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PAPER

Use of the Multispecies Freshwater Biomonitor to assess behavioral changes of *Poecilia reticulata* (Cyprinodontiformes: Poeciliidae) and *Macrobrachium lanchesteri* (Decapoda: Palaemonidae) in response to acid mine drainage: laboratory exposure

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The behavioral responses of guppy *Poecilia reticulata* (Poeciliidae) and prawn *Macrobrachium lanchesteri* (Palaemonidae) individuals exposed to acid mine drainage (AMD) were monitored online in the laboratory with a Multispecies Freshwater Biomonitor™ (MFB). These responses were compared to those to reference water acidified to the respective pH values (ACID). Test animals in the juvenile stage were used for both species and were exposed to AMD and ACID for 24 hours. The stress behaviors of both test animals consisted mainly of decreased activity in AMD and increased activity in ACID, indicating that the metals in the AMD played a role as a stress factor in addition to pH. The locomotor activity levels of guppies and prawns for the ACID treatment were higher than the locomotor activity levels for the AMD treatment with increasing pH value. For guppies, significant differences were observed when specimens were exposed to AMD and ACID at pH 5.0 and 6.0; the percentage activities were only 16% and 12%, respectively, for AMD treatment, whereas for ACID treatment, the percentage activities were 35% and 40%, respectively, similar to the value of 36% for the controls. Similar trends were also observed for prawns, for which the percentage activities were only 6% and 4%, respectively, for AMD treatment, whereas for ACID treatment, the percentage activities were 31% and 38%, respectively, compared to 44% in the controls. This study showed that both species are suitable for use as indicators for ecotoxicity testing with the MFB.

1. Introduction

Online biomonitoring instruments have been proven to have deterrent effects on polluters,[1] confirming the need to determine the effects of pollutants on aquatic organisms to be used as test species for online ecotoxicological water quality control. The study of biological responses allows the assessment of the toxicity of environmental pollutants while taking bioavailability into account.[2] These responses can be measured with online biomonitoring systems[3] which have the advantage of providing information on exposure to toxic compounds on a continuous real-time basis and recording a time series of behavioral data to establish effects based on exposure time.[4] Indigenous organisms are better biomonitors than standard test species because they play an ecological role in the aquatic ecosystems in which they occur, and indigenous species have been proven to be better

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Environmental impact

This study determined the sensitivity of behavioral changes in *Poecilia reticulata* and *Macrobrachium lanchesteri* individuals exposed to acid mine drainage using the new approach of online biomonitoring with the Multispecies Freshwater Biomonitor™ (MFB). Considering the average locomotor activity for each concentration in both the ‘ACID only’ and AMD treatments, both guppies and prawns were more active in ACID than in AMD treatments. Thus, metals in the AMD and the low pH affected the animals’ locomotion. As the pH decreases, the increase in H^+ cations competing with metals for organic and inorganic ligands raises the concentration of free metal ions. These free ionic species represent a more bioavailable form of the metal that is more readily taken up by aquatic organisms.

indicators for anthropogenic stressors,[5] hence providing more environmentally realistic results.[1] Currently, Malaysia uses only chemical and physical analyses of water to monitor and detect water pollution in surface and waste waters. The combined use of chemical and physical analyses and a biological early warning system will increase both the effectiveness and the reliability of monitoring programs for pollution control in Malaysia's water.

Biomonitoring should rely on sublethal endpoints rather than mortality alone. Behavior is a sublethal parameter that is readily altered by stress,[6] and changes in behavior may have ecological consequences; *e.g.*, avoidance behavior or decreased activity may influence populations by affecting migration or susceptibility to predation.[7] Behavioral endpoints are more sensitive than mortality tests,[8] reflecting different levels of susceptibility of organisms to environmental stimuli and are therefore appropriate indicators for biomonitoring purposes.[9,10] Once a determined behavior can be quantified, it has the potential to be used as a biomarker in the assessment of stress.[6]

Previous studies have shown that mining is one of the most important sources of heavy metal contamination in the environment.[11–15] Heavy metals contained in residues coming from mining and metallurgical operations are often dispersed by wind and water after their disposal.[16] As a developing country, Malaysia is no exception and faces metal pollution caused by mining activities and abandoned mines. Among mining activities in Malaysia, tin mining was the most popular, and Malaysia was the world's largest producer of tin until 1985. In 1979, Malaysia was producing almost 63 000 tons, accounting for 31% of the world output. Apart from tin, other minerals such as gold, iron ore, bauxite, coal and kaolin were also mined.[17]

Abandoned mines can impact their surrounding environment because of the chemical nature of their effluents. Mining activities often focus on ores rich in heavy metals and expose metal sulfides to oxidation with the consequent release of acid mine drainage (AMD).[18] In addition, the pollution of soil and groundwater by dissolved heavy metals has mainly been associated with AMD, one of the most serious environmental hazards of the mining industry. AMD is generated by the oxidation of sulfide-containing minerals exposed to weathering conditions, resulting in low-quality effluents characterized by acidic pH, a high level of dissolved metals (*e.g.*, As, Cd, Cu, and Zn), and a high concentration of anions (*e.g.*, sulfates and carbonates).[16]

