Biomonitoring with *Gammarus pulex* at the Meuse (NL), Aller (GER) and Rhine (F) rivers with the online Multispecies Freshwater Biomonitor®†

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Biological early warning systems represent a set of tools that may be able to respond to certain chemical monitoring requirements of recent European legislation, the Water Framework Directive (WFD2000/60/EC), that aims to improve and protect water quality across Europe. *In situ* biomonitoring was performed along the rivers Meuse (NL), Aller (GER) and Rhine (F) within the frame of the European Union-funded Project SWIFT-WFD. *Gammarus pulex* was used as a test organism during the evaluation of the Multispecies Freshwater Biomonitor® (MFB), an online biomonitor to quantitatively record different behaviour patterns of animals. At the river Meuse *G. pulex* reacted to pulse exposure of either a mixture of trace metals or of several organic xenobiotics, by showing up to 20% decreased locomotory activity (already at the 1st pulse) and increased mortality (at 2nd or 3rd pulse only). *G. pulex* deployed within the MFB system were observed to survive well at the monitoring station on the Aller (100%) and monitoring did not result in the measurement of chemical irregularities. In contrast, deployment at the monitoring station on the Rhine river demonstrated that the test organism was able to detect chemical irregularities by up to 20% decreased locomotory activity in the animals. The MFB proved to be an alert system for water quality monitoring at sensitive sites and sites with accidental pollution.

Introduction

The objectives of the Water Framework Directive (WFD2000/60/EC) are to improve, protect and prevent further deterioration of water quality across Europe. Different types of monitoring are demanded: surveillance monitoring to assess long-term water quality changes and for the generation of baseline data on river basins, operational monitoring for additional and essential data on water bodies at risk or those failing environmental objectives of the WFD and investigative monitoring to determine causes of such failures. For each type, monitoring of a number of quality elements such as biology, hydromorphology, physico-chemistry and chemistry (in/organic priority substances) and adequate techniques are required.[1] Within the EU-funded project SWIFT-WFD (Screening methods for Water data InFormaTion in support of the implementation of the Water Framework Directive) one important focus was on the evaluation and validation of existing and emerging tools and technologies for water quality monitoring in various case studies[2] and to establish a link between information provided by chemical sensors and by biological methods. Biological techniques that were evaluated included biological early warning systems that enable real-time monitoring of changes in water quality.[2]

Since *Gammarus pulex* is a detritus feeder and commonly-found or frequent inhabitant of European streams,[3] it was chosen as test organism for the present work. Until now, this species has never been used within another online biomonitor system; the use of the Multispecies Freshwater Biomonitor® (MFB) (LimCo International, Ibbenbueren, Germany) with Gammarids has been the focus of many studies, both in the laboratory and *in situ* (*e.g.* ref. 3–6). Importantly, this species has repeatedly been proposed as a standard test organism for ecotoxicological tests worldwide.[3]

The Multispecies Freshwater Biomonitor® (MFB) allows the quantitative behavioural recording of a variety of animal species in water, soil and sediment in a fully automated manner.[5] The MFB has been at the centre of numerous laboratory- or field-based ecotoxicological studies (*e.g.* ref. 2, 3, 7–9), such as on the river Rhine[3] or at a sewage treatment plant,[10] however *in situ*

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applications are currently limited. In the present study, the MFB was evaluated in three European river basins and more specifically in the rivers Meuse (Eijsden, NL), Aller (GER) and Rhine (F).

The MFB presents a number of advantages when compared with other *in situ* online biomonitoring systems:

(1) No requirements for filtration or pre-treatment of the water under evaluation. This allows the realistic validation of the effects of both dissolved and bioavailable but particle-bound pollutants on the test organism. This is especially important since detritus serves on the one hand as food for numerous stream organisms such as *G. pulex* and on the other hand contributes to the sediment dynamics (deposition, remobilisation); thus the "sediment" component is included towards an integrated approach in online biomonitoring.

(2) The MFB also allows the measurement of sediment inhabitants directly in their substrate. Organisms are exposed in flow-through measurement chambers that may be filled with sediment since sediments do not interfere with the non-optical measurement principle.[11]

In order to validate the MFB for *in situ* application with *G. pulex* as new indicator species, three different field sites were chosen with different characteristics in order to answer the following research questions:

(1) Is *G. pulex* able to survive in clean unfiltered surface water with detritus as food source in the MFB?

