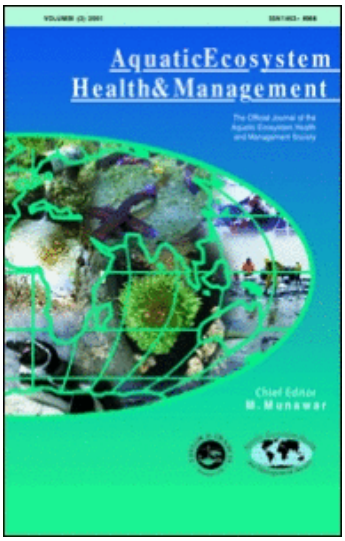

Aquatic Ecosystem Health & Management
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713393886

Quality Control of Drinking Water from the River Rhine with the Multispecies Freshwater Biomonitor

A. Gerhardt [a]; L. Janssens de Bisthoven [a]; E. Penders [b]
[a] LimCo International, Ibbenbüren, Germany. [b] Water Transport Company Rhine Kennemerland (WRK), Nieuwegein, The Netherlands.

Online Publication Date: 01 April 2003

To cite this Article Gerhardt, A., de Bisthoven, L. Janssens and Penders, E.(2003)'Quality Control of Drinking Water from the River Rhine with the Multispecies Freshwater Biomonitor',Aquatic Ecosystem Health & Management,6:2,159 — 166
To link to this Article: DOI: 10.1080/14634980301466
URL: http://dx.doi.org/10.1080/14634980301466

Aquatic Ecosystem Health and Management Society

Taylor & Francis
Taylor & Francis Group

Quality control of drinking water from the River Rhine with the Multispecies Freshwater Biomonitor

A. Gerhardt,[1]* L. Janssens de Bisthoven,[1] and E. Penders[2]

[1]*LimCo International, Ibbenbüren, Germany*

[2]*Water Transport Company Rhine Kennemerland (WRK), Nieuwegein, The Netherlands*

**Corresponding author: LimCo International, An der Aa 5, D-49477 Ibbenbüren, Germany; E-mail: limco.int@t-online.de*

The Multispecies Freshwater Biomonitor (MFB) based on quadropole impedance conversion technique is a "biological early warning system" (BEWS) for online water quality biomonitoring. The aim of this study was to test the MFB with two crustaceans (Gammarus pulex *and* Daphnia magna) *in a drinking water processing plant at the River Rhine: 1) Sensitivity of the test species to short-term acid pulses and alarm-responses in the MFB were studied in the laboratory. 2) Long-term monitoring with* Gammarus pulex *and* Daphnia magna *in the MFB was performed* in situ*. A decrease in pH, especially below pH 6.8, resulted in a significant behavioural response of both species according to the Stepwise Stress Model. After a first escape response of* D. magna*, both species reacted similarly with decreased activity and* G. pulex *additionally with increased ventilation.* G. pulex *survived better than* D. magna*. The behavioural effects remained in the recovery phase. Long-term monitoring showed stable locomotory behaviour and better survival of* G. pulex *when compared to* D. magna*. The MFB can be recommended for drinking water control using* G. pulex *as test species.*

Keywords: biological early warning system, online biomonitoring, *Gammarus pulex*, *Daphnia magna*, biotest, Multispecies Freshwater Biomonitor, drinking water

Introduction

The River Rhine has been one of the most polluted European Rivers, as almost one fifth of all chemical production in the world takes place in its drainage basin. Numerous efforts have been undertaken to reduce the pollution and improve water quality in the last decades. Over 20 million people are still getting their drinking water from the Rhine (Friedrich and Schulte-Wülwer Leidig, 1996). Estimates of chemosynthetic compounds which occur in the Rhine range from 30,000 to 50,000, of which only about 150 to 200 substances are analysed by routine analyses (Botterweg et al., 1989). After serious pollution accidents in the past, an international association of waterworks in the Rhine basin has been established to define water quality norms for relevant chemicals in the discharge control. As a risk of serious water pollution remains, a Rhine Alarm Model has been developed to provide predictions of pollution concentrations along the river. Online biomonitors, serving as "biological early warning systems" for acute pollution pulses, are based on rapid pollution induced changes in behavioural and physiological parameters of selected test species, e.g. crustaceans like *Daphnia* sp., mussels like *Dreissena polymorpha* and different fish and algae species. During the last decades, these single species biomonitors have been installed in a battery at different monitoring stations along the River Rhine. However, due to false alarms, differences in sensitivity of various test species and differences in the test parameters, operational problems of the biomonitor instruments *in situ*, challenges in further research and development still exist (Gerhardt, 1999). Reliable and ecologically

Aquatic Ecosystem Health & Management, 6(2):159–166, 2003. ISSN: 1463-4988 print / 1539-4077 online
DOI: 10.1080/14634980390199626

relevant biomonitors should be developed, especially according to the new EU-legislation, which stresses cleaner (drinking) water based on: 1) the precautionary principle, 2) prevention of pollution at the source, 3) the polluter-pays principle, 4) the sustainability principle and 5) increased international cooperation in water issues.

