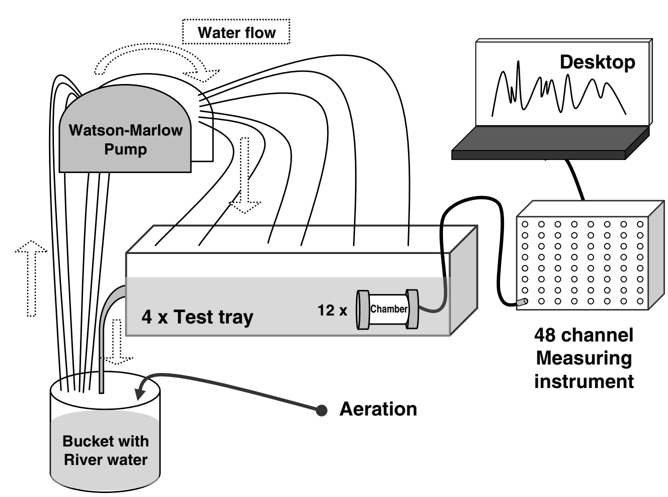

Fig. 1. Constituents of the exposure semistatic system of the experiment and the MFB (chambers, measuring instrument and desktop).

spectroscopy" (ICP-MS). When the values were less than 30 μg l^{-1} the results from ICP-MS were used. For most elements the correspondence between values determined by these alternative methods was excellent. The following elements were only analysed by ICP-AES: Ca, Fe, Mg, Na and S. Cl was analysed by ion chromatography.

2.2.1. Behavioural responses

The MFB construction and functioning is described elsewhere (Gerhardt et al., 1998; Gerhardt, 2001). Briefly it is based on the quadropole impedance technique and consists of a desktop computer, a measuring instrument with 48 channels, and the respective 48 chambers (Fig. 1). The chambers are made of plexiglas pipe (2 cm in diameter and 4 cm long) with two pairs of stainless steel electrodes attached oppositely to the inner walls and capped with 1 mm mesh in both ends. In one pair of electrodes a high frequency alternating current is applied and the movements of the organism create an impedance to the current that is measured by the other pair of non-current-carrying electrodes. The signal measured is then sent to the desktop where the data are registered with the MFB software. The result of the organisms' movements is plotted as a graph of current (V) over time, that can be transformed by a discrete fast Fourier transformation (FFT) with the Hamming function into a frequency histogram, that includes the relative amounts of the low frequency behaviours (0–4 Hz) (*e.g.* locomotion, feeding behaviour) and high frequency behaviour (4–8 Hz) (*e.g.* ventilation) (Gerhardt, 2000b) (Fig. 2). These measurements are recorded every 10 min, for 4 min, while the MFB is online. The MFB was online in this experiment from 1728 of d 0 to 1614 of d 5, *i.e.* a total of 116 h.

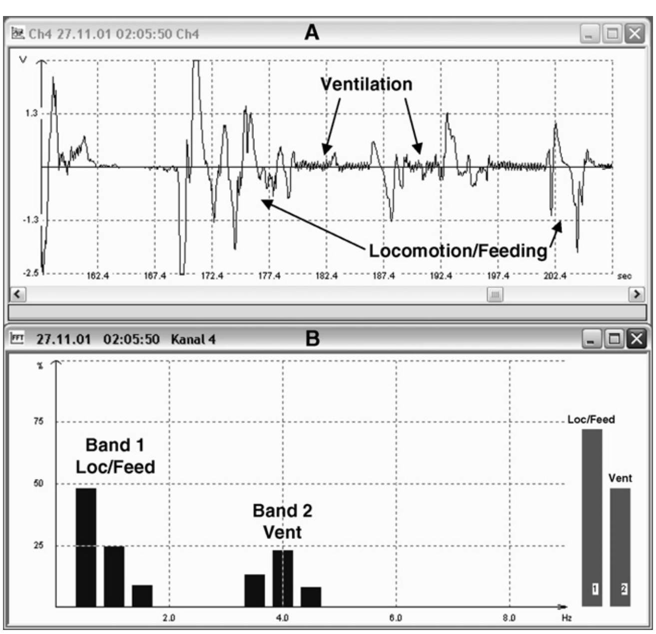

Fig. 2. (A) Characteristics of the signals of locomotion/feeding and ventilation. (B) FFT diagram.