The Aller (D), a small, relatively unpolluted stream, allowed the operation of the MFB test system without alarms or disturbances, *i.e.* the undisturbed base operation. The Aller and its adjacent land areas are announced as Fauna Flora Habitat (FFH) areas, parts are Special Protection Areas (SPA) under the Birds Directive.[12]

(2) Is *G. pulex* able to react to a cocktail of metals or organic xenobiotics applied as pulse pollution in concentrations relevant to those occuring under accidental circumstances?

The Meuse River (Eijsden, NL) represents natural river conditions with average water quality conditions, such as those found in many river basins across Europe. A manned monitoring station is present on the Meuse River between Liège and Maastricht.[13] There, many chemical and biological parameters are recorded online, and in the past were proved to be able to detect pollution accidents. However, biomonitors used there are based on video techniques and require filtering of the original water as well as changes in water velocity. Accidental Cd pollution levels in the Meuse have been reported before.[14]

(3) After demonstrating that *G. pulex* is a pollution-sensitive, robust and easy to handle indicator species, the final question is to test the MFB in combination with *G. pulex* during a long-term evaluation at a location with frequent pulse pollution and changes in water quality.

The Rhine river flowing through Huningue and Basel is a waterway heavily used for navigation, and with many nearby chemical industries. Therefore, this portion of the Rhine river is at risk and monitoring is required. Water quality of the Rhine is monitored through 29 national and international monitoring stations.[15] The monitoring station at Huningue, located at the border of Switzerland, France and Germany, has been working since 1986, the year of the Sandoz incident that resulted in the contamination of the Rhine with organophosphorus pesticides and mercury compounds.

Materials and methods

Maintenance of test organims

G. pulex was sampled from an unpolluted reference stream flowing through agricultural and forested areas a few days before the tests (Eijsden: Aa, Germany, 11.04.05; Langlingen: Aa, Germany, 15.11.05; Huningue: Kander, Germany, 14.05.06) and brought to the respective measurement stations in Eijsden (Meuse), Langlingen (Aller) and Huningue (Rhine) and kept in aerated stream water with detritus as a food source until use.

Multispecies Freshwater Biomonitor® (MFB)

The Multispecies Freshwater Biomonitor® (MFB) allows fully automatic, online, real-time based quantitative recordings of the whole behavioural pattern of all aquatic in/vertebrates. It consists of test chambers with usually one animal in each, that can be placed *in situ* or in a tank (recirculation or flow-through), a recorder (impedance converter instrument) and a PC-unit (Laptop or PC). Organisms are exposed continuously in the test water. A quantitative recording of the behaviour (swimming (0.5–2 Hz), ventilation (2.5–8 Hz), inactivity (zero line)) is conducted automatically every 10 min and lasts for 4 min. Data recording and analyses (*via* a time series model) are fully automated.[7,16] Since the MFB is based on a non-optical recording principle—the tetrapole impedance conversion—it is suitable for applications both in laboratory and *in situ* with unfiltered raw water. It is a modular test system (8–96 channels), which allows a high replication, as well as the monitoring of several species at the same time. The control of the system is done by the monitoring of an empty chamber with leaves (without *G. pulex*) for the detection of signal disturbances due to physico-chemical changes in the water or man-made disturbances. Alarm calculation was based on a moving average of time series models as well as a series of jump detectors, such as the Hinkley detector, the double sigma detector and the slope detector.[15]

Experimental setup and design

***In situ* monitoring in the Aller.** Several chemical and physico-chemical parameters (*e.g.* temperature, pH, conductivity, oxygen content) are continuously monitored at the Langlingen monitoring station at the Aller River. *In situ* monitoring with the MFB was performed from the 17th of November to the 1st of December 2005.

Experimental tank tests with Meuse river water. While the manned monitoring station in Eijsden allows near continuous monitoring of a wide range of physico-chemical characteristics, it is difficult to predict changes in water quality or levels of trace pollutants under field conditions or to incorporate these into field studies. Therefore, a series of 5 day-long tank tests were performed to enable the evaluation of a number of water quality monitoring tools under more controlled trace pollutant concentrations that, otherwise, would be impossible in field studies.[13,17–19] The experiments were performed in the spring, 14th to 26th of April 2005. In this time, pulsed spiking with metals and organics was performed.