In the Netherlands, the Water Transport Company Rhine Kennermerland (WRK) is one of the largest water supplier for drinking water. The company takes surface water from the Rhine and the Ijsel Lake, applies pretreatment procedures, such as acidification and sedimentation with $FeCl_3$, followed by a neutralisation step before the water is transported to the dunes for infiltration, from where the drinking water companies supply Amsterdam and the province of North Holland. In addition, WRK supplies certain large industries (steel, paper) with process water.

The aim of this study was to test the Multispecies Freshwater Biomonitor (MFB) with pretreated water in laboratory simulations of acid stress and *in situ* long-term operation with the standard test species *Daphnia magna* and the local species *Gammarus pulex*.

Methodology

Multispecies Freshwater Biomonitor

The Multispecies Freshwater Biomonitor (MFB) is based on quadropole impedance technology (Gerhardt et al., 1994). The individual test organism is placed in a flow-through cylindrical test chamber with two pairs of stainless steel-plate electrodes, attached at the opposite chamber walls (Figure 1). One pair generates a high frequency alternating current, the other non-current carrying electrode pair senses impedance changes due to the movements of the organism in the electrical field. Different types of behaviour generate characteristical electrical signals (Gerhardt, 1999, 2000). The electrical signals are processed by a discrete Fast Fourier Transformation and generate a histogram of the occurrence of all frequencies in % (summarized in intervals of 0.5 Hz from 0 to 10 Hz), hence yielding a "fingerprint" of the behavioural pattern of the organism. For alarm recognition the following settings were made in the software: if the actual value of a certain frequency or a group of frequencies in a behavioural signal derives 10% from the prognosis value, which is calculated as moving average of the last 5 values, the system gives a warning on the screen. A mortality alarm is generated if during one hour 50% of the organisms have proven "inactive," i.e. generated no behavioural signals. The

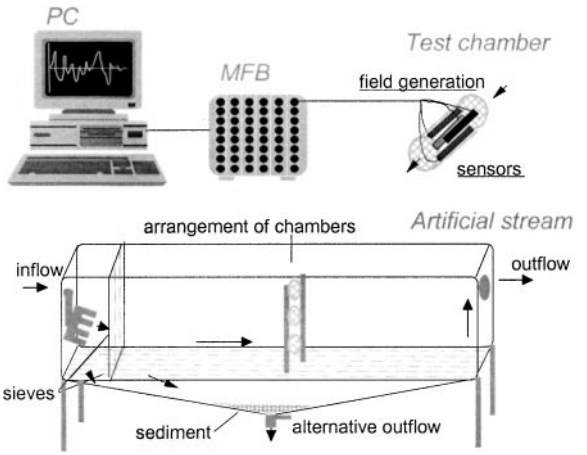

Figure 1. Experimental setup for laboratory acid pulses and *in situ* long-term experiments consisting of a PC, the Multispecies Freshwater Biomonitor (MFB) and test chambers arranged in an artificial stream.

MFB has been successfully used in several laboratory and *in situ* experiments with different local and standard test species in Sweden, Belgium, Northern Ireland, Portugal, Germany, Bolivia, South Africa and China (Gerhardt, 1999, 2000).

Test species

Daphnia magna *Straus*

Daphnids have often been used in toxicological studies and environmental monitoring of aquatic systems due to their sensitivity to toxins, ease and economy of culture (Tomasik and Warren, 1996). *D. magna* proved to be most resistent to metals compared to *Ceriodaphnia reticulata* and *D. pulex* (Tomasik and Warren, 1996). Due to the inability to withstand fish predation, *D. magna* is often absent from fish-inhabiting lakes. In consequence, *D. magna* is seldom an indigenous species (Koivisto, 1995).

Gammarus pulex *(L.)*

Gammarus sp. is abundant all over the year in many European streams of different sizes, playing a key role in the aquatic foodweb and being an indicator for trend biomonitoring methods (Garmendia Tolosa and Axelsson, 1993; Böhmer et al., 1999; Rinderhagen et al., 1999). *Gammarus pulex* has been the basis of the development of different biotest methods (feeding activity: Taylor et al., 1993; precopula separation: Pascoe et al., 1994, scope for growth: Maltby et al., 1990; drift: Taylor et al., 1994; Stepwise Stress Model: Gerhardt, 1999) and has been recommended as test organism in ecotoxicology (Williams et al., 1984).

Experimental setup

Laboratory experiments

The experiments in the laboratory were set up in an artificial acrylglass stream system (volume: 100 l, length: 103 cm, height: 31 cm, width: 28 cm with a bottom part of 16 cm depth arranged as a slideway in an angle of 30°) (Figure 1). The artificial stream received processed Rhine water from a tap with a renewal time of 10 min. No food was added during the short-term acid pulse experiments with pretreated Rhine water. During the long-term monitoring at the water intake, it was anticipated that the Rhine water contained enough particles and algae as food source for the test species. The organisms were placed in cylindrical acrylglass test chambers (3 cm long, 2 cm in diameter), which were closed on both sides with nylon nets (250 m). The test chambers were attached in flow direction in the artificial stream (Figure 1). *Gammarus pulex* was collected *in situ*, two days old *Daphnia magna* were taken from a laboratory culture. The two acid pulse experiments consisted of an acclimation time of 7 h, followed by an acid pulse of 6 hrs (1st exp., 1 M HCl) and 9 h (2nd. exp., 10 M HCl) with stopped flow, and ending with a recovery phase of about 6–9 h., where pretreated pH-neutral Rhine water was pumped through the artificial stream at a flow rate of 10 l/min. For each experiment, eight chambers with five *Daphnia magna* and 14 chambers with one *Gammarus pulex* were used.

