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# New Online Biomonitoring System for *Gammarus pulex* (L.) (Crustacea): *In Situ* Test Below a Copper Effluent in South Sweden

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Online biomonitors register biological effects of toxic discharges on selected indicator species and allow for fast, continuous, and ecological relevant water quality control. This paper presents a new online biomonitor based on a quadropole impedance conversion technique that records simultaneously several behavioral parameters of *Gammarus pulex*, a new biomonitoring test species. The behavior of two different populations of *G. pulex* was compared; one population originated from an anthropogenically unpolluted stream, and the other population lived at the copper-polluted study site, where the biomonitor was placed. Responses to simulated copper pollution peaks of 70 $\mu$g of $Cu^{2+}$/L were registered in the biomonitor and compared to the natural drift. *G. pulex* was the most abundant species in the natural drift. A nocturnal drift maximum was found in the natural drift and for both populations in the biomonitor. Mortality was high in the biomonitor; the local population survived slightly better than the reference population. The reference population showed significantly less activity than the local population measured as number of active organisms per day and time spent on locomotion and ventilation. Copper pollution pulses provoked increases in number of active organisms and time spent on locomotion in the biomonitor; however, no significant changes in the natural drift were registered.



## Introduction

The basic idea of the use of automated biological sensor systems for water quality management was first proposed by Cairns (*1*). Since the Sandoz incident in Switzerland in 1986, where the river Rhine was polluted due to a fire in an industrial storage building, the development and installation of biological early warning systems (BEWS) has seriously been pushed forward in Europe, especially in Germany, the Netherlands and United Kingdom. At the same time, several BEWS have been used in the United States (*2*).

The principle of a BEWS is that test organisms react immediately to pollution peaks with changes in their physiology and behavior. These systems are used to protect freshwater ecosystems by giving early warning of toxic discharges, providing data about possible permit violations and the need for secondary treatment. They also allow for field validation of laboratory toxicity bioassays (*3*). In the last decade, several continuous biotests have been tested and evaluated along the river Rhine; however, none proved to be satisfactory (*4*).

The aim of the present paper is to introduce a new online biomonitoring system based on a quadropole impedance conversion technique for *Gammarus pulex* (L.), a new biomonitoring test species. The system has been tested *in situ* below an electronics factory with two different populations of amphipods, one population from a reference stream and one local population. Behavioral responses to simulated copper pollution peaks have been measured in the biomonitor and simultaneously validated in the field.

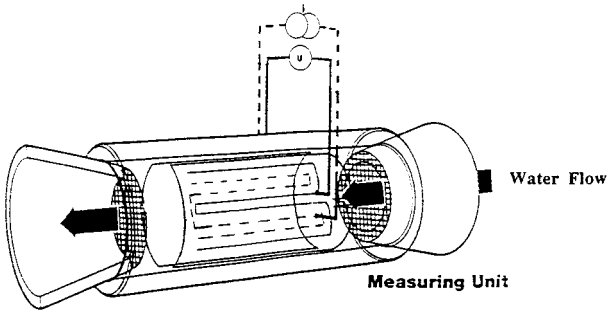


**FIGURE 1. Cylindrical test chamber as measuring unit of the biomonitor. The arrows show the water flow through the Plexiglas tube and the rubber stoppers covered with nylon net (500 $\mu$m). One pair of electrode coil (full line) on both opposite chamber walls produces an alternating current (u), the other pair of platinum coil (dashed line) measures the impedance changes (i) in the chamber.**

## Description of the Biomonitor

**Hardware.** The measuring principle of the biomonitor is based on a quadropole impedance conversion technique (*5*). Each test organism is placed in a water-filled cylindrical flow-through chamber made of Plexiglas pipe (3.2 cm in diameter, 9 cm long, 144.7 mL volume), where it can move freely between two pairs of platinum electrodes placed as interweaved coils at the opposite chamber walls (Figure 1). One pair of electrodes produces an alternating current of 50 kHz over the chamber; a second pair of non-current-carrying electrodes measures the impedance changes across the chamber due to movements of the organism in the electrical field (*5*). In order to prevent the organisms from touching the electrodes, the chamber walls are covered with nylon netting (500 $\mu$m). The inflow and outflow of the Plexiglas tube are closed by pierced conical rubber stoppers, the aperture covered with nylon netting (500 $\mu$m). These "tube chambers" are cheaper in construction; they have a more homogeneous water flow and less possibilities of hide for the organisms as compared to previously described rectangular test chambers (*5*). The chambers are arranged in a flow-through Plexiglas experimental channel. Eight chambers are connected to one measuring impedance converter. Up to seven impedance converter systems are available and can be synchronized by an external controlling system (multiplexer). Via an A/D converter, the signals are transformed and processed in a Mac LCIII computer using software implemented in LabView 2.2.1 (National Instruments).

