Research Articles

Behavioural Early Warning Responses to Polluted Water

Performance of *Gammarus pulex* L. (Crustacea) and *Hydropsyche angustipennis* (Curtis) (Insecta) to a Complex Industrial Effluent

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Abstract

Behavioural early warning responses to polluted surface water from an industrial effluent have been measured in two freshwater macroinvertebrate species using the four electrode impedance conversion technique. Specimens of *Gammarus pulex* (L.) and *Hydropsycho angustipennis* (Curtis) were caught from reference streams and exposed to water from above and below the factory and to reference water for about 1 h with different kinds of behaviour being registered on-line every 10 min, such as time spent on locomotion, number of "high swimming peaks", number of activity phases, cleaning, time spent on ventilation, ventilation frequency and netspinning. Additionally, *G. pulex* was exposed *in situ* above and 100 m, 500 m and 1000 m below the factory for 24 h.

G. pulex reacted to water pollution within 1 h with less time spent on locomotion, fewer "high swimming peaks" and lower number of activity phases ($p < 0.01$). The same results were found after the *in situ* exposure ($p < 0.05$) and the pollution gradient mirrored survival and behavioural performance of the organisms.

H. angustipennis reacted to water pollution within 1 h with decreased time spent on ventilation ($p < 0.001$), no change in locomotion and netspinning during daytime. During the night, exposure to water pollution resulted in increased locomotion ($p < 0.0001$).

Simultaneous multispecies on-line biomonitoring of industrial effluents is recommended for reliable risk assessment.

Key words: Biomonitoring; risk assessment; surface water, polluted; industrial effluents; macroinvertebrate species; aquatic organisms; *Gammarus pulex* L.; Crustacea; *Hydropsyche angustipennis*; Insecta; water pollution

1 Introduction

Benthic macroinvertebrates have been attractive targets for pollutant risk assessment and biomonitoring, because they are a diverse group reacting strongly and predictably to aquatic pollution (CAIRNS & PRATT 1993), they are important links in the aquatic foodweb (GARMENDIA-TOLOSA & AXELSSON 1993) and their different responses have an increasing currency in assessing and predicting freshwater pollution (ROSENBERG & RESCH 1993) as well as in on-line biomonitoring in biological early warning systems (GERHARDT 1995a). Biological early warning responses are fast (within a few hours) and sensitive (at low exposure levels) reactions to pollutant stress beyond homeostasis causing impairment of the organisms health. As long as these responses are compensatory the disability of the organism is low. Beyond the compensatory range, however, disability increases exponentially (DEPLEDGE 1989).

The concept of bioindicators implies that there are specific species in an aquatic community which react in a sensitive manner to pollutants and which at the same time mirror the "health" of the community. This concept assumes a community to be seen as a "supraorganism", which is affected by a pollutant if the indicator species is affected (MORIARTY 1988). This model can be true in case that the chosen indicator species is a keystone species or an engineer species *sensu* LAWTON (1994). In those cases the decrease in abundance of such a species due to pollution would strongly affect the ecosystem processes. It is, however, more reliable for biological risk assessment to test the responses of different test species, *e.g.* from different trophic levels and with different life history strategies to get an impression how the whole community could be affected by pollution.

As the pollutants affect individuals in the first place, the individual organisation level is an appropriate biomonitoring tool for biological early warning systems. If ecological relevant responses of the individuals are chosen, which have consequences on the population level, predictions and risk assessments for ecosystem health are more realistic and results are less likely to be affected by chance and unrepresentative measurements such as extrapolations from LC_{50} bioassays.

Behavioural responses are amongst the first and most sensitive reactions to chemical stress (WARNER 1967; BLAXTER

& HALLERS-TJABBES 1992). Behaviour is based on biochemical responses (*e.g.* altered chemoreception), controlled by neurological and hormonal mechanisms on the suborganism level. It can indicate ecological consequences at the level of the organism (*e.g.* reduced performance), the population (*e.g.* impaired reproductive success) and of the community (*e.g.* predation). The integrative nature of behavioural parameters allows them to be sensitive, ecological relevant and non-destructive biomarkers for pollution (SCHERER 1992; DEPLEDGE & FOSSI 1994) and hence appropriate for biological early warning systems.