In situ *test with the Multispecies Freshwater Biomonitor (MFB)*

Twice during one month, the MFB and the artificial stream system were tested with unfiltered surface water at an intake in a monitoring station along the Rhine in order to define long term reliability, survival of test species, handling and maintenance efforts. Twelve respectively 15 chambers with each five two day old *Daphna magna* and 17 resp. 22 chambers with one *Gammarus pulex* were used.

Statistical analysis

One-way repeated measurement ANOVAs were used to analyse differences between test chambers, treatments and activity levels of the two species. They were applied on arcsin squareroot transformed data of the percentages of band 1 (0.5–4 Hz: locomotion) and band 2 (4–8 Hz: ventilation and other high frequency behaviour). The long-term monitoring data were analysed as to "alarm generation" according to the alarm algorithm based on ARIMA-models in the MFB software.

Results

Behavioural pattern

Typical behavioural signals of *Gammarus pulex* consisted of locomotion (body stretching and leg movements) and pleopod undulation (ventilation) as described by Gerhardt et al. (1994), Gerhardt (1995), Gerhardt et al. (1998) and Gerhardt (1999). Typical behavioural signals of *Daphnia magna* consisted of locomotion (big movements with the antennae at 0.5–1.5 Hz) and ventilation (small movements with the phylopods within the carapax at >2 Hz) (Gerhardt et al., 1994, 2001). Comparing both species, the planktonic *D. magna* was significantly more active (locomotion) than the benthic *G. pulex* independently of the acid exposure ($p = 0.001$, $F(2, 1297) = 412.3$). *Daphnia magna* showed significant differences in activity (band 1, 2) between different test chambers ($p = 0.01$, $F(6, 7) = 6.8$), which might be due to the fact that there were five organisms per test chamber. *Gammarus pulex*, however, behaved more equally with no significant differences between different test chambers.

Simulation of acid pulses

In a first experiment, called "short acid pulse," a decrease from pH 7.8 to 6.8 resulted for *Daphnia magna* in a significant decrease in ventilation ($p = 0.008$, $F(1, 599) = 6.9$) and locomotion ($p = 0.02$, $F(1, 1202) = 6.4$). *Gammarus pulex* reacted with increased ventilation ($P = 0.001$, $F(1, 1202) = 57.8$). In the post-exposure recovery phase with increasing pH, the observed behavioural effects persisted and even increased (Figure 2). Locomotion of *Daphnia magna* ($p = 0.001$, $F(1, 656) = 30.6$) and especially of *Gammarus pulex* ($p = 0.001$, $F(1, 1202) = 184.3$) decreased further compared to the preexposure and exposure phases. Ventilation of *D. magna* ($p = 0.02$, $F(1, 656) = 5.4$) and especially of *G. pulex* ($p = 0.001$, $F(1, 1297)$: 55.4) were significantly lower than in the preexposure and exposure phases. Simultaneously, the variances of the behavioural responses increased (Figures 3 and 4).

In a second experiment, called "long acid pulse," the pH decreased from 7.5 to 3.4. The activity level of *D. magna* increased during the acid pulse (Figure 2) ($p = 0.001$, $F(1, 871) = 32.4$), whereas no change in ventilation behaviour was found. *G. pulex* did not show significant differences in locomotion and ventilation during the acid pulse. Only at the end of the acid pulse, ca. 3 h after pH 3 was reached, clear