2.2.2. Feeding inhibition responses

The feeding test was adapted from the methodology proposed for *Gammarus pulex* feeding inhibition bioassays (Naylor et al., 1989; Maltby et al., 1990a). Individual *E. meridionalis* were deployed in the MFB chambers. Twelve chambers were allocated per treatment (trays), nine of them containing one adult male amphipod (3.32 ± 0.54 mg) and a ration of five preweighed alder leaf discs (10 mm diameter); three chambers containing only leaf material were used to control leaf weight loss due to physical or microbial degradation. Tests were based on male gammarids, obtained by the disruption of precopula pairs, to avoid possible differences in the feeding rates and sensitivity to AMD between males and females. Leaves were rehydrated in the solutions four d before the beginning of the experiment. After the 116 h exposure period (*ca.* five d), animals and leaf material were washed and dried at 60 °C for four d and then weighed.

Feeding rate (FR), expressed in terms of dry weight of food (mg d^{-1}), was calculated using the equation: $FR = ((P_i \times C_f) - P_f)/d$, where P_i is the initial dry weight of leaf discs, P_f is the final (after 5 d) dry weight of leaf discs, d is the elapsed time in d, and C_f is the leaf weigh change correction factor and is given by the mean of the quotient of the final to initial weight of control leaf discs i.e. deployed in the control cages (adapted from Maltby et al., 2002).

2.3. Statistics

The behavioural responses were analysed using one-way ANOVA with the SigmaStat statistical package (SPSS, 1995) and pair-wise multiple comparison procedures with the Student-Newman–Keuls method whenever significant differences between treatments were found. Statistical analysis was carried out with a significance level of $p < 0.05$. Behavioural frequencies were arcsine transformed to ensure normality and homoscedasticity of data (Zar, 1996). For each tray, the average locomotion/feeding and ventilation were calculated resulting of four values for the locomotion/feeding and four values for the ventilation

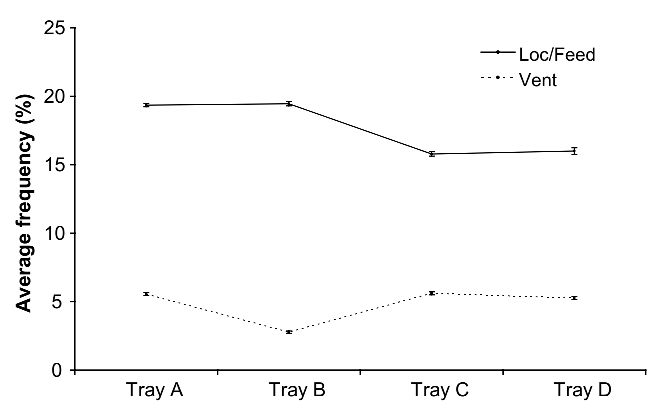

Fig. 3. Average frequency (%) (±standard error) of the locomotion/feeding and ventilation activities, for each tray.