Tank set-up for dosing experiments. Two tank tests were undertaken for a period of five days each by using 200 L tanks filled with fresh natural river water from the Meuse. Recirculating (plastic tank) and flow-through (stainless steel tank) systems were used for the simulation of fluctuating trace metals and organic pollutants, respectively (Fig. 1). River water from the Meuse was used in the system and spiking with a mixture of metals or organic pollutants allowed to evaluate a wide range of tools under controlled conditions. Mixing was by a carousel housing a number of *in situ* monitoring devices, such as passive sampling devices and the flow-through systems that enable control over variations in pollutant concentrations with time.

In short, spiking solutions were prepared in nitric acid and methanol for metals and organics, respectively, and were added by direct addition for metals or with the use of a peristaltic pump for organic pollutants and volumes were optimised in order to ensure minimal influence of spiking media on the performance of the tools evaluated. Fortification with trace metals included Cd, Cu, Ni, Pb and Zn and addition of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and a range of polar and hydrophobic pesticides was undertaken (for concentrations see Results and discussion section). Water samples were collected at regular intervals in order to determine total organic pollutant concentrations or total, filtered (0.45 μm) and ultrafiltered (5 kDa) metal concentrations.[17] A total of 16 triplicate and 20 replicate samples were collected during the tank test, with fortification with metals and organic pollutants, respectively. Sample analysis was undertaken by an accredited commercial laboratory.

***In situ* monitoring at the Rhine.** Several parameters (physicochemical, trace metals, organic matter and toxicity) were continuously monitored at the water monitoring station of Huningue. This site is particularly important since it allows the monitoring of episodic pollution events and the control of a lock to isolate a canal downstream of the river in order to protect groundwater from contamination. At this particular point, canal water infiltrates into the groundwater, that is mainly used as drinking water or for agricultural activities in the area. The test systems were located inside the monitoring station in Huningue, at the border of Switzerland, France and Germany. The measurements of toxicity were carried out by two BEWS systems (Multispecies Freshwater Biomonitor® and Mosselmonitor® (www.mosselmonitor.nl). The measurement of total organic carbon (TOC) was carried out by an online UV spectrophotometer (STAC-Secomam). General toxicity was measured at the station by a biosensor, the Fluotox device that exploits the photosynthetic activity of algae cells. In principle, the presence of pollutants (such as pesticides) inhibits photosynthesis and thus induces an increase in fluorescence emission.[2] Monitoring with the MFB and *G. pulex* was conducted from the 18th of May to 29th of June 2006. Fig. 2 shows the experimental setup for the measurements at Aller and Rhine.

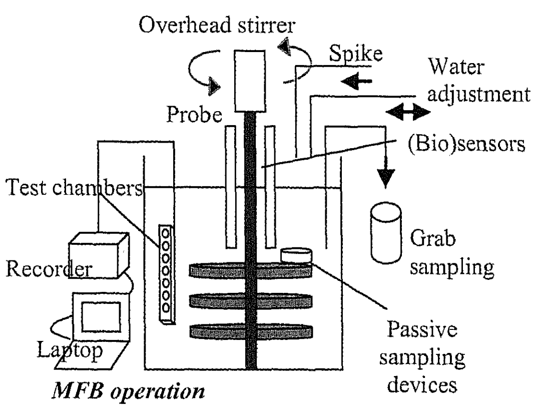

Fig. 1 General diagram of the set-up for the tank tests conducted with Meuse river water for the evaluation of a wide range of water quality monitoring tools (off, online sensors, biosensors, passive sampling devices, chemical test kits and biological early warning systems).

Handling of the test organisms and behaviour measurements. The animals (size: *ca.* 5–8 mm) were carefully and separately placed in the measurement chambers (length: 4 cm, diameter: 2 cm, mesh width: 1 mm). Additionally, a piece of conditioned leaf (size: 1 cm) was introduced into the chamber as food. The chambers were arranged in a flow-through aquarium (flow velocity: 40 mL s^{-1}, Aller test; >10 mL s^{-1}, Meuse test; approx. 200 mL s^{-1}, Rhine test) parallel to the direction of flow and fixed. For the test, a measuring device with 8 (Aller, Rhine) or 9 (Meuse) measurement channels was used with 7–8 Gammarids and 1 empty chamber as a control. The lead time including assembly of the measurement device and insertion of the animals lasted 1–2 h. The MFB was then left to operate without disturbances for two weeks (Aller) without maintenance and for 1.5 months at the Rhine (Fig. 2).