Sungai Lembing (3°54′23″N and 103°2′30″E) is a tin mining town located 34 km northwest of Kuantan in Pahang, Peninsular Malaysia. It is an old mining town that once had the biggest tin mine on Earth. Sungai Lembing was founded 100 years ago during the British colonial days. After 80 years of mining activities, it became the largest, longest and deepest underground tin mine in the world. Until the 1970s, Sungai Lembing was a major producer of underground tin. The town of Sungai Lembing was developed in the 1880s when the British set up the tin mining industry, although the history of mining in this area extends much further back in time. From 1891, the Pahang Consolidated Company Limited, which was under British control, had a 77 year lease to mine the area. The pit mines were closed in 1986 due to high operational costs and low yields, but during their heyday, these mines were among the largest and deepest in the world. The total tunnel length is 322 km, with a depth ranging between 610 m and 700 m. The Sungai Lembing mine kept its waste material from the treatment plant at a predetermined site near the bank of the Kenau River.[17] Currently, water from precipitation still flows out from this abandoned mine; this water has a very low pH level and elevated metal concentrations.[19]

The purpose of this study was to determine the sensitivity of locomotion and ventilation behavioral changes in *Poecilia reticulata* and *Macrobrachium lanchesteri* individuals exposed to acid mine drainage by using the new approach of online biomonitoring with the Multispecies Freshwater Biomonitor™ (MFB).

2. Materials and methods

2.1. Test species

The guppy is one of the most popular freshwater aquarium fish species in the world. It is a small member of the Poeciliidae family (female ±3.5 cm long and male ±2 cm long). Guppies feed on zooplankton, small insects and detritus.[20] The optimum temperature for the guppy is between 23.9 and 29.4 °C, and the optimum pH is approximately 6.8 to 7.2.[21] In this study, guppies were collected from a small stream on the university campus. The sizes of the guppies were approximately 1.5 to 2.0 centimeters.

M. lanchesteri prawns were purchased from an aquarium shop in Bangi, Selangor, and the sizes of individuals used in this study were approximately 1.5 to 2.5 centimeters. *M. lanchesteri* is common in still or slowly flowing waters such as reservoirs, ponds, irrigation ditches and other artificial enclosed freshwater bodies.[22,23] This species has been used in several toxicity testing and environmental monitoring programs.[24–26]

Before the experiment started, the test species were acclimated for four days to the laboratory conditions with a controlled temperature of 28 ± 2 °C and cycles of 12 hours of light and 12 hours of dark. During the acclimation period, they were fed by Aquadine® fish pellets. All experiments were performed in compliance with the relevant laws and local guidelines.

2.2. Experimental design

AMD was collected from the abandoned mine at Sungai Lembing, Pahang, Malaysia (N 03°54.662′, E 103°01.878), and had a pH 2.17. The pH and other water quality parameters (temperature, dissolved oxygen and conductivity) were measured using a multi-parameter water quality meter (Hydrolab Quanta®). Total dissolved heavy metal concentrations (filtered with 0.45 μm membrane filters (Whatman International ltd., Maidstone, England)) were measured in all experimental waters/dilutions using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Perkin-Elmer ELAN 9000). Diluted nitric acid: HNO_3, 70% (Merck, Germany) was used as acidified control water (ACID) as a reference against which to compare the behavioral changes of test organisms exposed to AMD. Each species was exposed to 5 concentrations of AMD and ACID (pH 2, 3, 4, 5 and 6) and a control (pH 7.15 ± 0.12) of dechlorinated tap water (filtered through several layers of sand and activated carbon; T.C. Sediment Filter® (TK Multitrade, Seri Kembangan, Malaysia)).

It was decided to use dilutions of AMD from the field site to create a realistic chemical environment for the following reasons: (1) in nature, populations are exposed to mixtures of chemical contaminants and (2) dilutions of such a mixture occur downstream, resulting in a pollution gradient.[42] The AMD and ACID were diluted to the required pH using dechlorinated tap water from the laboratory. The lowest pH AMD sample was the original, undiluted sample from Sungai Lembing. The dilutions of test solution were prepared by adding dechlorinated tap water to the original AMD solution and to the ACID until the desired pH was reached. The experiment was conducted in 10 L plastic basins (HDPE) filled with 5 L of test solution, a static-water test design (without any renewal).

Only healthy and active organisms (tested by direct observation using the oscilloscope function in MFB™ software) were used and transferred carefully into the test chambers. The test organisms were placed individually in every test chamber with Nylon mesh screwed at both ends (seven replicates per treatment plus one blank chamber as a control for signal disturbance). Subsequently, the chambers were placed horizontally in the plastic basin. The remaining air bubbles in the chambers were removed with a pipette to avoid signal disturbances.