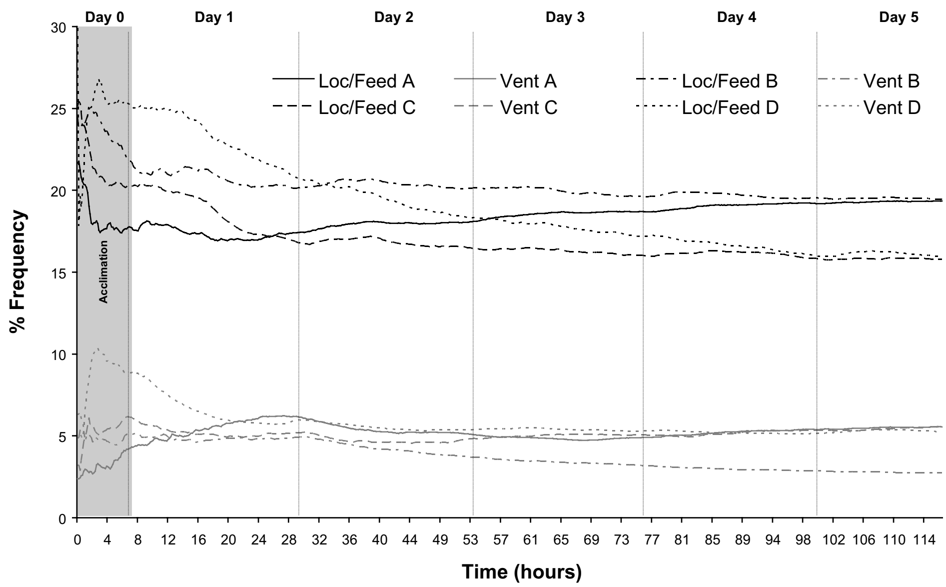

Fig. 4. Baselines for the behaviours' frequency (%), in the trays, during the 116 h experiment. The first 8 h were considered as the acclimation period. The lines above 15% represent the locomotion/feeding (Loc/Feed) in trays A–D; the lines below 10% represent the ventilation (Vent) in trays A–D.

(Fig. 3). The different behaviours were also integrated over the 116 h of the experiment, by creating sets of locomotion/feeding and ventilation lines for the trays. These lines are presented in the form of baseline activities obtained from the average of every measurement registered and the first measurement, hence overcoming the high variability of these responses (Fig. 4). For the feeding bioassays, one-way ANOVA were performed using the SigmaStat statistical package (SPSS, 1995) and whenever significant differences between treatments were found, Dunnett's method was used as post-hoc testing to determine statistical differences between treatments and the control. Statistical analysis was carried out with a significance level of $p < 0.05$. Feeding rates were square root transformed to ensure normality and homoscedasticity of data (Zar, 1996). Dead organisms were excluded from data analysis for both behavioural endpoints and feeding inhibition.

3. Results

3.1. Physical and chemical data

The physical and chemical parameters taken during the experiment are registered in Table 1. Although the pH was adjusted during the experiment, it increased in every tray with initial pH values for the trays A 7.6, B 7.0, C 6.4, and D 5.8, reaching 8.2, 8.0, 8.0, and 7.8, respectively. From tray A to tray D there was an increase in the conductivity due to an increase in the concentration of electrolytes (ions) as more AMD was added to lower the pH. The air saturation was around 80–83% in the trays. The average temperature in the four trays ranged between 18.7 and 19.4 °C. The analysis of the total concentration of metals in the trays showed an overall tendency to increase with the increasing levels of AMD, from tray A to tray D, except for Cl, K and Pb, which did not conform to this overall pattern. For each of these elements, the concentration in the AMD collected at the S. Domingos mine (Cl 90 mg l^{-1}; K 20 mg l^{-1}; Pb 51.8 μg l^{-1}) was higher than that recorded from the river Lena, and therefore the addition of AMD would theoretically lead to their increase in the treatment trays. For some heavy metals such as Cd, Cu and Zn, there was an increase in concentration of several orders of magnitude, as the concentration of AMD rose, respectively, from 0.03 to 17.0, 3.7 to 260, and 35.1 to 1641 μg l^{-1}.

3.2. Behavioural data

As the AMD increased in concentration from tray A to tray D, pH decreased and was associated with behavioural changes in exposed animals (Fig. 3). Considering the average locomotion/feeding activity for each tray, two groups were formed: A/B and C/D (respectively, $19.35 \pm 0.12\%/19.46 \pm 0.15\%$ and $15.78 \pm 0.16\%/16.00 \pm 0.24\%$). A statistically significant decrease in the average locomotion/feeding was evident between tray B and tray C ($F_{3,2744} = 149.485$; $p < 0.001$). The ventilation behaviour in tray B ($2.77 \pm 0.08\%$) was significantly lower ($F_{3,2744} = 235.620$; $p < 0.001$) compared with the other treatments (A: $5.55 \pm 0.11\%$; C: $5.61 \pm 0.12\%$; D: $5.26 \pm 0.10\%$).

In the graphic of Fig. 4, the variation of the behaviour is integrated over 116 h experiment in the form of baselines. As the baselines are obtained from the medium of two measurements, they do not represent the exact percentage of frequency but a trend of the behaviour at each measurement point. The first 8 h were considered as the acclimation period of *E. meridionalis* and were not included in data

Table 1
Physical and chemical parameters, and metal contents in the four trays during 116 h experiment; pH is presented in the form of range of the values registered; air saturation (O_2), conductivity (Cond.), temperature (T), and total metal concentration are the average values (± standard error) measured; metal contents and pH in the AMD from S. Domingos mine