Changes in behaviour due to aquatic pollution have been observed in the laboratory and in the field including increased downstream invertebrate drift (BRITTAIN & EIKELAND 1988), avoidance in several fish species (BEITINGER 1990), changes in gill ventilation and locomotion (WINGARD & SWANSON 1992; GERHARDT 1995b; GERHARDT & JANSSENS DE BISTHOVEN 1995), changes in valve movements of mussels (BORCHERDING & VOLPERS 1993).

2 Objectives

The main aim of the present study was to test differences in the behavioural early warning responses of two invertebrate species differing in habitat selection, functional feeding groups and life history strategies during short-term exposure to polluted surface water. It was hypothesized that test organisms exposed to polluted water react with altered locomotion, gill ventilation, cleaning and netspinning behaviours compared to exposures in unpolluted water. Moreover, the responses were expected to be species specific, and behavioural responses were expected to be more sensitive than survival as endpoint parameter.

3 Material and Methods

3.1 Test species

Gammarus pulex L. is widely distributed in fast-flowing brooks and streams in Northwestern Europe (MEIJERING 1971). It is an important allogenic engineer species (*sensu* LAWTON 1994) in the aquatic foodweb and lives epigeic (PLENET 1995) as a shredder on decomposing leaves, a main food source in mountain streams. It is prey for predatory insects (*e.g.* Trichoptera, Coleoptera, Plecoptera, Odonata) and fish (*e.g. Salmo* sp., *Barbus barbus*) (GARMENDIA-TOLOSA & AXELSSON 1993). The life history is characterised by overlapping iteroparity, *i.e.* a female breeds several times a year and no distinct breeding seasons exist so that all size classes can be found throughout the year, which is advantageous for continuous biomonitoring purposes. *G. pulex* is sensitive to a wide range of toxicants (WILLIAMS *et al.* 1984) and has been recommended for use in toxicity bioassays both in the laboratory and in the field (PASCOE *et al.* 1994). It has been widely used in biotic indices for organic pollution (MALTBY 1995). Recent development of behavioural toxicity tests with endpoints such as feeding behaviour (TAYLOR *et al.* 1993), precopula separation (PASCOE *et al.* 1994) and scope for growth (MALTBY *et al.* 1990) contribute to establish *G. pulex* as a toxicity test reference organism such as *Daphnia magna*.

Hydropsyche angustipennis has an univoltine annual life cycle (GEORGIAN & THORP 1992). It is common in European streams, often the dominant hydropsychid species in polluted streams (PETERSEN 1986). It is living in a guild, *i.e.* congeric with the closely related species *H. pellucidula* and *H. siltalai*, with *H. pellucidula* being the keystone species (PETERSEN 1986). It covers two functional feeding groups as the young larvae are mostly filter feeders, while the older larvae are predatory (WALLACE & MERRITT 1980). As the species builds nets and lives in cages at least during daytime it can be considered as temporary hemisessil. The preferred habitats are lake outlets due to high seston content (CUSHING 1963). *Hydropsyche angustipennis* has not been used for toxicity bioassays, however responses to metals included changes in competition behaviour (VUORI 1994), growth and mortality (VUORI 1995), colour and structure of the tracheal gills and anal papillae (VUORI & PARKKO 1995), anomalies in the capture net structure (PETERSEN & PETERSEN 1984; DECAMPS *et al.* 1973) and changes in guild structure (PETERSEN 1986). These responses indicate a battery of potentially sensitive toxicity parameters.