2.3. Behavioral response

The MFB construction and function are described elsewhere.[3,4] This system is based on the quadrupole impedance technique and consists of a desktop computer, a measuring instrument with 8 channels, and the respective 8 test chambers. The chambers are made of a Plexiglas pipe (2 cm in diameter and 5 cm long) with two pairs of stainless steel electrodes attached on opposite sides of the inner walls and capped with 1 mm mesh at both ends.[9] A high-frequency alternating current is applied to one pair of electrodes, and the movements of the organism create impedance to the current that is measured by the other pair of non-current-carrying electrodes. The signal measured is then sent to the computer where the data are registered with the MFB software. The results of the organisms' movements are plotted as a graph of current (V) over time, which can be transformed by a discrete fast Fourier transformation (FFT) into a frequency histogram that includes the relative amounts of the low-frequency behaviors (0–2 Hz) (*e.g.*, locomotion, feeding behavior) and high-frequency behaviors (2.5–8 Hz) (*e.g.*, ventilation).[27] These measurements were recorded every 10 minutes for a duration of 4 minutes. The MFB recorded data over 24 hours for each concentration of AMD and ACID for both species.

2.4. Statistical analysis

Statistical analysis was carried out with a significance level of $p < 0.05$ and was conducted using the non-parametric Friedman Test with the statistical software Minitab 15 to analyze the significant differences between pH treatments or AMD dilutions compared to the control/reference. In the case of significant differences, post-hoc pairwise tests (Wilcoxon test) were performed to further define the differences with respect to the control or between AMD and ACID at the respective pH level.

3. Results

3.1. Physical and chemical data

The physical and chemical parameters recorded during the experiment are presented in Table 1. Although the pH for every concentration was adjusted at the start of each experiment, it increased during the experiment for every pH concentration. From pH 2.0 to pH 6.0, there was a decrease in the conductivity due to a decrease in the concentration of electrolytes (ions) as more dechlorinated water was added to AMD and ACID to increase the pH (Table 1). The air saturation was approximately 81–85% for all exposures. The average water temperature for all experiments ranged between 26 and 28 °C.

The total dissolved metal concentrations in AMD and ACID are presented in Table 2. According to the data, the abandoned Sungai Lembing tin mine is contaminated with Fe > Al > Cu > Zn > Mn > Ni > Cd > Pb in non-diluted AMD. The analysis of the total dissolved metals in AMD showed an overall tendency to decrease with increasing pH from pH 2.0 to pH 6.0, due to dilution with dechlorinated water containing low metal concentrations.

3.2. Behavioral data analysis

Behavioral signal responses of *P. reticulata* and *M. lanchesteri* show that both species were active during the entire 24 hour period in the control treatment, with more than 40% of the time spent on locomotion during the first 2 hours. Two of the highest peaks of fish behavior observed during the 24 hour exposure period were approximately 7 to 8 o'clock in the morning and 7 to 8 o'clock in the evening (Fig. 1). These are the times when the light was switched on and off in the laboratory to mimic the natural environmental conditions. Similar behavior patterns were found for guppy behavior when exposed to ACID at pH 5 and 6 (Fig. 2). For the prawns, there was no specific behavioral trend in the signal and they showed similar locomotor activity during the 24 hour period.

As the AMD pH increased from pH 2.0 to pH 6.0, behavioral changes in exposed animals were observed (Fig. 2). At pH 2 and pH 3 for both AMD and ACID treatments, 100% mortality was observed. Considering the average locomotor activity for each concentration in both the ACID and AMD treatments in Fig. 3, both guppies and prawns were more active in ACID than in AMD treatments with increasing pH. Guppies were less active in AMD at pH 5.0 (16%) and 6.0 (12%) than in the ACID treatments at the same pH (35% and 40% activity, respectively). Prawns were less active at pH 5.0 (6%) and pH 6.0 (4%) in AMD

Table 1 Physical and chemical parameters of the AMD and ACID solutions[a]

	pH	DO (%)	Cond (mS cm^{-1})	T (°C)
AMD				
pH 2.0	2.05–2.25	92.43 ± 7.98	1.56 ± 0.07	25.59 ± 0.27
pH 3.0	2.92–2.99	91.81 ± 6.23	0.43 ± 0.04	27.66 ± 0.54
pH 4.0	3.99–4.15	87.26 ± 3.22	0.19 ± 0.01	27.37 ± 0.56
pH 5.0	4.98–5.28	90.68 ± 4.74	0.15 ± 0.00	28.04 ± 0.48
pH 6.0	6.00–6.17	88.82 ± 4.91	0.14 ± 0.00	27.57 ± 0.52
ACID				
pH 2.0	2.07–2.31	97.23 ± 1.17	1.02 ± 0.02	27.20 ± 0.62
pH 3.0	2.93–3.08	97.77 ± 1.56	0.29 ± 0.01	27.15 ± 0.29
pH 4.0	3.96–4.68	91.97 ± 6.52	0.16 ± 0.01	26.99 ± 0.59
pH 5.0	4.98–5.94	81.44 ± 23.52	0.14 ± 0.00	27.05 ± 0.51
pH 6.0	6.00–6.10	90.80 ± 0.00	0.14 ± 0.00	26.83 ± 0.00
Control	7.00–7.29	81.78 ± 4.44	0.12 ± 0.00	27.10 ± 0.72

[a] DO – dissolved oxygen, Cond – conductivity, T – water temperature.