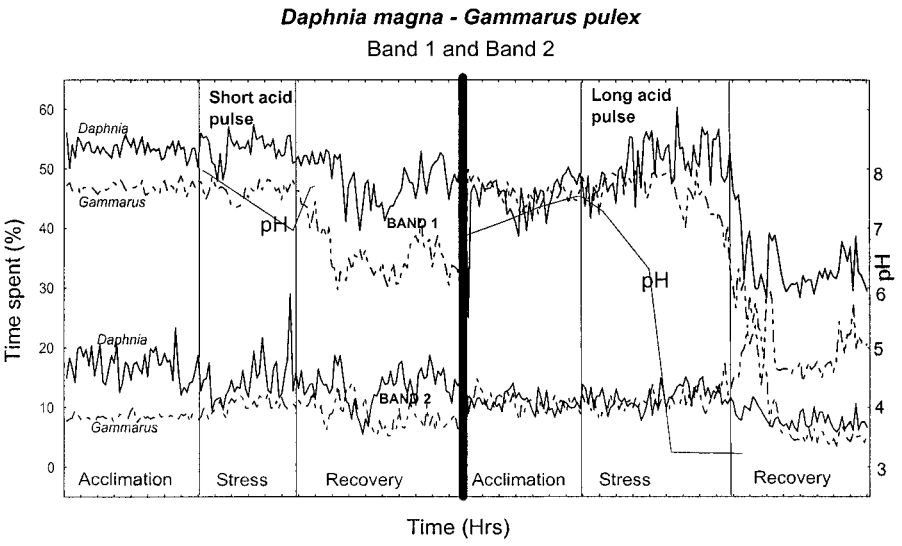

Figure 2. Behavioural responses of *D. magna* and *G. pulex* to acid pulses. Time spent on locomotion (band 1) and ventilation (band 2).

responses, which persisted in the post-exposure phase were found: *G. pulex* showed decreased locomotion ($p = 0.001$, $F(1, 1202) = 837.4$) and increased ventilation ($p = 0.001$, $F(1, 1202) = 139.6$). *D. magna* showed decreased locomotion ($p = 0.001$, $F(1, 907) = 187.2$) and decreased ventilation ($p = 0.001$, $F(1, 907) = 63.6$ (Figure 2). The variances in the behavioural responses of both species increased during and especially after the acid pulse (Figures 3 and 4).

Both crustaceans showed increased mortality after the acid pulse. *Daphnia magna* was more sensitive during the weak acid pulse (56%, 24 h) compared to *G. pulex* (22%, 24 h). However, the mean time-to-death (TTD) was the same for both species (14 h), with the ranges being smaller for the age-synchronous cultured *D. magna* (min: 10, max 19 h) than the natural population of *G. pulex* (min. 7, max. 31 h). In the second acid pulse experiment of pH 3.4, 100% *D. magna* and 81% *G. pulex* died within 24 hrs, with mean TTD decreasing to 8 h (min. 8, max. 9 h) for *D. magna*, whereas it remained similar to the weak acid pulse for *G. pulex* (mean. 14 h, min. 8, max. 22 h).

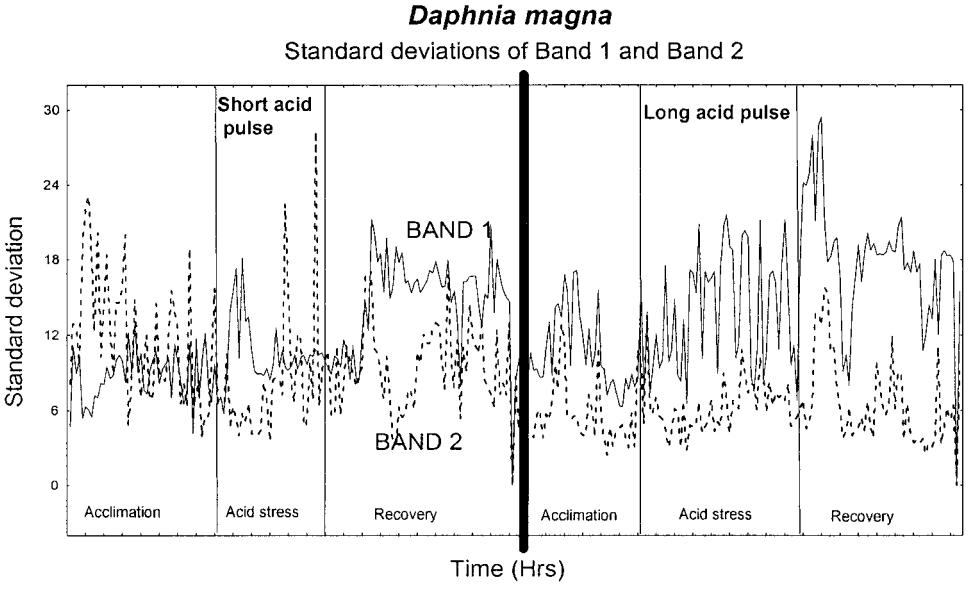

Figure 3. Behavioural responses of *D. magna* to acid pulses. Variability of time spent on locomotion (band 1) and ventilation (band 2).