Tray	pH	O_2 (%)	Total metal concentration							
			As ($\mu g\,l^{-1}$)	Ca ($mg\,l^{-1}$)	Cd ($\mu g\,l^{-1}$)	Cl ($mg\,l^{-1}$)	Co ($\mu g\,l^{-1}$)	Cr ($\mu g\,l^{-1}$)	Cu ($\mu g\,l^{-1}$)	Fe ($mg\,l^{-1}$)
A	7.6–8.2	81 ± 5	0.6 ± 0.0	66 ± 5	0.03 ± 0.0	38 ± 9	0.2 ± 0.0	0.7 ± 0.3	3.7 ± 0.5	31 ± 18
B	7.0–8.0	83 ± 4	2.7 ± 0.6	74 ± 3	1.5 ± 0.3	3 ± 2	11.7 ± 0.8	0.6 ± 0.1	50.2 ± 12.0	306 ± 99
C	6.4–8.0	83 ± 5	11.0 ± 4.3	77 ± 1	7.6 ± 1.7	26 ± 2	43.4 ± 3.6	1.2 ± 0.4	215 ± 88.0	1.6 ± 0.7
D	5.8–7.8	82 ± 5	11.3 ± 5.8	78 ± 1	17.0 ± 2.1	29 ± 3	85.2 ± 2.6	1.2 ± 0.5	260 ± 139	1.6 ± 0.9
AMD	2.4	–	971± 3	86 ± 0.4	427 ± 0.2	90 ± 18	$1.4 \times 10^3 \pm 0.0$	89.2 ± 0.5	$37.5 \times 10^3 \pm 9.2 \times 10^2$	314 ± 9
	Cond ($\mu S\,cm^{-1}$)	T (°C)	K ($mg\,l^{-1}$)	Mg ($mg\,l^{-1}$)	Mn ($\mu g\,l^{-1}$)	Na ($mg\,l^{-1}$)	Ni ($\mu g\,l^{-1}$)	Pb ($\mu g\,l^{-1}$)	S ($mg\,l^{-1}$)	Zn ($\mu g\,l^{-1}$)
A	424.8 ± 23.03	18.7 ± 0.1	17 ± 8	5.4 ± 0.1	4.3 ± 1.6	9.3 ± 0.1	1.1 ± 0.2	9.1 ± 2.6	3.6 ± 0.1	35.1 ± 10.2
B	475.0 ± 8.25	18.8 ± 0.0	1663 ± 0.2	6.6 ± 0.1	152 ± 8.9	9.7 ± 0.2	5.0 ± 0.3	4.0 ± 0.7	16 ± 0.7	145 ± 33.5
C	534.2 ± 1.38	19.1 ± 0.1	1.5 ± 0.1	8.9 ± 0.1	490 ± 13.9	10 ± 0.1	33.3 ± 2.1	5.3 ± 2.1	39 ± 0.8	747 ± 267
D	588.2 ± 2.40	19.4 ± 0.1	1.6 ± 0.2	11 ± 0.1	823 ± 13.9	13 ± 0.3	53.2 ± 1.7	5.3 ± 4.1	59 ± 1.1	1641 ± 427
AMD	–	–	20 ± 20	92 ± 0.3	$12.4 \times 10^3 \pm 3.8 \times 10^2$	46 ± 0.2	437 ± 1.5	51.8 ± 4.7	1127 ± 29	$81.7 \times 10^3 \pm 2.0 \times 10^3$

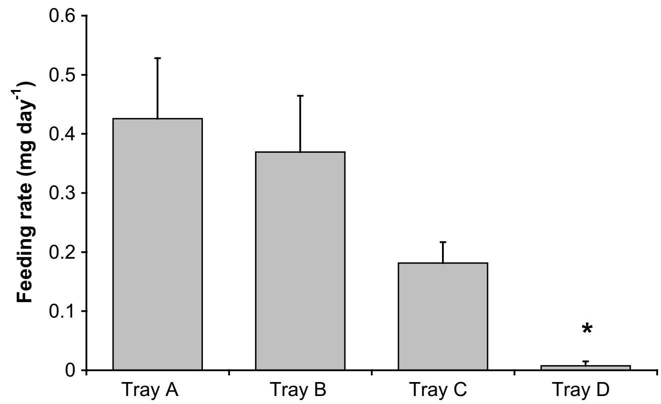

Fig. 5. Mean feeding rate (±standard error); ∗ denotes significant differences, Dunnett method $p < 0.05$.

analysis. In trays C and D, locomotion/feeding behaviour showed a decreasing trend and reached the lowest frequencies (around 15%) of all trays in spite of the fact that activity in tray D was the highest in the beginning of the test. Locomotion/feeding activity in trays A and B ended at values of *ca.* 20%, having started in tray A lower than in other trays but with an increasing tendency after 26 h. In tray B ventilation decreased over time (ending with 2.8% frequency), whilst ventilation recorded from the other trays were comparable in value and remained stable over time apart from tray D, where ventilation demonstrated a higher initial value but stabilised at a frequency of 5% after 22 h.