The survival of the animals was checked at the Aller and the online data analysed after the 2 week exposure. During the Rhine test, survival was checked after 2 weeks and dead animals were replaced and new food provided. Three weeks

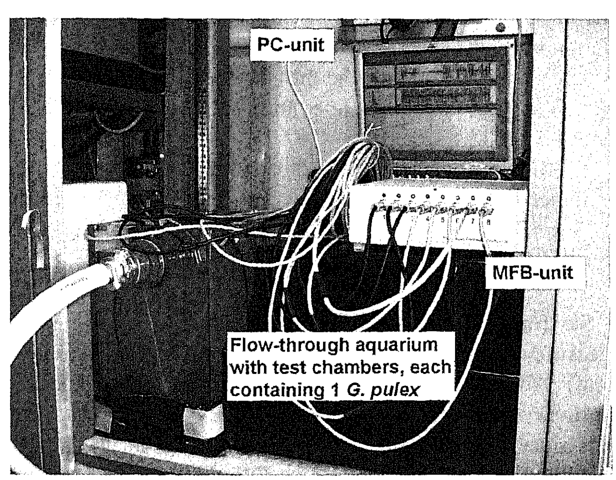

Fig. 2 Experimental setup for the Aller and the Rhine experiments.

later, the constitution of the animals was checked once more and the online data was analysed.

Data analysis. Data analysis consisted of the calculation of the percentage of time the animal spent on different behaviours, characterised by their signal frequencies, such as locomotion ($\leq$2 Hz) and ventilation ($>$2 Hz). If the measured value deviated by more than 20% from the predicited value (mean of five last records) on three subsequent recording occasions, a warning was given (light grey bars, see Fig. 4) and if more than 50% of the animals were immobile, an alarm for mortality was given (dark grey bar, see Fig. 5).

Results and discussion

In situ monitoring at the Aller

Chemical analysis. Continuous chemical measurements revealed a constant decrease of the water temperature over time especially in the second week of exposure (from 6.7 to 3.8 °C), while oxygen, pH and conductivity levels remained mainly constant (oxygen saturation: 91–101%; pH: 7.46–7.59; conductivity: 810–830 μS cm^{-1}). Chemical analysis of the Aller water (01.12.05) showed detectable concentrations of isoproturon (<1 ng L^{-1}) as well as metals (Al, Cr, Mn, Co, Ni, Cu, Zn, As, Cd, Pb) in the μg L^{-1} range. The water contained 90 mg Cl L^{-1} and 146 mg SO_4 L^{-1}. The TOC concentration was 6.8 mg L^{-1} while dissolved organic carbon (DOC) ranged around 5 mg L^{-1}. No chemical irregularities concerning organic compounds (pesticides, PAHs, PCBs) were detected.

Behaviour measurements. No alarms based on behaviour were detected by the MFB during the entire two week measurement period and the Gammarids were observed to have survived well in these conditions (100% survival after 2 weeks exposure).

Behaviour pattern over time. The animals showed constant activity, consisting of swimming movements and crawling in the measurement chambers. However, in the second week a slight decrease in activity occurred. This could most easily be explained by the decrease of water temperature (from 6.7 °C in the beginning to 3.8 °C). The empty chamber demonstrated that no outside disturbances occurred during the exposure, which would have led to the measurement of a signal in that chamber (constant zero line and dark grey mark means "no signals").

The double sigma detector did not reveal any alarms, but exhibited some non-significant variations, particularly during the second week. This may be attributed to the short inactivity phases of the animals.

Survival. All 7 animals were observed to survive in the measurement chambers and to eat the food they were given (leaf). Additionally, they received detritus as food and substrate *via* sedimentation of detritus in the measurement chambers since approximately 1/4 of each measurement chamber was filled with mud. This is desirable since it reflects natural conditions without disturbing the measurement signals.