3.2 Streams

The Ybbarpsån in the northern part of the province Scania is a mesosaprobic brownwater stream flowing through mixed forest vegetation on silicate rock. It receives effluents from an industrial area (factory complex) including manufacturing plants containing metals (Al, Zn, Cu, Cr, Mo) and organic xenobiotics, *e.g.* low soluble long-chained fatty acids and alcohols, phenols, formalin, toluene (Perstorp kommun, pers. comm). A few km below the discharge points, *G. pulex* and *H. angustipennis* have been found, however in low densities.

The organisms for the toxicity bioassay were caught in unpolluted streams, where they could be found in high abundances. The Skäralidstream is an oligosaprobic clearwater mountain stream with gneiss gravel and rocks covered with coarse organic detritus as well as aquatic mosses, thus being the optimal habitat for *Gammarus pulex*. The Övedsån is a lake outflow rich in seston in an agricultural area, thus creating eutrophic and mesosaprobic conditions. The streambed contains gneiss gravel and rocks covered with aquatic mosses and algae. *Hydropsyche angustipennis* was found in high densities. The chemical parameters of the water from the three streams used for the experiments have been analysed by ICP-AES (metals, total-P and total-N) and colorimetric methods (NO_3), *(→ Table 1)*.

4 Experimental Design

4.1 Laboratory study

The test organisms were caught in the respective species specific unpolluted sites and kept in the laboratory in small aquaria (30 x 20 x 15 cm^3 with 30 organisms in each) in

Table 1: Chemical parameters of the stream water for the experiments

Stream	°C	pH	Lf	P	NO_3	Ca	Al	Fe	Cd	Cu	Pb	Zn
	–	–	µS/cm	µg/l	mg/l	mg/l	mg/l	mg/l	µg/l	mg/l	mg/l	mg/l
Ybbarpsån (above: 56°8′36″ N, 13°27′54″ W; below: 56°7′25″ N, 13°22′50″ W)												
above	16	5.5	112	60	0.7′	*	0.2	0.6	–	–	0.05	0.01
below	25	6.4	250	199	2.0′	*	0.1	0.3	3	0.01	0.02	0.03
Skäralidb. (56°2′18″ N, 13°15′0″ W)												
	10	6.0	*	–	44.4	19.0	0.2	0.8	–	–	–	0.03
Övedsån (55°41′30″ N, 13°38′40″ W)												
	*	8.1	*	60	350.0	62	<0.01	0.5	–	0.07	–	0.004

′ = Ntot; – = below detection limit (Al: 10 µg/l; Cd: 1 µg/l; Cu: 7 µg/l; Pb: 20 µg/l)
* = not analysed

water and sediment from the respective sampling site. *H. angustipennis* was fed with *D. pulex*, powder of *Urtica dioica* and traditional fish food (Tetramin) and *G. pulex* was provided with leaves from the sampling site. As *H. angustipennis* is rheophil a flow was created by recirculating the water in the aquaria. The water in all aquaria was aerated and kept at 10 +/– 1 °C and a 12 h : 12 h photoperiod.

After at least two days acclimation time a series of parallel behavioural measurements started. Three randomly chosen organisms of one species were placed in their test chambers, one filled with water from the reference site, one with water from above the factory and one with water from below the factory and acclimated for 10–15 min. Afterwards a behaviour recording (110 sec. for *G. pulex*, 280 sec. for *H. angustipennis*) was performed every 10–15 min. for a period of about one to one and a half hour. The recording time for *H. angustipennis* was longer, because that species was less active than *G. pulex*. At the same time the organisms were observed visually and the different kinds of behaviour protocolled manually, while being plotted on the computer screen in real time. These measurements were repeated with 30 to 34 organisms for water from the reference stream, 30 to 34 organisms for water from above the factory and 30 to 64 organisms for water from below the effluent as increased variation of the behaviour was expected in polluted water (McNICHOL & SCHERER 1991). As that was not found for *G. pulex*, only 30 organisms of *H. angustipennis* were used in the subsequent experiment.

Moreover, diurnal activity was measured every hour over 24 h with *H. angustipennis* in water from above (n = 6) and below (n = 6) the effluent in the test chambers with recirculating stream water (10 ml/min).