**Software.** Online data collection allows for choice of trace length, recording intervals, and sampling frequency. The electrical signals from all chambers are plotted in real time

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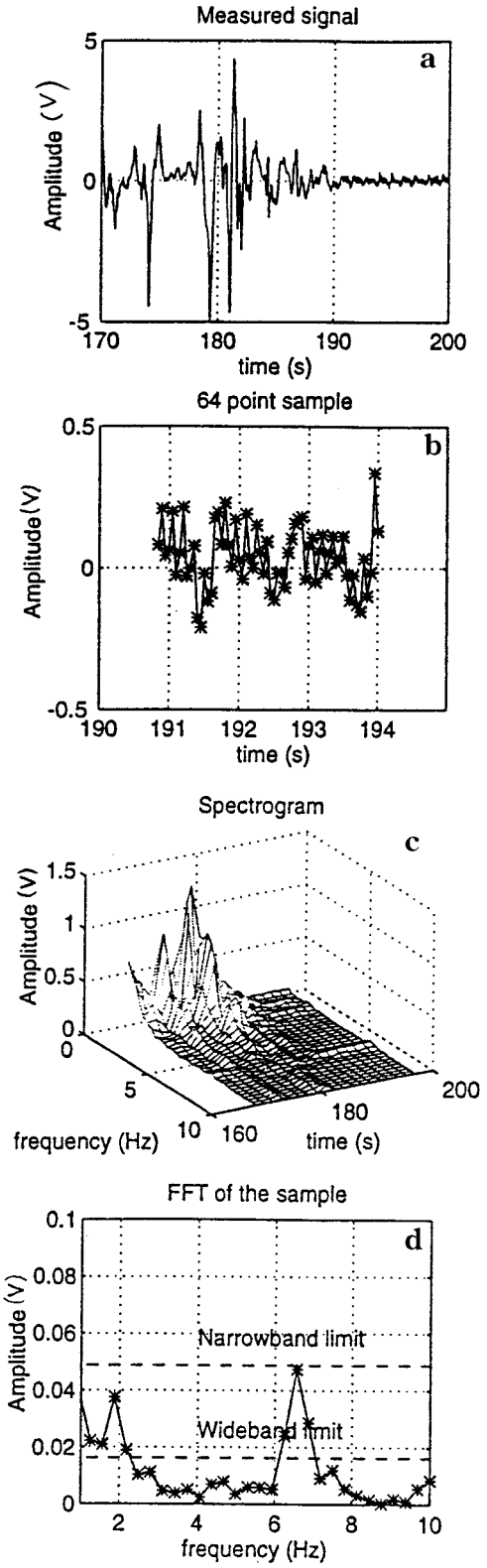


**FIGURE 2. Example of a typical locomotion signal (a), data analysis with disctrete Fourier transform (64 points) (b), the resulting spectrogram (c), and the pattern matching procedure to distinguish between noise (below wideband limit) and behavior (above narrowband limit) (d).**

on screen and stored in a file with a time stamp and name of the species. The analysis algorithm is based on the spectrogram for impedance variations, which is calculated by splitting the signal in intervals of 64 samples and calculating the discrete Fourier transform with the Hamming

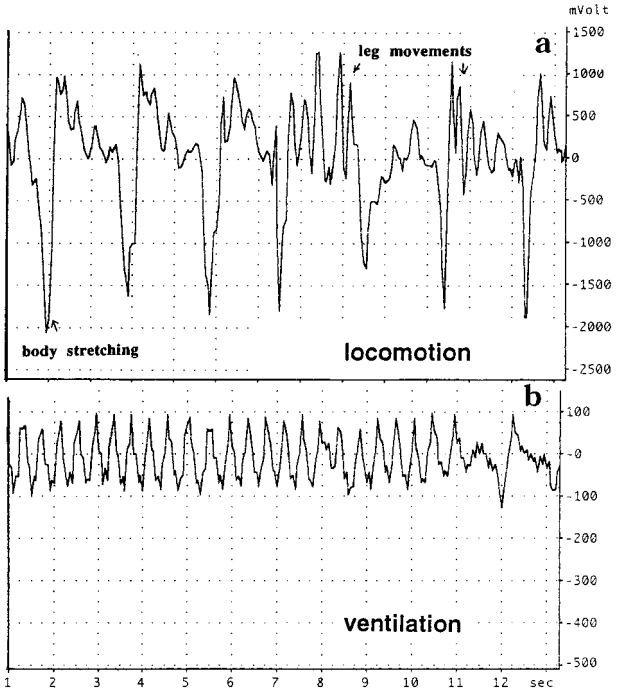


**FIGURE 3. Behavioral signals of *Gammarus pulex*: (a) locomotion and (b) ventilation.**

function for each of the intervals (Figure 2). The discrete Fourier transform (DFT) $S(k)$ of a signal $s(j)$ is defined by