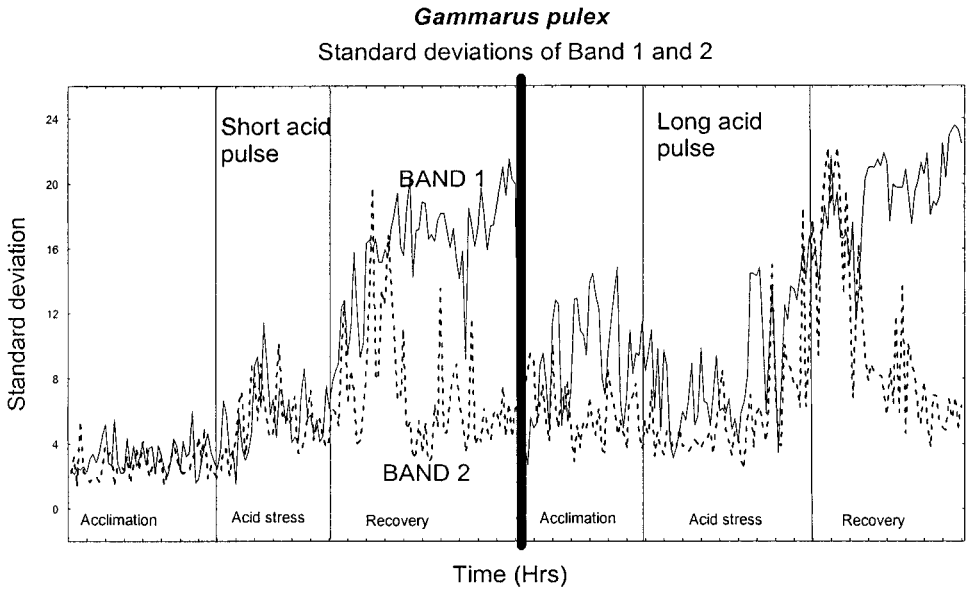

Figure 4. Behavioural responses of *G. pulex* to acid pulses. Variability of time spent on locomotion (band 1) and ventilation (band 2).

In situ long-term operation of the MFB

During the first 20 days of the first experiment both species showed a stable baseline for locomotion (band 1) without behavioural alarms and diurnal activity patterns (Figure 5). However, afterwards activity decreased gradually for 60% of *D. magna* and 50% of *G. pulex*. This gradual decrease over several days did not lead to a water quality alarm. Overall mortality of *D. magna* was 30% with a mean TTD of 25 days. *G. pulex* showed a better performance, overall mortality was 14%, with a mean TTD of 25 days. *Gammarus pulex* showed a periodically significant increase in ventilation every 3–5 days, which released

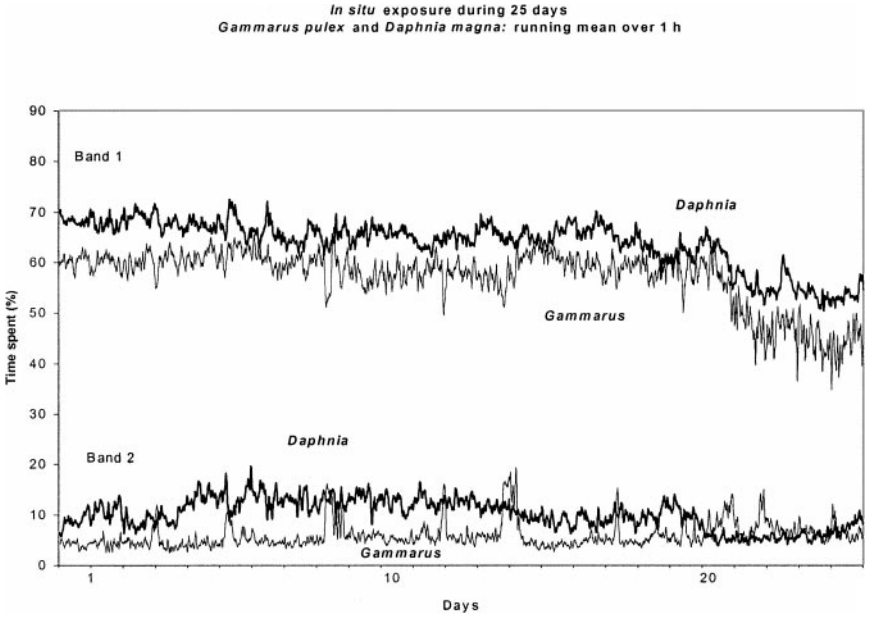

Figure 5. *In situ* exposure of *G. pulex* and *D. magna* at the drinking water intake (River Rhine): Running mean data over 60 min. for band 1 (locomotion) and band 2 (ventilation and other high frequency behaviour).

an alarm (when set at 10% deviation) that might be due to variations in turbidity or bacterial content in the test water, which may vary 1–2 orders of magnitude (Penders, pers. comm). Unfortunately WRK had no chemical online data to match with the biological alarms. In the 2nd *in situ* experiment, *Gammarus pulex* survived better (80%) and remained active for 32 days, the ventilation periodicity remained, but was just below the preset alarm-threshold of 10% deviation. However, activity and survival of *Daphnia magna* declined after already one week and daphnids had to be replaced often.

Discussion

Responses of *G. pulex* and *D. magna* to acid stress

The pretreated Rhine water is expected to be free from toxic chemicals and particles, so that the measured responses should exclusively represent acid stress. Both acid pulses resulted in significant persistent behavioural effects in both species. The slight decrease in pH during the first acid pulse showed parallel curves of the running means for locomotion and ventilation for both species. However, the more dramatic acid pulse revealed *D. magna* to react first with increasing activity, which might be seen as escape trial. The next response was decreased activity in both species, followed by another response of *G. pulex*, which consisted of increased ventilation. Both species showed two subsequent stress responses and thus followed the Stepwise Stress Model (Gerhardt 1999, 2000). Even though *D. magna* was first to show a response to acid stress, it is regarded less appropriate for the biomonitor, because of the high variability in its normal behaviour. Regarding the course of the standard deviations of the two types of behaviour we conclude *G. pulex* to show the more clear and consistent response pattern, similar in shape for both acid pulses, whereas the variability in the behaviour of *D. magna* might lead to false alarms.