Table 2 Total dissolved metal contents in the AMD and ACID solutions

	Cd (ppb)	Cu (ppb)	Mn (ppb)	Fe (ppb)	Pb (ppb)
AMD					
pH 2.0	88 ± 1.19	19 047.67 ± 141.52	2930.99 ± 146.03	27 342.00 ± 12 521.85	15.01 ± 10.35
pH 3.0	18.92 ± 0.70	4068.92 ± 200.42	608.40 ± 62.00	3894.29 ± 1312.57	3.59 ± 1.97
pH 4.0	7.61 ± 1.58	1583.87 ± 379.16	265.36 ± 88.08	158.73 ± 30.22	0.83 ± 0.07
pH 5.0	4.69 ± 0.54	987.25 ± 38.45	172.57 ± 44.10	128.78 ± 6.57	0.37 ± 0.05
pH 6.0	2.92 ± 0.82	632.39 ± 193.06	109.89 ± 43.10	113.90 ± 16.27	0.11 ± 0.09
ACID					
pH 2.0	0.05 ± 0.04	47.27 ± 16.04	8.78 ± 0.70	150.05 ± 1.04	1.00 ± 1.19
pH 3.0	0.06 ± 0.01	36.49 ± 27.05	9.08 ± 1.03	172.47 ± 46.25	1.02 ± 0.19
pH 4.0	0.07 ± 0.01	23.20 ± 14.87	8.75 ± 1.02	158.10 ± 22.85	0.85 ± 0.12
pH 5.0	0.13 ± 0.08	26.64 ± 3.39	5.90 ± 2.19	163.43 ± 14.14	0.58 ± 0.12
pH 6.0	0.05 ± 0.01	10.96 ± 11.96	3.91 ± 3.74	121.11 ± 46.46	0.17 ± 0.24
Control	0.07 ± 0.01	11.28 ± 11.17	5.54 ± 0.06	122.51 ± 16.47	0.00 ± 0.00

	Zn (ppb)	Al (ppb)	Ni (ppb)	Ca (ppb)	Mg (ppb)
AMD					
pH 2.0	8200.34 ± 206.55	26 018.28 ± 156.60	114.02 ± 3.72	20 749.34 ± 2017.90	7678.83 ± 307.64
pH 3.0	2111.28 ± 202.14	5312.80 ± 197.49	26.98 ± 0.29	10 738.42 ± 2484.92	2220.62 ± 241.59
pH 4.0	874.04 ± 239.74	1857.24 ± 572.33	12.42 ± 1.22	8778.96 ± 2445.21	1319.77 ± 202.80
pH 5.0	568.66 ± 107.57	536.63 ± 38.65	9.15 ± 0.38	8186.02 ± 2179.79	1117.17 ± 115.99
pH 6.0	373.34 ± 127.47	127.96 ± 25.74	7.13 ± 0.10	7732.98 ± 1882.10	961.30 ± 135.17
ACID					
pH 2.0	71.74 ± 11.86	162.73 ± 55.93	2.33 ± 0.60	8977.72 ± 1387.48	834.14 ± 67.69
pH 3.0	73.17 ± 1.72	145.40 ± 33.52	3.06 ± 0.21	9116.06 ± 1048.48	832.05 ± 62.22
pH 4.0	83.70 ± 12.06	120.99 ± 23.15	3.43 ± 0.02	9948.44 ± 93.92	928.88 ± 36.90
pH 5.0	87.03 ± 12.57	91.23 ± 36.79	2.98 ± 0.66	9816.93 ± 366.97	823.24 ± 140.98
pH 6.0	82.58 ± 14.15	61.27 ± 49.92	1.78 ± 1.58	9485.89 ± 74.12	830.47 ± 112.85
Control	43.00 ± 9.92	62.05 ± 4.59	3.75 ± 0.04	6455.62 ± 74.97	723.06 ± 10.98

treatments compared to ACID treatments, for which the activity levels were 31% (pH 5.0) and 38% (pH 6.0). Thus, for both species, metals in the AMD and low pH affected the animals' locomotion more than low pH alone (Table 2).

Statistical analysis using the Friedman test showed that there were highly significant differences between all treatments and controls for all treatments with AMD and ACID. Relatively, the mean ranks of the control for the AMD treatments for fish and prawn were the highest among all treatments. Friedman test analyses showed that there were significant differences between treatments [(X^2 (5, $N = 145$) = 600.78, $P < 0.01$) and (X^2 (5, $N = 145$) = 537.23, $P < 0.001$)]. A post-hoc pairwise test (Wilcoxon test) showed that there were significant differences between prawns and guppies for all respective treatments ($p < 0.001$) except at pH 2.0 ($p > 0.05$).