$$S(k) = \frac{1}{N}\sum_{j=0}^{N-1} s(j) e^{(i2p/N)jk}$$

The actual frequency represented by a point in the DFT spectra can be calculated from the expression $fk = fs \times k/N$ where $f_s$ is the sampling frequency, $N$ is the total number of points, and $k$ falls in the range $0-N/2$, so that the FFT gives a frequency spectrum from 0 to $f_s/2$ Hz.

The spectrogram is analyzed with a simple pattern matching to distinguish between inactivity/noise and activity of the organisms (Figure 2). The pattern matching is based on comparing the spectrum values with an absolute limit and counting the number of points in the spectrum that are larger than this limit. If this number is within a specified range, it is considered a match. For example, if at least one but not more than three spectrum values are at least 45% of the RMS value (root mean square, a measure of variation in an FFT), the signal is regarded as "narrowband", *i.e.*, the signal contains strong periodical variation. Similarly, "wideband" and "noise" patterns are matched against the signal spectrum. The exact parameters have to be determined empirically for each test species. If the narrowband criterium is fulfilled and the amplitudes of the signal are above the noise level (50 mV), the organism is considered to be "active".

The result output offers one option for single-channel analysis where the following parameters are plotted: the electrical signal, the spectrum components, the RMS values, and the histograms for frequencies in Hertz (Hz) and amplitudes in volt (V). The alternative is a table with means, standard deviations, and medians for eight chambers of each impedance converter for time spent on activity, which is further specified as time spent on different frequency and amplitude ranges. For example, "swimming" is characterized by high amplitudes and low frequencies, whereas "ventilation" shows low amplitude and high frequency. Swimming consists of body stretching followed by leg movements (Figure 3).

**TABLE 1. Chemical Characteristics of Streams, Where Two Populations of *Gammarus pulex* Were Collected in Summer 1995**

| parameter | Skäralidbäcken | Ståstorpsån |
|---|---|---|
| pH | 6.00 | 6.50−7.00 |
| temp (°C) | 10.00 (d)[a] | 20.00 (d) |
| total hardness (mmol/L) | 0.08 | 2.20−2.90 |
| phosphate (mg/L) | 0.07 | 0.90−1.50 |
| nitrate (mg/L) | 0.05 | 1.10−2.00 |
| oxygen (mg/L) | 7.30 | 3.10−9.50 |
| Cu ($\mu$g/L) | 2.00 | 2.50−360.00 |
| Pb ($\mu$g/L) | 1.00 | 20.00 |

[a] d, day maximum.

## Test Species

*Gammarus pulex* (L.) (Crustacea, Amphipoda) has been chosen due to its wide distribution, abundant occurrence in streams, and availability in all size classes throughout the year (*6*). *G. pulex* is an important key species in aquatic food webs as it contributes in decaying coarse detritus as well as being prey for fish and predatory invertebrates. *G. pulex* is sensitive to a wide range of toxicants (*7*) and has been recommended for standard toxicity tests (*8*). Several new toxicity bioassays with *G. pulex* are under development with end points such as feeding activity (*9*), precopula separation (*8*), and scope for growth (*10*). *G. pulex* has shown behavioral responses to lead, copper, and complex effluents within 1 h (*11*, *12*).

One population of *G. pulex* was collected from an unpolluted first-order mountainous forest stream (Skäralidbäcken), another from a lowland stream affected by agricultural pollution, effluents from a water purification plant and an electronics factory (Ståstorpsån). Compared to Ståstorpsån, Skäralidbäcken was characterized by low temperature, hardness, and metal concentrations (Table 1). Whereas the macroinvertebrate fauna in Skäralidbäcken contained high diversity and some rare clearwater bioindicators such as the plecopteran *Dinocras cephalotes*, the fauna in Ståstorpsån was impoverished containing high densities of a few species such as *G. pulex*, Chironomidae, snails, and Oligochaeta (*13*).