In general, daphnids were more prone to death, probably due to ageing, as they have a short life cycle. However, the mean TTD were similar for both crustaceans, probably due to a common physiological factor, such as impaired ion-regulation and respiration due to acid stress. As crustaceans, both *Daphnia magna* and *Gammarus pulex* are highly pH-sensitive. Acid pulses of 6–9 h were sufficient to show persistent behavioural effects and mortality. The natural occurrence of *G. pulex* is restricted to pH > 6 (Kelso et al., 1982). Avoidance responses in *Gammarus pulex* have been observed in experiments within the pH range of 6.4 to 9.6, where the amphipods definitively preferred the alkaline side (Costa, 1967). Increased activity of *Gammarus pulex* was found at pH 5.4 during an exposure of 10 days (Taylor et al., 1994). Effects of acidification on zooplankton have often been described, species begin to disappear at pH < 6.0, with extreme loss at pH < 5.5–5.0 (Malley et al., 1982). However, interspecific differences exist: according to Havas (1987), *D. pulex* was considerably more acid tolerant than *D. magna*. Intraspecific differences were found as well: pale daphnids died more quickly than red daphnids. Acid stress was accompanied by a rapid clearing of the gut, reducing filtering activity, swelling of the heart muscle, a rapid fading on hemoglobin and a net loss of both sodium and chloride (Havas, 1987). Many species sensitive to low pH have problems with maintaining internal concentrations of Na and Cl, for example in fish, planktonic crustacea, crayfish and insect larvae (Havas, 1981). For *D. magna*, the rate of Na loss at pH 3 was 3 times higher (64% per h) than at neutral pH (Potts and Fryer, 1979). It appears that a slight lowering of the pH stimulates the uptake of Na around pH 5, maybe due to penetration of H^+ ions across the membrane (Potts and Fryer, 1979). *Daphnia* sp. has shown the ability to recover from a brief exposure to low pH. At pH levels around 3.0 the blood pH in fish dropped from pH 7.4 to 6.9. This small changes had lethal consequences (Havas, 1981). Post-moult individuals of crayfish were more sensitive to low pH than inter-molt organisms and calcification was slower at pH < 6 than at neutral pH (Havas, 1981). At pH < 5 additional Na regulation stress occurred, at pH < 4 additional respiration stress occurred (Havas 1981).

Test species

Daphnia magna is a standard organism for toxicity tests and much literature exists on responses to different types of toxins. However, this species is regarded inappropriate for online biomonitoring of water quality in surface waters: 1) it does not occur in many natural habitats, 2) due to reproduction, offspring can produce disturbances, although in some biomonitors this can be excluded, 3) different clones might have different response times and response thresholds, 4) laboratory clones might be more sensitive than natural populations and thus give "false" alarms, and 5) some of the measured parameters, such as number of turnings or inconstancies in swimming measured with image analysis, might be due to the size of the chamber, thus "artificial" and not relevant for detecting a toxic response.

6) the water needs to be filtered in *Daphnia* biomonitors, which are based on optical detectors. This removes particle bound toxins and food particles, thus affecting toxin bioavailability and behaviour, as starved daphnids proved to be more active than fed ones. 7) the life cycle of daphnids is short and during the one month duration of the *in situ* test, many daphnids died due to normal ageing, hence giving false alarms. 8) in a *Daphnia* biomonitor the short-lived animals must be replaced more often (every 1–2 weeks) compared to a *Gammarus pulex* biomonitor (every 3–4 weeks). It thus requires more maintenance effort, especially when food sources have to be added. Maintenance costs are increasing. All daphnids should have the same age, as 7-d old organisms showed higher activity than 1-d old ones in the dynamic waterflea biomonitor based on infrared light detection of swimming activity (Matthias and Puzicha, 1990). The mortality of *G. pulex* in the MFB was much lower than that of *D. pulex* in the short-term and long-term experiments. Survival in the test chambers might even be optimized by provision of even larger test chambers. To optimize survival and sensitivity of *D. magna* in the MFB daphnids should be placed individually in the chambers (Gerhardt et al. 2001).

G. pulex showed better survival and more consistent activity in the long-term monitoring experiment, both important prerequisites for online biomonitoring. Moreover, *G. pulex* showed periodically ventilation peaks, which might be due to changes in turbidity or other water quality parameters, hence sensitive alarm responses. Unfortunately online chemical data were missing. However, the annual mean values of the WRK water were below the limits for drinking water as for pH, sulphate, nitrate, ammonium, phosphate, arsenic, cadmium, chromium, copper, lead, organochlorine pesticides and polar pesticides. For AOX (adsorbable organochlorine compounds) the values of 0.024 mg/l surpassed the limits (0.01 mg/l). Also the contents of bacteria (thermotolerant coliformes (180–2 bacteria/ml), faecal streptococci (98–1 bacteria/ml)) and turbidity (33–0.04 FTU) varied and were sometimes above the limits (Penders pers. comm).