3.3. Feeding data

The average feeding rate (Fig. 5) was 0.426, 0.369, 0.181 and 0.007 mg d^{-1} in trays A, B, C and D, respectively. Inhibition of feeding was observed in all tested concentrations of AMD (13.3% reduction in feeding in tray B, 57.4% in tray C and 98.2% in tray D) with *E. meridionalis* feeding rate significantly reduced ($F_{3,27} = 3.598$; $p < 0.05$) at the highest AMD concentration (tray D).

4. Discussion

As pH decreases, the increase in H cations competing with metals for organic and inorganic ligands raises the concentration of free metal ions (Niyogi and Wood, 2003). These free ionic species represent a more bioavailable form of the metal that are more readily taken up by aquatic organisms (Depledge et al., 1993). In the present experiment the AMD with a pH 2.4 and enriched in heavy metals, was diluted by river water at pH 7.6 with hardness of 203 mg $CaCO_3\,l^{-1}$. As AMD was added to the tray it was dissolved in the hardwater, at circumneutral pH, and most of the metals complexed with other metals, complexed with the tray and chamber surfaces or formed a precipitate on the bed of the tray. This may have been the case of Fe and Pb that tend to co-precipitate (Hakansson et al., 1989). Hence the chemical nature of river Lena mitigated the toxicity of the AMD. The high buffering capacity, with alkalinity of 146 mg $CaCO_3$ l^{-1} in addition to the aeration process may explain the difficulty in obtaining lower pH values in the trays (as shown in Table 1), thus reducing the physiological deleterious effects associated with high concentration of H^+ ions (Havas, 1981). Also, due to the high water hardness, both Ca^{2+} and CO_3^{2-} may have acted to diminish the AMD's toxicity by affecting metal speciation. Cd and Zn, and at a less extent Cu are considered to be amongst the most available metals (Alonso et al., 2004). According to the biotic ligand model (Di Toro et al., 2001; Niyogi and Wood, 2003), Ca^{2+} is expected to compete with Cd, Zn and Cu cations for binding with the biotic ligand that is the site of toxic action on the organism. In addition CO_3^{2-} tends to complex with metals transforming them into less bioavailable forms. Therefore, as the proportion of river water increases, and AMD becomes more dilute, toxicity to *E. meridionalis* is reduced.

In previous experiments with the MFB, the behaviour of aquatic invertebrates was studied to assess the effects of AMD. In short-term 48 h bioassays, the crustacean species *Atyaephyra desmaresti* (Gerhardt et al., 2004; Janssens de Bisthoven et al., 2006) and *Daphnia magna* (Gerhardt et al., 2005b), and also the insects *Choroterpes picteti* (Gerhardt et al., 2005a) and *Chironomus* (Janssens de Bisthoven et al., 2004) showed an overall decrease in locomotion with increasing AMD concentration (and decreasing pH). Results from this study corroborate previous research as shown in Fig. 3, with a significant decrease of locomotion from tray B to C. However, differences in the AMD concentration were not detected between trays A and B, and C and D by this simplistic approach, *i.e.* considering only the average behaviour. Mortality provides further evidence of the increasing toxicity from tray B to C; it was only observed in trays C (22%) and D (67%). For relevant toxic heavy metals in solution such as Cd and Zn, LC_{50} (96 h) for *E. meridionalis* – LC_{50} 44.15 μg l^{-1} Cd, and LC_{50} 6.67 mg l^{-1} Zn (Pestana, unpublished data) – is higher than the concentrations observed in tray D where mortality was above 50%. The presence of several metals in solution may lead to synergistic and/or antagonistic effects (Depledge et al., 1993) although in this case when comparing the LC_{50} values with metal concentrations suggests that synergistic effects occurred. Furthermore, the pH level below 6 in tray D (at least when the pH was adjusted) was of major importance as it represents the survival threshold for many gammarid species (Hargeby and Petersen, 1988). The effect of AMD must, therefore, be interpreted by a holistic consideration of heavy metals and the acidity due to the complexity of chemical interactions taking place in this effluent and their respective and combined toxicity effects.