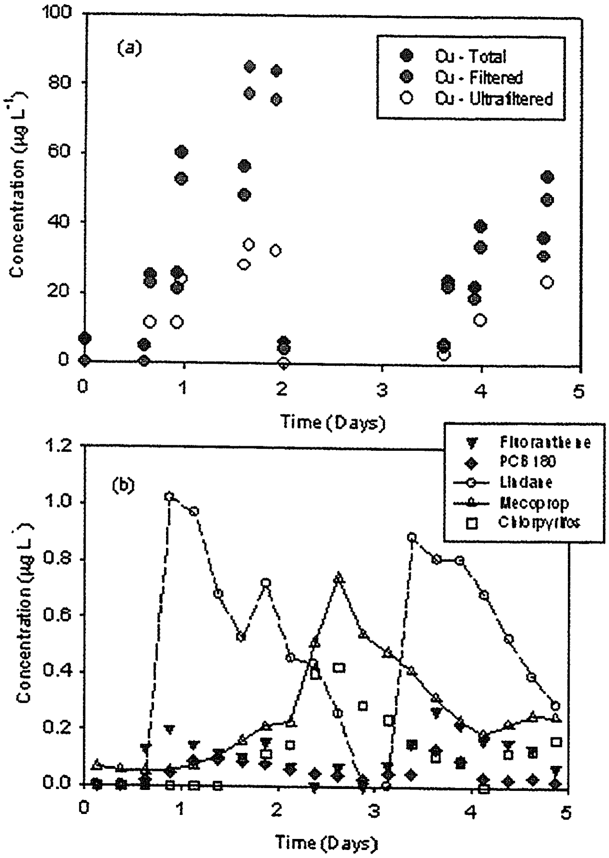

Fig. 3 Examples of changes in (a) total, filtered (0.45 μm) and ultrafiltered (5 kDa) Cu concentrations and (b) fluoranthene, PCB 180, lindane, mecoprop and chlorpyrifos concentrations (μg L^{-1}) with time, during the two tank tests.

Experimental tank test with Meuse river water

Main physico-chemical parameters. For the tank experiments with metals, most parameters remained stable over time. The water temperature ranged between 13 and 16 °C, the pH was ~8, conductivity lay between 490 and 500 μS cm^{-1} and dissolved oxygen ranged between 9 and 10 mg L^{-1}. The turbidity showed a constant decrease after stirring at the beginning of the measurements (14.04.05) and after adding fresh Meuse water (16.04.05).

Concerning the tank experiments with organic pollutants, most parameters were also relatively stable, with a temperature between 15 and 17.5 °C, a conductivity around 500 μS cm^{-1} and a pH of ~5. However, there was a significant drop of dissolved oxygen (from ~5.5 to 0 mg L^{-1}) and an increase in the redox potential (from ~40 to ~150 mV).

Temporal variations in metal and organic pollutant concentrations. Fluctuations in metal concentrations were generated during the course of the experiment and an example of these variations can be observed in Fig. 3a.

Peaks of metal concentrations were successfully obtained and reached a maximum of 69, 85, 58, 69 and 140 μg L^{-1} for Cd, Cu, Ni, Pb and Zn, respectively. While the filtered fraction of Cd, Cu, Ni and Zn varied between 80 and 90% of total concentrations, for Pb this was close to 45%. Ultrafiltration at

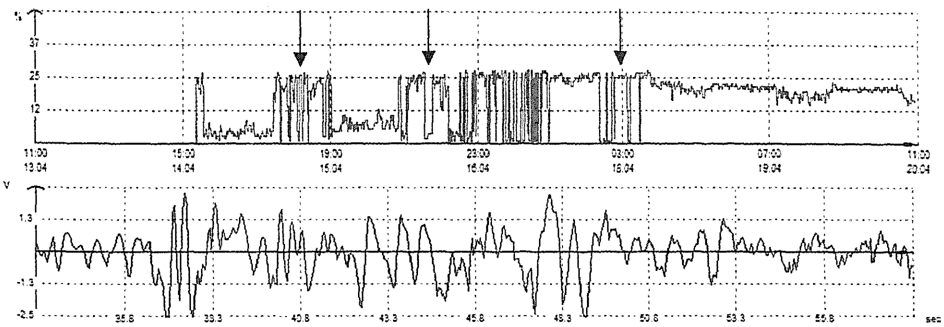

Fig. 4 Experiment 1. Top: locomotory activity of *G. pulex* ($n = 7$) in response to the subsequent metal pulses (percentage activity time [%] *versus* time [hh:mm]). Light grey marks in grey bar: behavioural warning responses (decreased locomotion). Black arrows: metal pulses. Bottom: typical movement pattern of *G. pulex* consisting mostly of locomotion signals (amplitude [V] *versus* time [s].