Online biomonitors

Except for the MFB, there is no other biomonitor using *Gammarus pulex*. Therefore, comparisons can only be made for *Daphnia magna* in the MFB and in other *Daphnia* biomonitors. There are two types of *Daphnia* biomonitors, first the ones measuring the summation response of up to 20 organisms per chamber, recorded by the number of passages through IR-light beams (dynamic waterflea assay, Knie, 1978, 82), and secondly the ones based on digital image processing technology, where the swimming speed, direction, position, turning rate, inconsistancy, etc. of individual daphnids is recorded (e.g. van Hoof et al., 1994, Blühbaum-Gronau and Hoffmann, 1997, Baillieul and Scheunders, 1998). In the present experiments, five daphnids per test chamber were used for the MFB, however, even individual daphnids per test chamber have been recorded by the MFB with good signal quality (Gerhardt et al. 2001). All approaches using the summation response of 5 (MFB, this study) to 20 organisms (different *Daphnia* biomonitors) are less suitable than approaches monitoring single individuals. Sensitivity and detection time are lower with 5–20 organisms per chamber, as the chance of having at least one insensitive specimen in each chamber is high, which by its activity might mask the decreased activity or even death of the other organisms in the same chamber. With one organism per chamber, the sensitivity distribution is spread over the chambers and the chance of having one insensitive specimen is inversely proportional with the number of test chambers.

Another reason for differences in biomonitor sensitivity is the choice of parameters to be recorded, such as number of IR-light beam passages, swimming speed, time spent on swimming, ventilation, number of turnings, etc. In order to find the most sensitive and reliable parameters, simultaneous comparisons of different *Daphnia* biomonitors are needed. Moreover, each biomonitor has different alarm recognition algorithms. Sometimes, differences between a treatment time series of data are compared with a control data series, recorded several hours/days earlier, are calculated, which may rather be due to the "time" effect of the data series than to the treatment. Time series modelling methods for data matrices (ARIMA), ANCOVA or "jump detectors" (if distinguishable from outliers and artefacts), neuronal networks, Fuzzy logics, multivariate methods such as decision trees, etc. are regarded more appropriate. As long as data analysis methods, parameters and recording methods are not unified, comparisons between different types of biomonitors remain difficult. The advantage of the MFB is that several species can be measured with the same measurement principle and analysed with the same mathematical method, thus making comparisons easier. Considering the course of standard deviations instead of running means as additional alarm criterium might increase the sensitivity of the MFB.

References

Baillieul, M., Scheunders, P., 1998. On-line determination of the velocity of simultaneously moving organisms by image analysis for the detection of sublethal toxicity. Water Research 32(4), 1027–1034.

Blühbaum-Gronau, E., Hoffmann, M., 1997. Steigerung der Sensitivität eines kontinuierlichen Daphnientestes durch die Berücksichtigung einer Vielzahl von Verhaltensweisen. Vom Wasser 89, 163–173.

Böhmer, J., Rawer-Jost, C., Kappus, B., 1999. Ökologische Fliessgewässerbewertung: Biologische Grundlagen und Verfahren-Schwerpunkt Makrobenthos. In: C. Steinberg, H. Bernhardt, H. Klapper, (Eds.), *Handbuch Angewandte Limnologie*, 8/9, pp. 1–119. ecomed, Landsberg, Germany.

Botterweg, J., van de Guchte, C., van Breemen, L. W. C. A., 1989. Bio-alarmsystemen: een aanvulling op de traditionele bewaking van de waterkwaliteit. H20. 22, 788–794.

Costa, H. H., 1967. Responses of *Gammarus pulex* (L.) to modified environment. 2. Reactions to abnormal hydrogen ion concentrations. Crustaceana 13, 1–10.

Friedrich, G., Schulte-Wülwer Leidig, A., 1996. Der Rhein- das alte Sorgenkind. In: J. L. Lozan, H. Kausch, (Eds.), *Warnsignale aus Flüssen und Ästuaren. Wissenschaftliche Fakten*, pp. 65–75. Parey Verlag, Berlin, Germany.

Garmendia Tolosa, A. J., Axelsson, B., 1993. *Gammarus*, their biology, sensitivity and significance as test organisms. IVL-report B 1095, 88 pp. Swedish Environmental Research Institute, Stockholm, Sweden.

Gerhardt, A., 1995. Monitoring behavioural responses to metals in *Gammarus pulex* (L.) (Crustacea) with impedance conversion. Environ. Sci. & Pollut. Res. 2(1), 15–23.

Gerhardt, A., 1999. Recent trends in online biomonitoring for water quality control. In: A. Gerhardt, (Ed.): *Biomonitoring of Polluted Water-Reviews on Actual Topics*, pp. 95–119. Environmental Research Forum 9, TTP, Zürich, Switzerland.