4. Discussion

As the pH decreases, the increase in H^+ cations competing with metals for organic and inorganic ligands raises the concentration of free metal ions.[28] These free ionic species represent a more bioavailable form of the metal that is more readily taken up by aquatic organisms.[29] In previous experiments with the MFB, the behavior of aquatic invertebrates was studied to assess the effects of AMD. In short-term 48 hour bioassays, the crustacean species *Atyaephyra desmaresti*[30,31] and *Daphnia magna*[32] and the insects

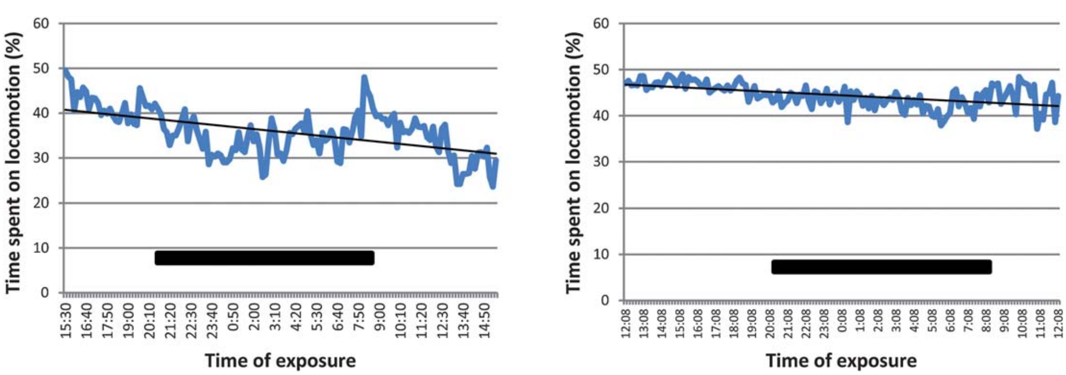

Fig. 1 Behavioral response for the control treatment and the trend line from the linear regression of *P. reticulata* (left) and *M. lanchesteri* (right); the black bar represents the dark period.

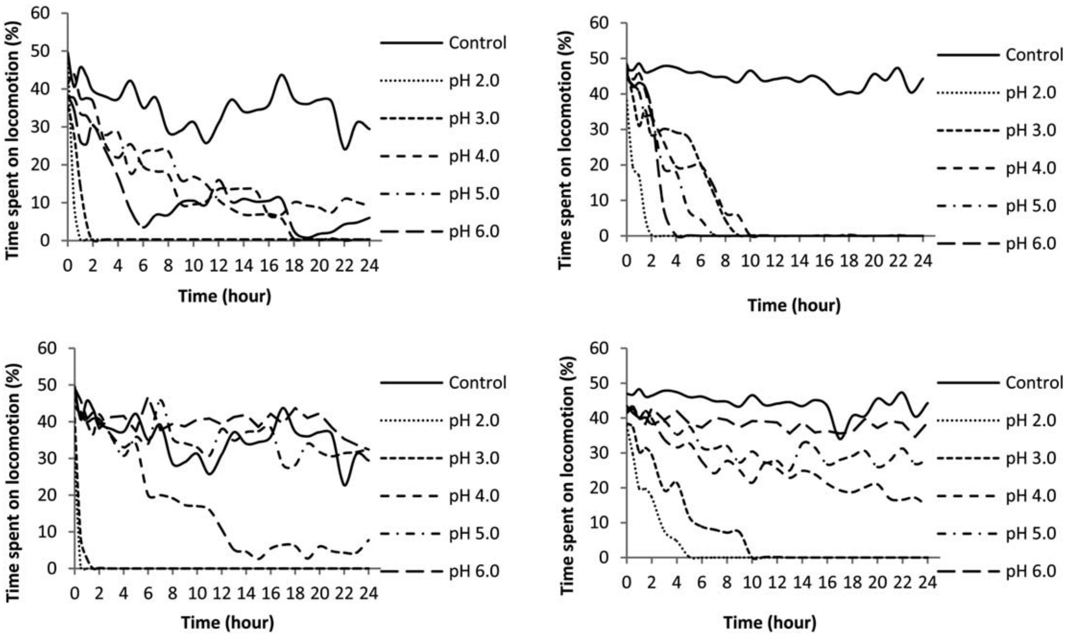

Fig. 2 Locomotor activity under the different AMD and ACID conditions (24 h). Values from the raw data are plotted ($N = 6$–7, mean presented, SD bars omitted for clarity, SD: 2–5%). Top left: exposure of guppies to the AMD treatment. Top right: exposure of prawns to the AMD treatment. Bottom left: exposure of guppies to the ACID treatment. Bottom right: exposure of prawns to the ACID treatment.

Choroterpes picteti[33] and *Chironomus* sp.[34] showed an overall decrease in locomotion with increasing AMD concentration (and decreasing pH), results that are in agreement with the findings of the present study.