The variation in *E. meridionalis*' behaviour shown in the graph of Fig. 4 permits the distinction of behaviour between trays (unlike the data from Fig. 3) but does not give a clear idea for the separate variation in low frequency behaviour feeding and locomotion, during the 116 h of the experiment. Nevertheless in some stages it seems possible to extrapolate the variation in locomotion and in feeding separately. In tray D the initial high activity in the locomotion/feeding behaviour probably corresponded to the efforts of the organisms to escape the deleterious conditions in this tray (Beitinger and McCauley, 1990; Gerhardt et al., 1998), and therefore, corresponding to locomotion. The trend decrease in this activity might have been higher if feeding had not occurred, which provided energy that could be used to restore homeostasis and offset the effects of the AMD. It would be informative in future experiments to distinguish these two activities separating a discrete band of frequencies corresponding to the feeding in the MFB software in order to get the information of the feeding activity over time. The locomotion/feeding activity in trays C and D was initially high but decreased after 14 h, which corresponded to the beginning of light period in the laboratory. It has been reported for other benthic invertebrates (Kratz et al., 1994; Gerhardt, 1996) that drifting activities occur mainly during the night, probably due to lower predation pressure. As soon as the light was turned on simulating daytime efforts to escape ceased and the low locomotion/feeding activity observed suggests that the animals that survived suffered sublethal effects.

In tray B the average ventilation was significantly lower than in the other trays (Fig. 3), and demonstrated an overall trend to decrease (Fig. 4). The uptake of metals via the water that passes through gills represents the dominant uptake pathway for aquatic organisms (Gerhardt, 1992) and due to the chemical conditions of tray B a decrease in organisms ventilation would act to decrease metal uptake. This compensatory response could have been maintained during the 116 h of the test in order to suppress the metal's pathway without jeopardising the organisms' homeostatic balance. In the other treatment trays, since this physiological parameter is highly resistant to toxic stress as an essential body function (Naylor et al., 1989; Maltby et al., 1990b; Gerhardt, 2000b), no variation occurred in relation to the control (tray A).

Feeding is in most cases one of the first responses to environmental perturbations (McLoughlin et al., 2000). The feeding rate of *E. meridionalis* was only significantly different from the control in tray D in this study, possibly as a result of the relatively few replicates employed that affected the power of statistical tests. Nevertheless, previously reported data on the effects of metals on the feeding behaviour using *G. pulex* have demonstrated significant effects (lower observed effect concentrations, LOEC) at low concentrations of heavy metals, 20.6 μg l^{-1} for Cd, 23.0 μg l^{-1} for Cu, and 0.5 mg l^{-1} for Zn (Maltby, 1994). Cadmium and zinc have also affected the feeding activity of *E. meridionalis* in lab trials with a LOEC of 6.5 μg l^{-1} for Cd and 0.4 mg l^{-1} for Zn (Pestana, unpublished data). From the metal concentrations in our trays (Table 1) the concentration of Cu was twice as high as the LOEC for the gammarid *G. pulex* in tray B, and in tray C the concentration of Cd and Zn was higher than the LOEC for the previous trials using *E. meridionalis*.

5. Conclusions

The analysis of the data obtained demonstrated an overall positive relation between AMD and toxicity to *E. meridionalis* with increase in AMD concentration, a decrease in pH, an increase in the concentration of most metals resulting in an increase in mortality, decrease in locomotion/feeding activity and inhibition of the feeding rate. However, the variation in the physical–chemical parameters was not always linearly related to these biological endpoints. No mortality occurred in tray B although pH was lower and metals were present in higher concentration than in the control. For the behavioural endpoints, the decrease in average locomotion/feeding was only significantly different between tray B and tray C, whereas the toxic effects of AMD on ventilation were observed in tray B although there was no evidence of an increased effect associated with the increasing in AMD concentration between trays C and D. Feeding rate was inhibited with increasing AMD concentration, but only decreased significantly in the treatment with higher AMD concentration (tray D).