5 kDa resulted in 90% of Cd and Ni in the filtrate and 40, 20 and 60% of Cu, Pb and Zn in the filtrate. During this tank test with metals, metal concentrations varied over time following the spiking scheme (Fig. 3a).[17]

Examples of scenarios of temporal variations in concentrations in PAHs, PCBs, organochlorine pesticides, carbamates or other phenoxy acetic acid herbicides may be found in Fig. 3b. Two peaks of concentration may be observed for lindane, and at a lower concentration, for fluoranthene and PCB 180. The total sum of concentrations of pesticide varied widely and approached 13 μg L^{-1} for the maximum peak concentration. While this value is highly unrealistic, concentrations for the various pesticides are generally relevant and this test was designed to evaluate a number of different tools and techniques. The sums of 16 PAHs and 7 PCB congeners approached a maximum of 3 and 1 μg L^{-1}, respectively.

Behaviour responses. During the tank test with metals, the first pulse (Fig. 4) was detected soon after the second dosing by 30% of the animals, while the third and fourth metal additions were detected by 90% of the animals. 30% of the animals died during the second pulse. The first pulse certainly weakened the animals that subsequently became more sensitive towards the second pulse.

When spiking with organic pollutants, the first dosing was detected by 10% of the animals (Fig. 5), while the second dosing was detected by 40% of the animals. After the third dosing 20% of the animals were observed to die, and 50% of the animals were dead after the fourth dosing.

In summary, *G. pulex* reacted very rapidly to the trace metal pulses. The behavioural warnings were detected within a few hours at sublethal levels, whereas mortality alarms occurred towards the end of the exposure and only after repeated pulses and stress. The response of *G. pulex* to episodic exposure to organic pollutants occurred in a slower way than for trace metals; however, behavioural warnings and mortality alarms were also recorded by the MFB, showing that the MFB is a reliable system for detection of rapid changes both in metals and organic pollutant concentrations.

In situ monitoring at the Rhine

Chemical analysis. At the Rhine monitoring station (Station d'Alerte de Huningue) several chemical irregularities could be detected (Fig. 7, see black arrows).

Behaviour measurements. No disturbances were observed with good zero baselines over the whole exposure period. Small sporadic signals (at background noise level) were observed but may be due to variations in flow conditions. Fig. 6 presents the behaviour of *G. pulex* from 18th of May to 29th of June and Fig. 7 presents the data available and provided by the station during the same period. Locomotion of *G. pulex* showed the typical "up and down signals" (*e.g.* 18 to 20.05.06). However, some phases showed a decrease in activity (*e.g.* 20–21.05.06, 30.05.–01.06.06, 05.06.06, 12–14.06.06 and 15–18.06.06). When the dates of these drops in activity are compared with chemical data provided by the station (Fig. 7), the following connections between chemical and behavioural data may be drawn:

20–21.05.06: slight increases in fluorescence (0.43 eq mg L^{-1} 20.05.06) and in copper concentration from 73 to 163 μg L^{-1} were observed. Hence, the drop in activity shown by the MFB may be linked to the increase in copper concentration in the water and/or oils present in the water.

30.05.–01.06.06: both an increase in fluorescence (0.4 eq mg L^{-1}, 30.05.06) and a slight increase in toxicity (29.05.06, 31.05.06) were observed. On these two occasions, organic

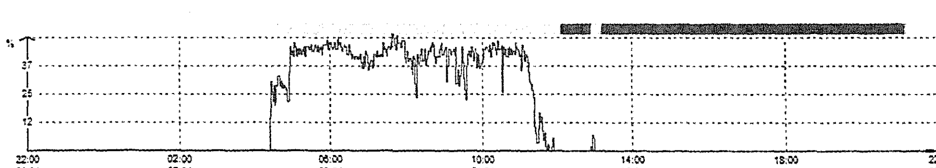

Fig. 5 Experiment 2. Locomotory activity of *G. pulex* in response to the subsequent organic xenobiotic pulses (percentage activity time [%] *versus* time [hh:mm]). Dark grey bar on top of graph: increasing mortality *versus* grey bar: alive animals (mean response of 7–8 animals).