Decreasing ventilation may be associated with the response of prawns to reduce the intake of metals. The metal intake in prawns takes place through the gills, and a decrease in ventilation might be an indirect avoidance behavior to reduce the intake of the metal.[35] A similar phenomenon was observed in the amphipod *Echinogammarus meridionalis* when exposed to acid mine drainage.[36] Similar results were also obtained when using municipal waste water and pharmaceuticals.[37] Gerhardt *et al.* (2002) explained that for the pelagic fish, the first response would be to escape, followed by an increase in ventilation, whereas for the benthic prawn, first it would increase ventilation, followed by a decrease in ventilation and then movement afterwards. Stress response cascades might thus be affected by the regulation and/or techniques available to species. Gerhardt *et al.* (2004) reported that the overall activity of the benthic prawn *Atyaephyra desmaresti* decreased with decreases in the pH of water samples from

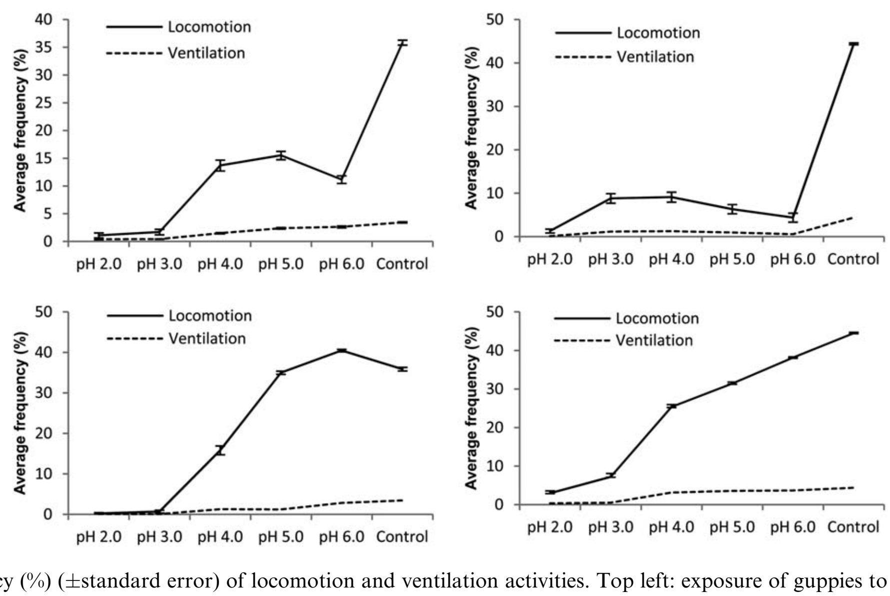

Fig. 3 Average frequency (%) (±standard error) of locomotion and ventilation activities. Top left: exposure of guppies to the AMD treatment. Top right: exposure of prawns to the AMD treatment. Bottom left: exposure of guppies to the ACID treatment. Bottom right: exposure of prawns to the ACID treatment.

acid mine drainage.[30] In addition, Janssens de Bisthoven *et al.* (2004) reported a reduction of locomotion and decreased ventilation of the benthic *Chironomus* sp. larvae exposed to reduced pH in acid mine drainage.[34]

The decreased ventilation of fish exposed to increasing concentrations of metal may be associated with attempts to remove metal ions that adsorb to the gill membrane that covers the metal binding site of Ca.[38] A similar observation was also reported with exposure of mayfly larvae *Adenophlebia auriculata* to Cu[39] and of the amphipod *Gammarus pulex* exposed to Cu (50 ppb) and Pb (>10 ppb).[7] The same results were also reported using the freshwater prawn *Atyaephyra desmaresti*; the time used for movement and ventilation were 25–35% and 3–10%, respectively.[31] Exposure of the prawn to the acid mine drainage indicated that the movement of prawns at a pH of 5.5 is lower than in the more acidic pH 5.0. It was concluded that this response was due to the metals in the water samples being more available at pH 5.5.[31] Janssens de Bisthoven *et al.* (2004) also reported similar observations for *Chironomus* sp. larvae in pond water (for control treatment) in which the time used for movement was between 15% and 28%, whereas the ventilation time was less than 6%.[34]

Heavy metals can enhance the toxicity of mine drainage and also act as metabolic poisons. Iron, aluminum, and manganese are the most common heavy metals which can compound the unpleasant effects of mine drainage. Heavy metals are generally less toxic at circumneutral pH. Trace metals such as zinc, cadmium, and copper, which may also be present in mine drainage, are toxic at extremely low concentrations and may act synergistically to suppress algal growth and affect fish and benthos.[43]

Cooper and Wagner (1973) studied the distribution of fish in Pennsylvania streams affected by acid mine drainage. They found fish species were severely impacted at pH 4.5 to 5.5; ten species showed some tolerance to pH 5.5 or less; 38 species were found at pH 5.6 to 6.4; and 68 species were found only at pH greater than 6.4. They found that a pH of 4.5 and total acidity of 15 mg L^{-1} accounted for complete loss of fish in 90% of streams studied. Although no concentrations of metals were taken into account, Cooper and Wagner indicated that the absence of fish in acidified waters can be related to dissolved metals at certain pH levels. They also indicated that sulfates, a major constituent of acid mine drainage, did not become toxic to fish until concentrations exceeded the saturation level of several thousand mg L^{-1}.[44]