## Methods

**Experimental Design.** The above described online biomonitoring system was positioned streamside in a mobile laboratory at Ståstorpsån, ca. 3 km below the effluent of the electronics factory, as the highest copper levels have not been found directly at the effluent outflow but a few kilometers further down, probably due to subterraneous water flows. *G. pulex* have been collected and placed individually in eight cylindrical chambers with a 1 $cm^2$ of coarse detritus from the stream as food source. The chambers were placed in a plastic aquarium of 10 L volume, where water from Ståstorpsån was pumped through at a flow rate of 1.5 L/min. After 1 day acclimation to the system, behavioral recordings of 24 s were performed every 30 min. Survival of the organisms was monitored twice a day; dead individuals were registered and replaced. The experiment lasted for 5−6 days and was performed twice simultaneously. Chemical parameters of the water such as pH, temperature, phosphate, nitrate, total hardness, oxygen, and $Cu_{tot}$ were measured daily.

One series of experiments was performed with *G. pulex* from Skäralidbäcken in order to test the behavior of a population from an unpolluted stream in the polluted water. In a second series of experiments, *G. pulex* was collected in Ståstorpsån ca. 100 m above the biomonitor. After 1 day acclimation, a single-dose copper pollution peak within previously observed concentration ranges (*11*) was simulated by pouring 1 L of a freshly prepared stock solution of copper sulfate (10 mg of $Cu^{2+}$/L) into the stream exactly where the water was pumped through the aquaria with the test chambers. Three Cu pollution experiments with peak concentrations of 69, 58, and 30 $\mu$g of $Cu_{tot}$/L in respective experiments were performed with *G. pulex* from the local population.

Drift measurements in the field were used to validate the activity measurements in the biomonitor as these two parameters are related (*14*, *15*). As many benthic invertebrates have diurnal activity patterns, drift measurements were performed during several 24-h cycles in 4-h intervals for periods of 10 min at a bridge ca. 100 m below the biomonitor, where about half of the streams' water body passed through a planktonnet (250-$\mu$m mesh size). Pollution peaks were simulated ca. 5 m above the bridge as described above.

**Statistical Data Analysis.** Nonparametric Friedman two-way analysis of variance by ranks was used to compare the two populations concerning mortality and different behaviors as described by selected frequency and amplitude ranges. For both populations, Spearman's correlation coefficients were calculated between number of active organisms per day and copper concentration in the water. Moreover, the means and maxima of the entire data series of 5−6 days were compared as proposed by Evans and Wallwork (*16*) to reveal differences between the populations for the different behavioral parameters (one-way ANOVA). The effects of simulated pollution peaks on *G. pulex* were analyzed by comparing different behavioral parameters for the first 2 h after the pollution with the same 2 h on the following day using Friedman tests.

Several behavioral parameters were analyzed such as number of active organisms, time spent on activity per recording (s), time spent on selected frequency ranges (measured as amplitude in the spectrogram, Figure 2), and time spent on different amplitude ranges (s), which were typical for certain behaviors, such as locomotion ($0.9 < x <$ 1.9 Hz) and ventilation ($2.8 < x < 3.7$ Hz) (*11*). Only the higher amplitude ranges of ≥0.3 V were analyzed, as they are typical for swimming activity of *G. pulex* (*11*), whereof it could be distinguished between small leg movements ($0.3 < x < 0.4$) and body stretching ($0.4 < x < 10$ V) (*11*).

Time series analyses were performed on unsmoothed raw data of means from eight organisms. An ARIMA ($p$,$q$) model expresses the current series value as a linear combination of the preceding $p$ values together with an error term for the current time and a linear combination of the $q$ most recent errors (*15*). If $q = 0$, the model is said to be an autoregressive process of order $p$ (AR($p$)), while if $p = 0$ it is a moving average model of order $q$ (MA($q$)). The order of the model refers to the number of recent values that is included in a future projection.

## Results and Discussion

**Water Quality.** Most of the chemical parameters in the Ståstorpsån, ca. 5 m above the biomonitor did not change remarkably in the course of the experiments. The pH varied between 6.5 and 7.0, and the total hardness varied between 2.15 and 3.00 mmol/L. The conductivity values were between 320 and 360 $\mu$S/cm, while the concentration of the nutrient anions varied from 0.91 to 1.49 mg/L for $P0_4^{3-}$ and from 1 to 2 mg/L for $N0_3^-$. The values of dissolved oxygen decreased from 9.5 mg/L in the beginning to 3.1 mg/L at the end of the experiment. The copper concentrations were low in the beginning (2.4 $\mu$g of $Cu_{tot}$/L) and increased toward the end of the test in coincidence with the end of the summer holidays (131 $\mu$g of $Cu_{tot}$/L). The copper values in the water did not indicate changes in mortality or behavior of the organisms as the Skäralid population was only exposed to <5 $\mu$g of