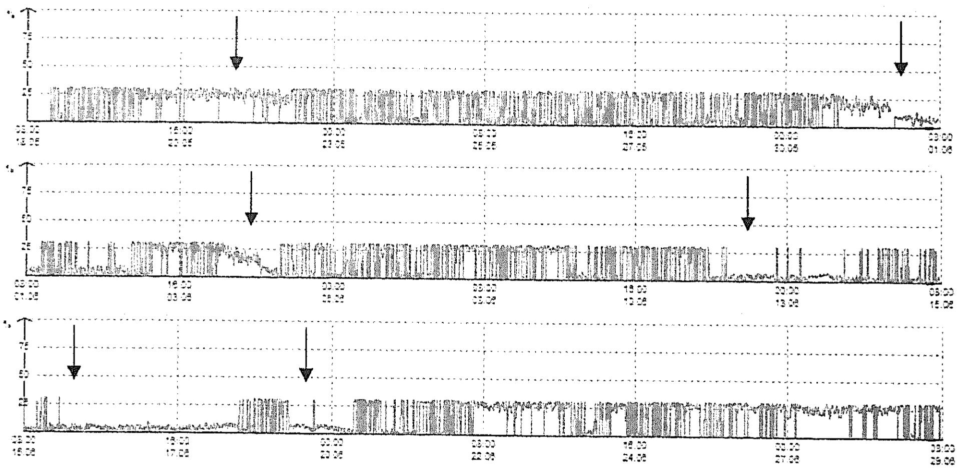

Fig. 6 Locomotory activity of *G. pulex* based on the mean of 8 individual recordings over the measuring period of 6 weeks (percentage activity time [%] *versus* time [hh:mm]). Arrows: chemical irregularities conform with behavioural alterations (decreased locomotory activity).

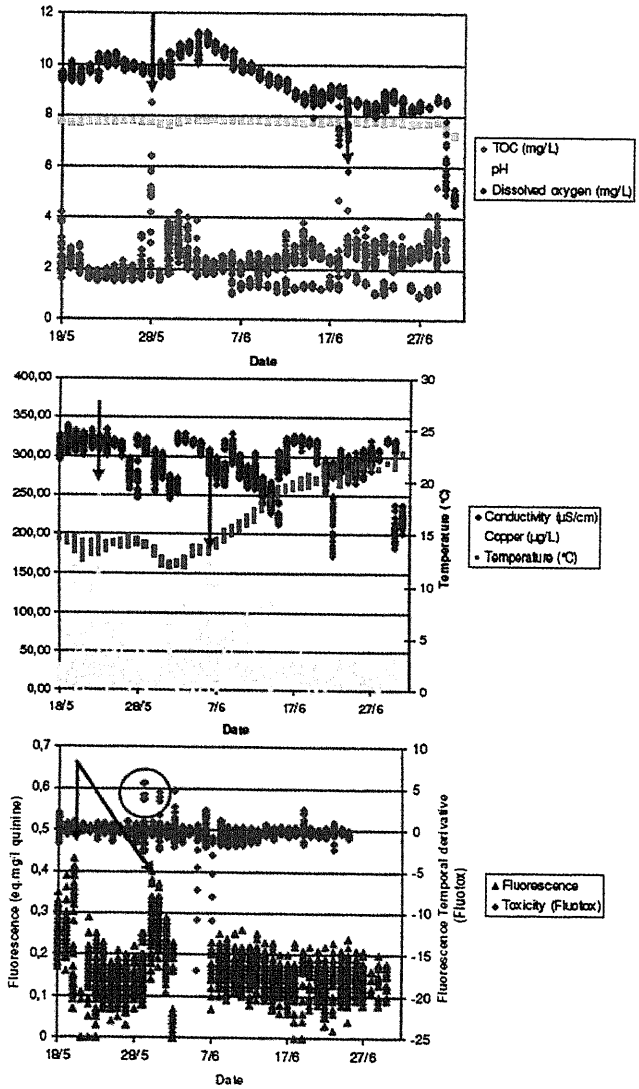

Fig. 7 Evolution of parameters measured by the Huningue Station during the deployment of the MFB. Black arrows: chemical irregularities.

pollutants such as oils and pesticides may be responsible for the decrease in activity of the Gammarids.