Gerhardt, A., 2000. A new Multispecies Freshwater Biomonitor for ecologically relevant supervision of surface waters. In: R. Butterworth, A. Gunatilaka, M. E. Gonsebatt, (Eds.), *Biomonitors and Biomarkers as Indicators of Environmental Change: A Handbook 2*, pp. 301–317. Kluwer/Plenum Publ., New York, USA.

Gerhardt, A., Clostermann, M., Fridlund, B., Svensson, E., 1994. Monitoring of behavioural patterns of aquatic organisms with an impedance conversion technique. Environment International 20(2), 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. Environm. Sci. Pollut. Res. 3:82, 63–70.

Gerhardt, A., Janssens de Bisthoven, L., Guhr, K., Soares, A. M. V. M., 2001. Water quality and toxicity assessment of a mine effluent: Short-term laboratory and *in situ* biotests with invertebrates, fish and macrophytes. (Abstract). Setac Europe, Madrid, Spain, 6–10 May, 2001.

Havas, M, 1981. Physiological response of aquatic animals to low pH. In: R. Singer, (Ed.), *Effects of Acidic Precipitation on Benthos*, pp. 49–65. Proc. Symp. Acidic Precipitation on Benthos, 1980, North American Benthological Society. Hamilton, NY.

Havas, M., 1987. Does hemoglobin enhance the acid-tolerance of *Daphnia*? Ecophysiology of acid stress in aquatic organisms: Intern. Conference, Antwerp, Belgium, 13–16 Jan. 1987.

Hoof, van F., Sluyts, H., Paulussen, J., Berckmans, D., Bloemen, H., 1994. Evaluation of a biomonitor based on phototactic behaviour of *Daphnia magna* using infrared detection and digital image processing. Water, Science and Technology, 30, 79–86.

Kelso, J. R. M., Love, R. J., Lipsit, J. H., Dermott, R., 1982. Chemical and biological status of headwater lakes in the Sault St. Marie District, Ontario. In: F. M. D'Itri, (Ed.), *Acid Precipitation: Effects on Ecological Systems*, pp. 165–209. Ann Arbor Science Publishers, Michigan, USA.

Knie, J., 1978. Der dynamische Daphnientest- ein automatischer Biomonitor zur Überwachung von Gewässern und Abwässern. Wasser und Boden 12, 310–312.

Knie, J., 1982. Der dynamische Daphnientest. Decheniana 26, 82–86.

Koivisto, S., 1995. Is *Daphnia magna* an ecologically representative zooplankton species in toxicity tests?. Environm. Pollution 90(2), 263–267.

Malley, D. F., Findlay, D. L., Chang, P. S. S., 1982. Ecological effects of acid precipitation on zooplankton. In: F.M. D'Itri, (Ed.), *Acid Precipitation: Effects on Ecological Systems*, pp. 297–327. Ann Arbor Science Publishers, Michigan, USA.

Maltby, L., Naylor, C., Calow, P., 1990. Effects of stress on a freshwater benthic detrivore: Scope for Growth in *Gammarus pulex*. Ecotoxicology and Environmental Safety 19, 285–291.

Matthias, U., Puzicha, H., 1990. Erfahrungen mit dem Dynamischen Daphnientest-Einfluss von Pestiziden auf das Schwimmverhalten von *Daphnia magna* unter Labor- und Praxisbedingungen. Z. Wasser- und Abwasserforschung 23, 193–198.

Pascoe, D., Kedwards, T. J., Maund, S. J., Muthi, E., Taylor, E. J., 1994. Laboratory and field evaluation of a behavioural bioassay-The *Gammarus pulex* (L.) precopula separation (GaPPS) test. Water Research 28(2), 369–372.

Potts, W. T. W., Fryer, G., 1979. The effect of pH and salt content on sodium balance in *Daphnia magna* and *Acantholeberis curvirostris* (Crustacea: Cladocera). J. Comp. Physiol. 129, 289–294.

Rinderhagen, M., Ritterhoff, J., Zauke, G. P., 1999. Crustaceans as bioindicators. In: A. Gerhardt, (Ed.), *Biomonitoring of Polluted Water-Reviews on Actual Topics*, pp. 161–194. Environmental Research Forum 9, TTP, Zürich, Switzerland.

Taylor, E. J., Jones, D. P. W., Maund, S. J., Pascoe, D., 1993. A new method for measuring the feeding activity of *Gammarus pulex* (L.). Chemosphere 26 (7), 1375–1381.

Taylor, E. J., Rees, E. M., Pascoe, D., 1994. Mortality and a drift-related response of the freshwater amphipod *Gammarus pulex* (L.) exposed to natural sediments, acidification and copper. Aquatic Toxicology 29, 83–101.

Tomasik, P., Warren, D. M., 1996. The use of *Daphnia* in studies of metal pollution of aquatic systems. Environm. Rev. 4, 25–64.

Williams, K. A., Green, D. W. J., Pascoe, D., 1984. Toxicity testing with freshwater macroinvertebrates: methods and application in environmental management. In: D. Pascoe, R. W. Edwards, (Eds.), *Freshwater Biological Monitoring*, pp. 81–93. Pergamon Press, London, UK.