Comparing the results for mortality, behavioural and feeding endpoints the dose of AMD above which deleterious effects were apparent seems to occur at AMD concentration that was between the concentration of tray B and C, where the toxic concentrations (metals and H^+) appeared to exceed the regulatory capacity of *E. meridionalis*.

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References

Alonso, E., Santos, A., Callejon, M., Jimenez, J.C., 2004. Speciation as a screening tool for the determination of heavy metal surface water pollution in the Guadiamar river basin. Chemosphere 56, 561–570.

Beitinger, T.L., 1990. Behavioral reactions for the assessment of stress in fishes. J. Great Lakes Res. 16, 495–528.

Beitinger, T.L., McCauley, R.W., 1990. Whole-animal physiological processes for the assessment of stress in fishes. J. Great Lakes Res. 16, 542–575.

Boult, S., Collins, D.N., White, K.N., Curtis, C.D., 1994. Metal transport in a stream polluted by acid mine drainage – The Afon Goch, Anglesey, UK. Environ. Pollut. 84, 279–284.

Depledge, M.H., Weeks, J.M., Bjerregaard, P., 1993. Heavy metals. In: Calow, P. (Ed.), Handbook of Ecotoxicology. Blackwell Scientific Publications, Oxford, pp. 79–105.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. 20, 2383–2396.

Gerhardt, A., 1992. Review of heavy metals on stream invertebrates with special emphasis on acid conditions. Water Air Soil Pollut. 66, 289–314.

Gerhardt, A., 1995a. Effects of metals on stream invertebrates. In: Department of Ecology – Chemical Ecology and Ecotoxicology. Lund University, Lund.

Gerhardt, A., 1995b. Monitoring behavioural responses to metals in *Gammarus pulex* (L.) (Crustacea) with impedance conversion. Environ. Sci. Pollut. R 2, 15–23.

Gerhardt, A., 1996. Behavioural early warning responses to polluted water. Environ. Sci. Pollut. R 3, 63–70.

Gerhardt, A., 2000a. Biomonitoring of Polluted Water. Trans Tech Publications Ltd., Zürich.

Gerhardt, A., 2000b. Recent trends in biomonitoring for water quality control. In: Gerhardt, A. (Ed.), Biomonitoring of Polluted Water. Trans Tech Publications Ltd., Zürich, pp. 95–118.

Gerhardt, A., 2001. A new multispecies freshwater biomonitor for ecologically relevant supervision of surface waters. In: Butterworth, F.M., Gunatilaka, A., Gonsebatt, M.E. (Eds.), Biomonitors and Biomarkers as Indicators of Environmental Change: A Handbook. Plenum, New York, pp. 301–307.

Gerhardt, A., Svensson, E., Clostermann, M., Fridlund, B., 1994. Monitoring of behavioral patterns of aquatic organisms with an impedance conversion technique. Environ. Int. 20, 209–219.

Gerhardt, A., Carlsson, A., Ressemann, C., Stich, K.P., 1998. New online biomonitoring system for *Gammarus pulex* (L.) (Crustacea): in situ test below a copper effluent in south Sweden. Environ. Sci. Technol. 32, 150–156.

Gerhardt, A., Janssens de Bisthoven, L., Soares, A.M.V.M., 2004. Macroinvertebrate response to acid mine drainage: community metrics and on-line behavioural toxicity bioassay. Environ. Pollut. 130, 263–274.

Gerhardt, A., Janssens de Bisthoven, L., Soares, A.M.V.M., 2005a. Effects of acid mine drainage and acidity on the activity of *Choroterpes picteti* (Ephemeroptera: Leptophlebiidae). Arch. Environ. Contam. Toxicol. 48, 450–458.

Gerhardt, A., Janssens de Bisthoven, L., Soares, A.M.V.M., 2005b. Evidence for the stepwise stress model: *Gambusia holbrooki* and *Daphnia magna* under acid mine drainage and acidified reference water stress. Environ. Sci. Technol. 39, 4150–4158.

Hakansson, K., Karlsson, S., Allard, B., 1989. Effects of pH on the accumulation and redistribution of metals in a polluted stream bed sediment. Sci. Total Environ. 87–88, 43–57.

Hargeby, A., Petersen, R.C., 1988. Effects of low pH and humus on the survivorship, growth and feeding of *Gammarus pulex* (L.) (Amphipoda). Freshwater Biol. 19, 235–247.