05.06.06: no significant modification of the water quality was observed according to the data available.

12–14.06.06: a slight increase in copper concentration and a slight decrease in oxygen were noticed.

15–18.06.06: a slight increase in TOC and a decrease in oxygen (5.84 mg L^{-1} 19.06.06) that often characterises the presence of organic pollutants were observed. The ventilation behaviour, however, did not show any serious irregularities.

In addition, the Alarmdetector software (LimCo & Hölle und Hüttner AG) detected several irregularities:

(1) H: Hinkley detector, locomotion: around 05.06.06, 19.06.06, 29.06.06.

(2) DS: double sigma detector, considering locomotion and ventilation gives even more irregularities, however a strong one around 18–19.06.06.

Survival. The survival of animals in the MFB was verified over the whole exposure period. Since the death of 3 animals was observed on the 31.05.06, these were replaced. The high sensitivity of the animals might be due to the fact that they originated from a clean location. It may also be the result of pollution induced by copper and/or organic compounds present in the water. Indeed between 18. to 31.05.06, we can notice an increase in copper concentration (250 µg L^{-1} 23.05.06), in TOC (8.5 mg L^{-1} 28.05.06) and in fluorescence (about 0.4 mg L^{-1}, the 20.05.06 and 30.05.06). At the end of the test (29.06.06), 3 animals were also dead. These deaths, at the end of the exposure, may be due to organic pollution (increase in TOC) and a decrease in oxygen that was observed at that time.

In summary, the MFB appeared suitable for the real-time detection of changes in water quality, with no occurrence of technical problems and with reasonable baselines during the entire six week exposure period. Deaths of *G. pulex* at the beginning of the test may be explained by an increase in organic compounds/matter and copper concentrations. *G. pulex* responded to sublethal copper concentrations around 100 µg L^{-1}, both in the laboratory[3] as well as *in situ*[7], with changes in locomotion and ventilation behaviour in previous experiments and the response appeared to be dependent on the origin of the test population.[7] Then, *G. pulex* responded to the Rhine water with several irregularities. These irregularities may sometimes be explained by an increase in concentration of pollutants such as copper and/or organic compounds (oils). At the end of the test, the deaths of animals might be due to organic pollution, pointed out by an increase in TOC and a decrease in dissolved oxygen.

Conclusions

During this evaluation at all three sites, the MFB performed well, without technical problems and with reasonable baselines obtained for all exposure periods in the Meuse, Aller and Rhine. *G. pulex* reacted to the changes in concentrations of metals and the organic pollutants during a series of tank tests undertaken with Meuse river water that resulted in stress behaviour and mortality. At the Aller river site, the undisturbed baseline operation of the MFB could be established. With regard to the Rhine water, *G. pulex* responded with several irregularities, with a particular event where the reaction coincided with a peak of oil pollution. The Alarm-detector software indicated this with both detectors, the Hinkley and the double sigma detector, and it can be verified in the original signals as shown in the MFB longterm graph (less activity).

The following conclusions can be drawn:

(1) *G. pulex* is suitable for water monitoring in the MFB because as a detritus feeder, it can also show particle bound effects of pollutants (all bioavailable contaminants).

(2) At the Aller, no alarms were detected; that is, no harmful effects of the water on *G. pulex* were recognized. However, *G. pulex* was able to detect several chemical irregularities at the Rhine monitoring station.

(3) The weekly exchange of the test animals (compared with bimonthly replacement in the present study) is standard and should therefore also be performed with the MFB.

(4) It is recommended to operate/run and calibrate the MFB further and for a longer period of time at monitoring stations *e.g.* in order to detect seasonal effects.

Our experiments showed that *G. pulex* were able to survive under the various conditions evaluated at all three monitoring stations and were able to react to changes in concentrations of various different types of pollutants. The MFB operation with a residential and ecologically relevant species such as *G. pulex* proved to be a reliable biomonitor system for surface water quality monitoring.

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Views presented here are those of the authors alone.

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