This study showed that prawns (*M. lanchesteri*) are more sensitive than fish (*P. reticulata*) to the AMD. The mortality in the AMD treatment for prawns was higher than for fish. The Friedman test showed that there were significant differences between the behavioral responses of the prawns and the fish ($P < 0.001$). Previous studies using fish (*Gambusia holbrooki*) and prawns (*Atyaephyra desmaresti*) exposed to acid mine drainage also found that the prawn *A. desmaresti* was more sensitive to heavy metals than the fish *G. holbrooki*.[30] Laboratory toxicity tests also showed that *M. lanchesteri* is more sensitive to Cd than *P. reticulata*; the 96 h LC_{50} values were 7.0 μg L^{-1} and 168.1 μg L^{-1}, respectively.[40] Gerhardt *et al.* (2002) stated that with respect to the relative values of the measured behavioral responses (with the MFB) of fish and prawns, the values for fish will usually be more depressed in the test chamber because they are pelagic organisms and are constantly swimming, whereas prawns are benthic organisms. This difference will lead to greater variance in behavior in the control and will make it difficult to distinguish the fish exposed to pollutants.[37] This explains why the fish are less sensitive than prawns. Pairwise comparisons with the Wilcoxon test also showed that there were significant differences between fish and prawns for all treatments ($P < 0.001$). Janssens de Bisthoven *et al.* (2006) also suggested the importance of prawns in the monitoring with MFB and have several other advantages such as high sensitivity, increasing the economic importance of prawns and prawn aquaculture and increasing the prawn's ecological importance, especially in subtropical and tropical ecosystems.[31] For this reason, freshwater prawns are a valuable option as a biological test organism.

This study also showed that the use of behavioral responses is more sensitive than mortality as a toxicity indicator. Laboratory toxicity tests showed that 24 h LC_{50} values for *M. lanchesteri* and *P. reticulata* exposed to Cd were 19.8 μg L^{-1} and 8205 μg L^{-1}, respectively.[40] In this study, the behavioral changes of animals exposed to AMD could be observed for 10 minutes only at the lower concentration levels. After 10 minutes of exposure of prawns to AMD, the percentages of locomotion decreased from 46% (pH 6) to 29% (pH 2) with increasing AMD concentrations. This result clearly shows the advantages of using behavioral responses in detecting contaminants in water and that the use of behavioral responses is suitable for monitoring water quality. Similar results were also obtained by Gerhardt *et al.* (2002), who showed that behavioral responses are more sensitive than mortality as a toxicity parameter by comparing the time for behavioral responses with time to death.[37] Previous studies using the same species also showed that the activity levels of *M. lanchesteri* and *P. reticulata* decreased when exposed to cadmium for 2 hours at concentrations as low as 1 ppb and 100 ppb, respectively;[41] the 96 h LC_{50} values are 7.0 ppb and 168.1 ppb, respectively.[40]

5. Conclusion

This study showed that for two freshwater species, the fish *Poecilia reticulata* and the prawn *Macrobrachium lanchesteri*, decreased locomotor activity was observed with increasing metal concentrations in AMD. This study also demonstrated that acid mine drainage is toxic not only because of very low pH level but also because of its high metal concentrations. In conclusion, *P. reticulata* and *M. lanchesteri* are both suitable species to be used as indicators in the MFB for online biomonitoring and the detection of AMD, acidity and other metal pollutants.