**TABLE 2. Diurnal Activity Pattern of *Gammarus pulex* in Biomonitor**

| time | % of active organisms from Skäralidbäcken | % of active organisms from Ståstorpsån | time | % of active organisms from Skäralidbäcken | % of active organisms from Ståstorpsån |
|---|---|---|---|---|---|
| 1600 | 25.0 | 50.0 | 500 | 25.0 | 25.0 |
| 1700 | 12.5 | 37.5 | 600 | 0 | 12.5 |
| 1800 | 12.5 | 12.5 | 700 | 25.0 | 37.5 |
| 1900 | 25.0 | 50.0 | 800 | 12.5 | 25.0 |
| 2000 | 0 | 25.0 | 900 | 25.0 | 25.0 |
| 2100 | 0 | 37.5 | 1000 | 12.5 | 75.0 |
| 2200 | 25.0 | 37.5 | 1100 | 0 | 50.0 |
| 2300 | 62.5 | 87.5 | 1200 | 50.0 | 50.0 |
| 2400 | 75.0 | 87.5 | 1300 | 37.5 | 12.5 |
| 100 | 37.5 | 50.0 | 1400 | 25.0 | 37.5 |
| 200 | 25.0 | 25.0 | 1500 | 25.0 | 37.5 |
| 300 | 12.5 | 50.0 | 1600 | 12.5 | 37.5 |
| 400 | 25.0 | 50.0 | | | |

**TABLE 3. Statistical Analyses and Models of Behavioral Time Series Data[a]**

| | behavior type | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | | 3 | | 4 | | 5 | | 6 | |
| statistic | rep 1 | rep 1 | rep 2 | rep 1 | rep 2 | rep 1 | rep 2 | rep 1 | rep 2 | rep 1 | rep 2 |
| **Skäralidbäcken (Population 1)** | | | | | | | | | | | |
| mean | 2.20 | 2.00 | 2.85 | 0.13 | 0.23 | 0.02 | 0.04 | 0.02 | 0.05 | 0.005 | 0.023 |
| SD | 1.70 | 1.55 | 1.21 | 0.16 | 0.16 | 0.09 | 0.13 | 0.05 | 0.08 | 0.04 | 0.09 |
| max | 7.00 | 8.90 | 6.10 | 0.83 | 0.78 | 1.20 | 1.04 | 0.35 | 0.55 | 0.50 | 1.00 |
| model | | AR(1) | AR(1) | AR(1) | AR(1) | mixed | AR(1) | mixed | mixed | mixed | mixed |
| MSE | | 1.90 | 0.90 | 0.03 | 0.03 | | 0.01 | | | | |
| **Ståstorpsån (Population 2)** | | | | | | | | | | | |
| exp 1 | | | | | | | | | | | |
| mean | 3.81 | 2.57 | 4.25 | 0.36 | 0.15 | 0.04 | 0.02 | 0.17 | 0.14 | 0.14 | 0.06 |
| SD | 1.64 | 1.72 | 1.37 | 0.25 | 0.15 | 0.11 | 0.10 | 0.15 | 0.17 | 0.30 | 0.29 |
| max | 7.00 | 9.70 | 8.60 | 1.50 | 0.90 | 0.80 | 0.90 | 0.90 | 1.00 | 3.00 | 3.40 |
| model | | AR(3) | AR(1) | mixed | AR(1) | AR(2) | mixed | AR(3) | AR(1) | mixed | mixed |
| MSE | | 1.71 | 1.24 | | 0.02 | 0.01 | | 0.01 | 0.02 | | |
| exp 2 | | | | | | | | | | | |
| mean | 3.44 | 1.98 | 3.99 | 0.34 | 0.17 | 0.18 | 0.44 | 0.44 | 0.31 | 0.36 | 0.45 |
| SD | 1.84 | 1.39 | 1.28 | 0.23 | 0.24 | 0.34 | 0.69 | 0.24 | 0.42 | 0.27 | 1.28 |
| max | 8.00 | 5.60 | 7.90 | 1.60 | 1.90 | 1.46 | 2.50 | 2.20 | 1.90 | 1.50 | 7.60 |
| model | | AR(1) | AR(1) | mixed | AR(1) | AR(1) | AR(1) | AR(2) | AR(1) | AR(3) | AR(1) |
| MSE | | 1.62 | 1.38 | | 0.04 | 0.06 | 0.14 | 0.03 | 0.03 | 0.05 | 0.53 |
| exp 3 | | | | | | | | | | | |
| mean | 4.38 | 5.38 | 4.26 | 0.32 | 0.23 | 0.28 | 0.09 | 0.44 | 0.14 | 0.43 | 0.13 |
| SD | 1.85 | 2.45 | 1.41 | 0.19 | 0.19 | 0.46 | 0.22 | 0.26 | 0.33 | 0.32 | 1.07 |
| max | 8.00 | 13.9 | 8.20 | 1.40 | 1.20 | 2.60 | 1.50 | 1.90 | 4.00 | 1.80 | 15.3 |
| model | | AR(1) | AR(1) | mixed | AR(2) | AR(2) | mixed | AR(1) | AR(1) | AR(2) | mixed |
| MSE | | 3.91 | 1.65 | | 0.03 | 0.10 | | 0.08 | 0.10 | 0.11 | |
| *p*(pairwise) | 0.005 (all) | 0.037 (1/2,3) | | ns | | ns | | ns | | 0.005 (1/2,2; 1/2,3) | |
| *p** | 0.003 | ns | | 0.054 | | ns | | 0.068 | | 0.05 | |