4.2 *In situ* study

Additionally to the exposure to polluted stream water in the laboratory, *G. pulex* was exposed *in situ* 4 km above and 100 m, 500 m, and 1000 m below the factory complex in net cages as described in GERHARDT & WESTERMANN (1995). The wooden framed cages (length: 20 cm, width: 10 cm, hight: 80 cm) contained a nylon net (1 mm mesh size) on all sides in order to allow for exchange of water and detritus between the cages and the stream. They were digged 5 cm into the sediment filled with previously dried (12 h: 110 °C) sediment from the respective exposure sites four days before the start of the *in situ* exposure experiment of 24 h. Thirty specimens of *G. pulex* from the reference stream were exposed in each cage without replicates. *H. angustipennis* was not used because of low recoveries in previous experiments, probably due to their predatory and cannibalistic behaviour. After the transport to the laboratory the behaviour of ten randomly chosen alive organisms from each cage was measured as described above for the laboratory bioassay in two replicates of 110 s each.

4.3 Behavioural measurements

The behavioural patterns of the test organisms were determined individually with the four-electrode impedance conversion technique (GERHARDT *et al.* 1994; GERHARDT 1995b and GERHARDT & JANSSENS DE BISTHOVEN 1995). The test organisms were situated in their chambers (10 x 3 x 2 cm^3) filled with stream water. One pair of electrodes placed at the chamber wall generated an alternating current, while another pair of electrodes measured changes of the impedance over the chamber due to the movements of the organism (GERHARDT *et al.* 1994).

Different kinds of behaviour could be defined according to the frequencies and the relative amplitudes of the electrical signals generated by the impedance converter at a sampling frequency of 50 Hz. The absolute amplitudes were not appropriate because they depended on the position of the organism in the chamber relative to the electrodes. The signals were transformed to digital signals in a Macintosh LC3 computer and analysed using Super Scope software.

4.3.1 Types of behaviour in *G. pulex*

Gammarus pulex performed locomotion, starting with stretching of the body (highest amplitude), which was considered as a so called "high swimming peak", followed by smaller leg movements (less than half of the highest amplitude). Locomotion signals were multifrequent (0.8–1.2 Hz) *(→ Fig. 1)*.

Repeated monofrequent signals between 2–7 Hz (ventilation) as observed in GERHARDT (1995b) could not be regis-