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References

1 A. Gerhardt, *Biomonitoring of Polluted Water*, Trans Tech Publications Ltd., Zürich, 2000.
2 J. A. Hoekkstra, M. A. Vaal, J. Notenboom and W. Slooff, *Bull. Environ. Contam. Toxicol.*, 1994, **53**, 98–105.
3 A. Gerhardt, A. Carlsson, C. Ressemann and K. P. Stich, *Environ. Sci. Technol.*, 1998, **32**, 150–156.
4 A. Gerhardt, in *Biomonitors and Biomarkers as Indicators of Environmental Change: A Handbook*, ed. F. M. Butterworth, A. Gunatilaka and M. E. Gonsebatt, Plenum, New York, 2001, pp. 301–307.
5 K. W. Pontasch and J. Cairns, Jr, *Arch. Environ. Contam. Toxicol.*, 1991, **20**, 103–112.
6 T. L. Beitinger, *J. Great Lakes Res.*, 1990, **16**(4), 495–528.
7 A. Gerhardt, *Environ. Sci. Pollut. Res.*, 1995, **2**, 15–23.
8 A. Gerhardt, *Effects of metals on stream invertebrates*, in Department of Ecology – Chemical Ecology and Ecotoxicology, Lund University, Lund, 1995.
9 A. Gerhardt, E. Svensson, M. Clostermann and B. Fridlund, *Environ. Int.*, 1994, **20**, 209–219.
10 S. Ulfstrand, *Oikos*, 1996, **77**, 183.
11 M. Benvenuti, I. Mascaro, F. Corsini, P. Lattanzi, P. Parrini and G. Tanelli, *Environ. Geol.*, 1997, **30**, 238–243.
12 R. Asklund and B. Eldvall, *Contamination of Water Resources in Tarkwa Mining Area of Ghana*, Royal Institute of Technology, 2005.
13 S. Wang and C. N. Mulligan, *Sci. Total Environ.*, 2006, **366**, 701–721.
14 M. C. Navarro, C. Perez-Sirvent, M. J. Martinez-Sainchez, J. Vidal, P. J. Tovar and J. Bech, *J. Geochem. Explor.*, 2008, **96**, 183–193.
15 P. Schwab, D. Zhu and M. K. Banks, *Bioresour. Technol.*, 2007, **98**, 2935–2941.
16 I. Razo, L. Carrizales, J. Castro, F. Diaz-barriga and M. Monroy, *Water, Air, Soil Pollut.*, 2004, **152**, 129–152.
17 W. W. H. Fuad, *Proceedings of the Regional Symposium on Environment and Natural Resources Kuala Lumpur*, Malaysia, 2002, 1, pp. 413–424.
18 S. Boult, D. N. Collins, K. N. White and C. D. Curtis, *Environ. Pollut.*, 1994, **84**, 279–284.
19 W. Zuhairi, N. Syuhadah and H. Abdil Mutalib, *Bull. - Geol. Soc. Malays.*, 2009, **55**, 15–20.
20 A. A. Fernando and V. P. E. Phang, *Aquaculture*, 1985, **51**, 49–63.
21 D. S. Johnson, M. H. H. Soong, *Proceeding of the 16th International Congress Zoology*, Washington D.C., 1963, 1, pp. 246–246.
22 D. S. Johnson, *Bulletin of the National Museum*, Singapore, 1963, 32, 5–30.
23 *Earl of Cranbrook*, ed. E. Cranbrook and J. I. Furtado, Malaysia, 1988, p. 225.
24 S. Suckcharoen, *Bull. Environ. Contam. Toxicol.*, 1980, **24**(4), 511–514.
25 M. Shuhaimi-Othman, A. Ahmad, S. Yap and M. Maziati, *J. Biol. Sci.*, 2006, **6**(4), 717–722.
26 M. Shuhaimi-Othman, Y. Nadzifah, R. Nur-Amalina and A. Ahmad, *Toxicol. Ind. Health*, 2011, **27**(6), 523–530.
27 A. Gerhardt, in *Biomonitoring of Polluted Water*, ed. A. Gerhardt, Trans Tech Publications Ltd., Zürich, 2000, pp. 95–118.
28 S. Niyogi and C. M. Wood, *Hum. Ecol. Risk Assess.*, 2003, **9**, 813–846.
29 M. H. Depledge, J. M. Weeks and P. Bjerregaard, in *Handbook of Ecotoxicology*, ed. P. Calow, Blackwell Scientific Publications, Oxford, 1993, pp. 79–105.
30 A. Gerhardt, L. Janssens de Bisthoven and A. M. V. M. Soares, *Environ. Pollut.*, 2004, **130**, 263–274.
31 L. Janssens de Bisthoven, A. Gerhardt, K. Guhr and A. M. V. M. Soares, *Ecotoxicology*, 2006, **15**, 215–227.
32 A. Gerhardt, L. Janssens de Bisthoven and A. M. V. M. Soares, *Environ. Sci. Technol.*, 2005, **39**, 4150–4158.
33 A. Gerhardt, L. Janssens de Bisthoven and A. M. V. M. Soares, *Arch. Environ. Contam. Toxicol.*, 2005, **48**, 450–458.
34 L. Janssens de Bisthoven, A. Gerhardt, K. Guhr and A. M. V. M. Soares, *Environ. Toxicol. Chem.*, 2004, **23**(5), 1123–1128.
35 A. Gerhardt, *Water, Air, Soil Pollut.*, 1992, **66**, 289–314.
36 J. A. Macedo-Sousa, J. L. T. Pestana, A. Gerhardt, A. J. A. Nogueira and A. M. V. M. Soares, *Chemosphere*, 2007, **67**, 1663–1670.
37 A. Gerhardt, L. Janssens de Bisthoven, Z. Mo, C. Wang, M. Yang and Z. Wang, *Chemosphere*, 2002, **47**, 35–47.
38 R. W. Winner and J. D. Gauss, *Aquat. Toxicol.*, 1986, **8**, 149–161.
39 A. Gerhardt and C. Palmer, *Sci. Total Environ.*, 1998, **215**, 217–229.
40 Y. Nadzifah, M. Shuhaimi-Othman, *Proceeding of 8th Graduate Colloquium*, Faculty of Science and Technology, UKM, 2008, 1, pp. 509–512.
41 M. Shuhaimi-Othman, M. Azmah and A. K. Ahmad, *Malays. J. Sci.*, 2010, **39**(4), 549–555.
42 D. T. Logan and H. T. Wilson, *Environ. Toxicol. Chem.*, 1995, **14**, 351–359; D. T. Logan and H. T. Wilson, *Environ. Pollut.*, 1994, **84**, 279–284.
43 R. C. Hoehn and D. R. Sizemore, *J. Am. Water Resour. Assoc.*, 1977, **13**, 153–160.
44 E. L. Cooper and C. C. Wagner, Environmental Protection Agency Report no. EPA-R3-73-032, 1973, p. 114.