[a] Behavior types: 1, no. of active organisms (max, 8); 2, time spent on activity (s); time spent on different frequency ranges measured as magnitude in the spectrogram, *e.g.*, 3, locomotion ($0.9 < x < 1.9$ Hz); 4, ventilation ($2.8 < x < 3.7$ Hz). Time spent on different amplitude ranges (s), *e.g.,* 5, leg movements ($0.3 < x < 0.4$ V); 6, body stretching ($0.4 < x < 10$ V). Rep: two replicates of eight organisms were performed simultaneously, except for behavior type 1. Exp: three experiments for 6 days performed with population 2. *p*, Friedman two-way analysis (time, population 1 and 2) for pairwise comparisons of the experiments 1−3. *p**, two-factor ANOVA of the means of the whole data series (populations, experiments).

$Cu_{tot}$/L and the local population should be adapted to pollution peaks up to 360 $\mu$g of $Cu_{tot}$/L (*11*).

**Survival in the Biomonitor.** Mortality of the amphipods in the biomonitor increased within 6 days up to 87.5% in the population from Skäralidbäcken and up to 72.9% in the local populations from Ståstorpsån. The high mortality might have been due to experimental handling stress of checking survival twice a day and operational problems with the pumping system. Organisms from the unpolluted stream survived slightly worse ($p = 0.08$) than the local organisms. This might be due to increased stress in the Skäralid population, as these organisms had to cope with increases in water temperature, hardness, and nutrients after transfer into the Ståstorpsån.

**Population Differences in Behavior.** Both populations showed a clear circadian activity pattern in the biomonitor, with maxima around midnight (Table 2). The second activity maximum in the morning might be due to handling stress as described above. Compared to the local population, *G. pulex* from the reference population was less active ("time spent on activity": $p = 0.037$; "number of active organisms": $p = 0.003$), performed less body stretchings ($p = 0.005$), swimming ($0.9 < x < 1.9$ Hz: $p = 0.054$), and ventilation ($2.8 < x < 3.7$ Hz: $p = 0.08$) (Table 3). *In situ* cage experiments with *G. pulex* from a reference population and a population exposed to metal pollution, both exposed in a metal-polluted stream, revealed higher mortality and sensitivity in the

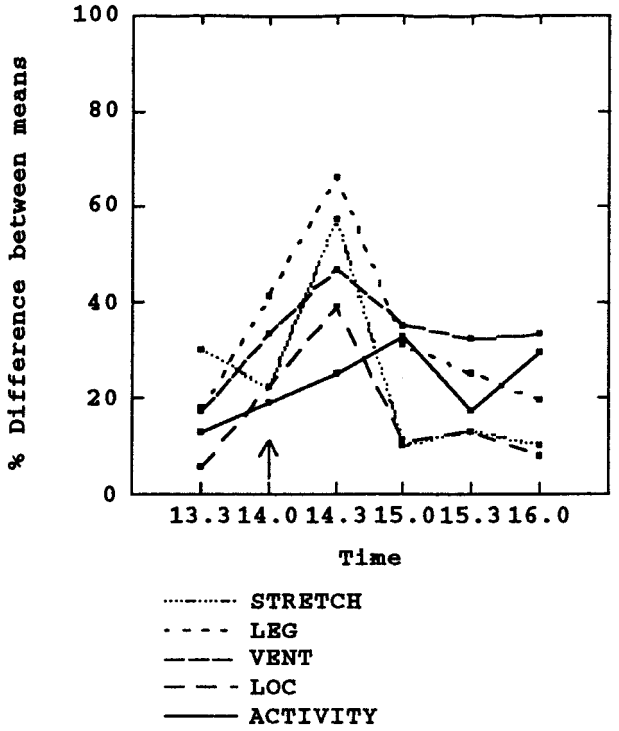


**FIGURE 4. Effect of simulated pollution peaks (<100 $\mu$g of $Cu^{2+}$/L) (arrow) on different behaviors of *Gammarus pulex*. Differences between the means from data series of directly after the pollution and data series of 24 h later are plotted for time spent on activity, locomotion, ventilation, leg movements, and body stretching.**