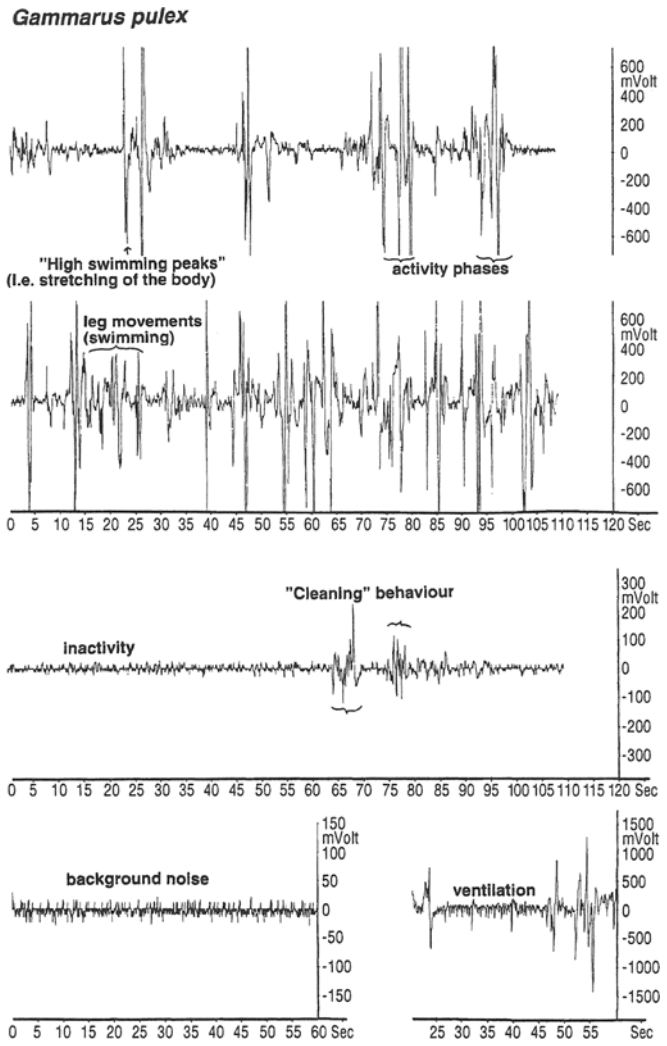

Fig. 1: Examples of different types of behaviour of *G. pulex*

tered quantitatively in this study because of the use of a bigger test chamber (10 x 3 x 2 cm^3 instead of 2 x 1 x 1 cm^3 in the previous study). The bigger the test chamber the more ecological relevant the behaviour, however, at the expense of resolution.

Cleaning behaviour consisted of a few repeated movements with the legs over the head and antennae while the organism was not swimming. As the signals for that behaviour were close to the noise level they could not be registered quantitatively. However, as the behaviour was rather scarce manual recordings were possible.

Signals lower than 50 mV were considered as background noise and thus a measure of inactivity.

4.3.2 Types of behaviour in *H. angustipennis*

Hydropsyche angustipennis was tested in the same chambers as *G. pulex*. Locomotion signals were multifrequent and of low frequencies (0.5–1.5 Hz) and of varying amplitude. There was no typical locomotion pattern as found for *G. pulex*. Ventilation signals were slow monofrequent movements with the whole abdomen (abdomen undulations) (0.9–1.2 Hz). Netspinning behaviour, *i.e.* movements with the head from one side to the other of the organism attached to a surface were below the noise level of the impedance converter and thus observed visually *(→ Fig. 2)*.

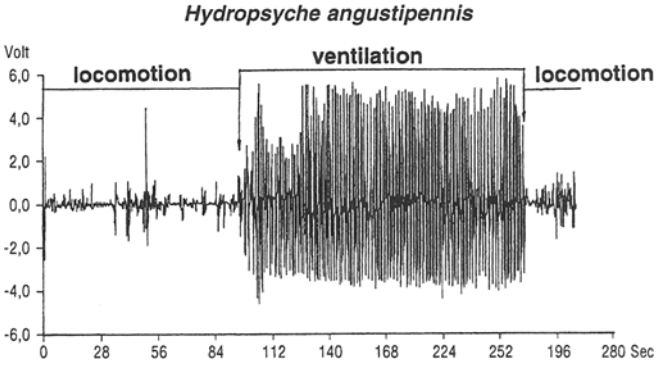

Fig. 2: Example of different types of behaviour of *H. angustipennis*

4.4 Statistics

All data were analysed by use of a repeated measurements ANOVA for the location of the factory complex (reference, above and below the effluent). Comparisons between two groups were done with pairwise Students't-tests for dependent samples.

5 Results

5.1 Early warning responses of *Gammarus pulex*

All results were characterised by a high variance typical for behavioural data, as some organisms react while others do not react at all to pollution, which in the worst case can result in dichotomous response characteristics (McNicHOL & SCHERER 1991). While the time the animals spent on locomotory activity remained high during the whole exposure period in the water from the reference stream, it decreased in water from Ybbarpsån below the effluent with time (Df: 6, 2, F: 3.04, p: 0.006) *(→ Fig. 3)*. Regarding the number of "high swimming peaks", *i.e.* the powerful stretching of the body at the beginning of each swimming, organisms in the reference water showed significantly more peaks than those in water from Ybbarpsån (above the effluent: Df: 6, 1, F: 6.75, p: 0.013; below the effluent: Df: 6, 1, F: 10.23, p: 0.0023) and organisms above the effluent showed significantly more such peaks than those below (Df: 6, 1, F: 10.23, p: 0.0023). In water from Ybbarpsån, the number of high swimming peaks decreased with exposure time (above the effluent: Df: 6, 1, F: 2.90, $p < 0.007$; below the effluent: n.s.) compared to specimens in reference water.