Havas, M., 1981. Physiological response of aquatic animals to low pH. In: Singer, R. (Ed.), Effects of Acidic Precipitation on Benthos. North American Benthological Society, Hamilton, pp. 49–65.

Hoekkstra, J.A., Vaal, M.A., Notenboom, J., Slooff, W., 1994. Variation in the sensitivity of aquatic species to toxicants. B. Environ. Contam. Toxicol. 53, 98–105.

Janssens de Bisthoven, L., Gerhardt, A., Soares, A.M.V.M., 2004. Effects of acid mine drainage on larval *Chironomus* (diptera, chironomidae) measured with the multispecies freshwater biomonitor. Environ. Toxicol. Chem. 23, 1123–1128.

Janssens de Bisthoven, L., Gerhardt, A., Guhr, K., Soares, A.M.V.M., 2006. Behavioral changes and acute toxicity to the freshwater shrimp *Atyaephyra desmaresti* Millet (Decapoda: Natantia) from exposure to acid mine drainage. Ecotoxicology 15, 215–227.

Kratz, K.W., Cooper, S.D., Melack, J.M., 1994. Effects of single and repeated experimental acid pulses on invertebrates in a high altitude Sierra Nevada stream. Freshwater Biol. 32, 161–183.

Logan, D.T., Wilson, H.T., 1995. An ecological risk assessment method for species exposed to contaminant mixtures. Environ. Toxicol. Chem. 14, 351–359.

Maltby, L., 1994. Stress, shredders and streams: using *Gammarus* energetics to assess water quality. In: Sutcliffe, D.W. (Ed.), Water Quality and Stress Indicators in Marine and Freshwater Systems: Linking Levels of Biological Organisation (Individuals, Populations, Communities). Freshwater Biological Association, Ambleside, Cumbria, UK, pp. 98–110.

Maltby, L., Naylor, C., Calow, P., 1990a. Effect of stress on a freshwater benthic detritivore: scope for growth in *Gammarus pulex*. Ecotox. Environ. Safety 19, 285–291.

Maltby, L., Naylor, C., Calow, P., 1990b. Field deployment of a scope for growth assay involving *Gammarus pulex*, a freshwater benthic invertebrate. Ecotox. Environ. Safety 19, 292–300.

Maltby, L., Clayton, S.A., Yu, H.X., McLoughlin, N., Wood, R.M., Yin, D.Q., 2000. Using single-species toxicity tests, community-level responses and toxicity identification evaluations to investigate effluent impacts. Environ. Toxicol. Chem. 19, 151–157.

Maltby, L., Clayton, S.A., Wood, R.M., McLoughlin, N., 2002. Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness, and relevance. Environ. Toxicol. Chem. 21, 361–368.

McLoughlin, N., Yin, D.Q., Maltby, L., Wood, R.M., Yu, H.X., 2000. Evaluation of sensitivity and specificity of two crustacean biochemical biomarkers. Environ. Toxicol. Chem. 19, 2085–2092.

McWilliam, R.A., Baird, D.J., 2002. Application of postexposure feeding depression bioassays with *Daphnia magna* for assessment of toxic effluents in rivers. Environ. Toxicol. Chem. 21, 1462–1468.

Naylor, C., Maltby, L., Calow, P., 1989. Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. Hydrobiologia 188, 517–523.

Niyogi, S., Wood, C.M., 2003. Effects of chronic waterborne and dietary metal exposures on gill metal-binding: implications for the biotic ligand model. Hum. Ecol. Risk Assess. 9, 813–846.

Økland, J., Økland, K.A., 1986. The effects of acid deposition on benthic animals in lakes and streams. Experientia 42, 471–486.

Oliveira, J.M.S., 1997. Algumas reflexões com enfoque na problemática dos riscos ambientais associados à actividade mineira. Boletim de Minas 39.

Pontasch, K.W., Cairns Jr., J., 1991. Multispecies toxicity tests using indigenous organisms: predicting the effects of complex effluents in streams. Arch. Environ. Contam. Toxicol. 20, 103–112.

SPSS, 1995. SigmaStat for Windows (version 2.03), IL.

Ulfstrand, S., 1996. Behavioural ecology and conservation biology. Oikos 77, 183.

Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall International Editions, Inc., New Jersey.