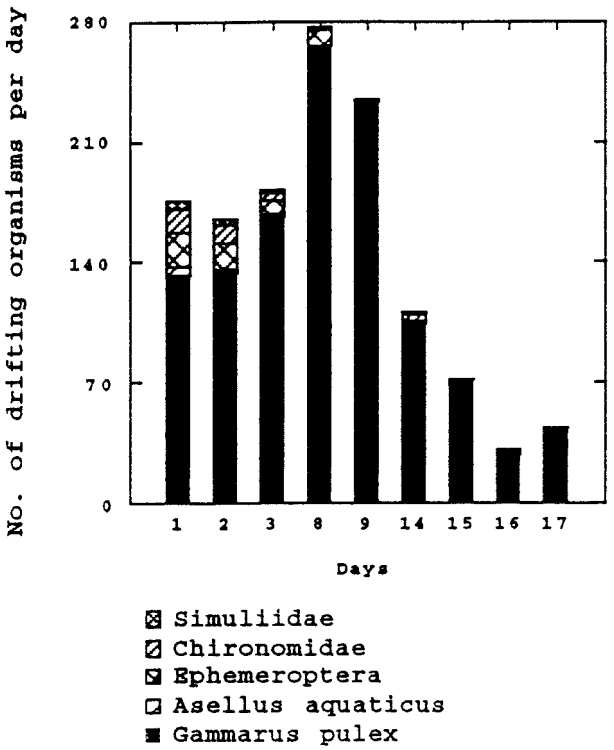


**FIGURE 5. Macroinvertebrate species in the natural drift during nine 24-h cycles.**

reference population (*18, 19*). *G. pulex* from a reference population formed less precopulas under fenvaleratstress (10 $\mu$g/L) as compared to a population suffering from agricultural pollution (*15*).

Most raw data series fitted a simple autoregressive model of order 1, AR(1), up to order 3 (Table 3) in concordance to Diamond *et al.* (*2*) for fish ventilation data. If no clear diagnostics was possible, a more complex ARIMA model seemed appropriate (mixed model).

**Pollution Responses.** A positive correlation between the number of active organisms per day and daily copper concentration was found (Skäralidbäcken: $r$, 0.63, $p < 0.001$; Ståstorpsån: $r$, 0.66, $p < 0.008$). During the first 2 h after a simulated Cu pollution peak, *G. pulex* from the local population reacted with an increased number of active organisms ($p = 0.005$). *G. pulex* spent also more time on leg movements ($p = 0.025$), body stretchings ($p = 0.005$), and ventilation ($p = 0.005$) as compared to their behavior at the same time on the following day. The differences between the mean responses during and 24 h after the pollution peak revealed response times within 30−60 min (Figure 4). As there was no significant increase in mortality after the simulated pollution peaks, the behavioral responses were clearly sublethal. The fast response time supports the finding that *G. pulex* can actively sense and avoid Cu at ≥63 $\mu$g/L (*20*). *Dreissena polymorpha* reacted within 3 min to 115 $\mu$g of Cu/L with increasing valve movements and a decreased percentage of open mussels by 20% (*21*). Within 4 h, *G. pulex* responded to phenolic compounds with a lack of escape behavior (*22*). *Gammarus duebeni*, an estuarine species, changed ventilation frequency and swimming patterns at ≥600 $\mu$g of Cu/L (*23*).

**Natural Activity Patterns and Responses to Cu Pollution.** The absolute number of drifting *G. pulex* per day varied immensely; however, *G. pulex* was always the dominant species in the drift, followed by ephemeropterans, chironomids, aselids, and simuliids (Figure 5). Trichopterans, coleopterans, snails, and hirudinea were found very rarely in the drift. A drift maximum occurred between 0.00 and 4.00 AM (Table 4). Nocturnal drift maxima for *G. pulex* and the closely related *G. pseudolimnaeus* have been reported earlier (*6, 24, 25*), especially during spring and summer when nocturnal drift activity was enhanced (*26*). The high day-to-day variations in drift rates during the present experiments might have been related to climatic factors, as the drift maximum was found in a night with a thunderstorm with heavy rain. Drift of *G. pulex* and *G. pseudolimnaeus* has earlier been found to depend on flow rates (*26, 27*).

**TABLE 4. Drift Responses of *Gammarus pulex* to Cu Pollution**

| | no. of drifting organisms[a] | | | | |
|---|---|---|---|---|---|
| time | 1 | 2 | 3 | 4* | 5 |
| 1200 | | 12 | 15 | 2 | |
| 1400 | | | 2 | 3 | 4 |
| 1600 | | 3 | 4 | 2 | 0 |
| 1800 | 4 | | 4 | 0 | 0 |
| 2000 | 5 | 4 | 0 | 6 | 0 |
| 2200 | 8 | | 5 | 8 | 4 |
| 2400 | 54 | 63 | 48 | 86 | 30 |
| 400 | 53 | 50 | 50 | 120 | 35 |
| 800 | 12 | 6 | 8 | 8 | 5 |
| 1200 | 8 | 5 | 5 | 10 | 0 |
| 1400 | | | 0 | 2 | 0 |
| 1600 | | | 3 | 3 | 2 |

[a] 1 and 2, natural drift; 3−5, drift after a simulated pollution pulse with 100 $\mu$g of $Cu^{2+}$/L. *Night with thunderstorm.

The simulated copper pollution peaks did neither cause any direct increase in the drift nor delayed increases of the nocturnal drift peaks (Table 4), probably due to adaptation of the local population to Cu concentrations up to 360 $\mu$g of $Cu_{tot}$/L (*11*). Increased "drift response" of *G. pulex* was found at concentrations ≥0.7 mg of Cu/L (*9*), ca. 10 times higher than the simulated pollution peaks (0.07 mg of $Cu^{2+}$/L) in the present experiment. Within 1−3 h, *G. pulex* reacted with

increased drift under exposure to permethrin (*28*), fenvalerat (*15*), and cypermethrin (*29*).

## Evaluation of the Biomonitor

**Survival Conditions.** *G. pulex* showed natural behavior in the test chambers such as locomotion, ventilation, and diurnal activity patterns similar to those in the field. Similar cylindrical test chambers have also been used by Morgan (*30*) for recording ventilation and locomotion of fish and mayflies. *G. pulex* was exposed to a flow rate of 1.5 L/min, which simulated a "flow-through pool". This seemed to be appropriate as in choice experiments *G. pulex* was mostly abundant in pools, in riffles only if cobbles or leaf packs were present, or when discharge decreased (*31*). Optimal flow rates for experiments with *G. pulex* of 25 cm/s were proposed by Williams and Moore (*32*). Hargeby (*33*) reared *G. pulex* successfully in trickle chambers with a flow rate of 1.7 L/min. Operational problems with the pumping system caused some irregularities in the water flow and possibly oxygen supply also, which might be avoided by using water supply from gravidity head tanks or submersed pumps (*30*). The advantage of the present biomonitor is the use of unfiltered streamwater without quality loss in the electrical signals. As many toxicants are bound to particles, filtration would diminish exposure and thus responses of the organisms in the biomonitor in an unrealistic way. The food supply of a leaf disc of 1 $cm^2$ seemed to be sufficient for 6 days (*34*).

**Data Collection.** In contrast to event-based data collection, the present biomonitor used the time-based collection method, providing a whole time series of data as a basis for statistical evaluation (*35*). Event-based data collection and analysis is fast and easy, but needs a preset threshold value and is therefore biased. The analysis of whole time series data is slower; however, it is more sensitive and reliable. The data collection interval used in the experiments was 30 min, the maximum recommended by van Schalie (*36*). The most frequent data recording scheme in a biomonitor mentioned in the literature was a trace of 3 min followed by a pause of 1 min (*30*). The choice of the interval and the length of a recording are a trade-off between disc space, number of potential pollution discharges, response time of the test species, etc. Traces of at least 2 min followed by pauses of a maximum of 10 min were used in the Dreissena monitor (*37*, *38*) or the ultrasonic fish monitor (*3*). The present biomonitor records different behaviors simultaneously such as number of active organisms, time spent on activity, time spent on different frequency, and amplitude ranges typical for different behaviors (*e.g.*, locomotion, ventilation) depending on previous definition for each species. Multiparameter registration enhances the reliability of the biomonitor and probably also the sensitivity to pollution pulses as compared to biomonitors using a single behavior based on binomial information, *e.g.*, pass/not pass a light beam (Dynamic Daphnia test), mussel open/closed (Dreissena monitor), or fish drifting or not (fish-rheotaxis test). Many systems use tolerant (*Leuciscus idus* fish test) or locally ecologically irrelevant species and create many false alarms (*4*). For biomonitoring of the water quality of a stream ecosystem, we highly recommend using local species, *e.g.*, from several kilometers above the point pollution source. This eliminates false alarms due to reactions to water quality parameters unrelated to the pollution. Moreover, this eliminates "oversensitivity" of the test organisms to the pollution, providing too low thresholds and leading to "overprotection" of the ecosystem. This also decreases mortality in the biomonitor and thus maintenance efforts.

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