The number of activity phases, *i.e.* how often the organisms change between different behaviours, *e.g.* swimming, sitting, swimming, sitting *etc.* was higher in the reference stream than in water from Ybbarpsån above the effluent (Df: 6, 1, F: 18.26, p: 0.0008). The animals exposed to reference water cleaned themselves more often than those ex-

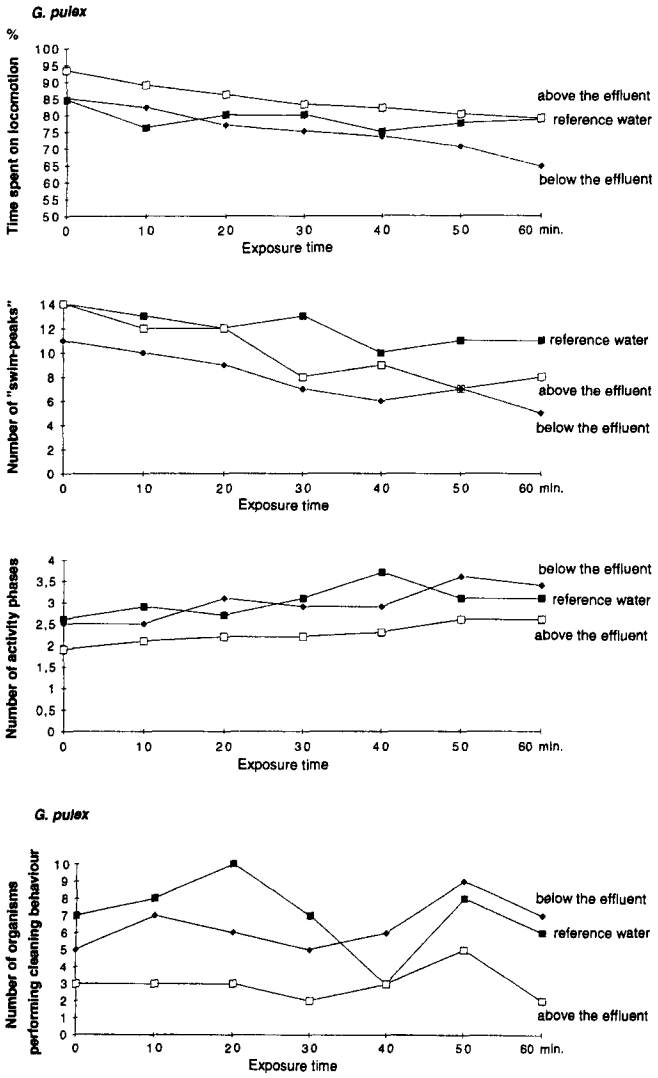

Fig. 3: Changes in different types of behaviour of *G. pulex* due to different water quality (reference, above, below effluent) and exposure time (60 min.):
a) Time spent on locomotion, b) Number of "swim-peaks", c) Number of activity phases, d) Cleaning

posed to water from Ybbarpsån above the effluent (Df: 6, 1, F: 4.64, p: 0.035).

5.2 Effects of short-term exposure *in situ*

After an *in situ* exposure of *G. pulex* in the Ybbarpsån (4 km above, 100 m, 500 m and 1000 m below the effluent) for 24 h, survival and behaviour of the organisms demonstrated clearly the toxicity of the factory effluent *(→ Fig. 4)*. Hundred meters below the effluent, no survivors were found, while 500 and 1000 m below survival was increasing until the levels of above the factory. The time the organisms spent on locomotory activity (p = 0.036, t-test) and the number of "high swimming peaks" (p = 0.007, t-test) at 500 m below the factory was significantly lower than above the effluent, whereas the behaviour of the organisms exposed 1000 m below the effluent was not different from those exposed above the factory.

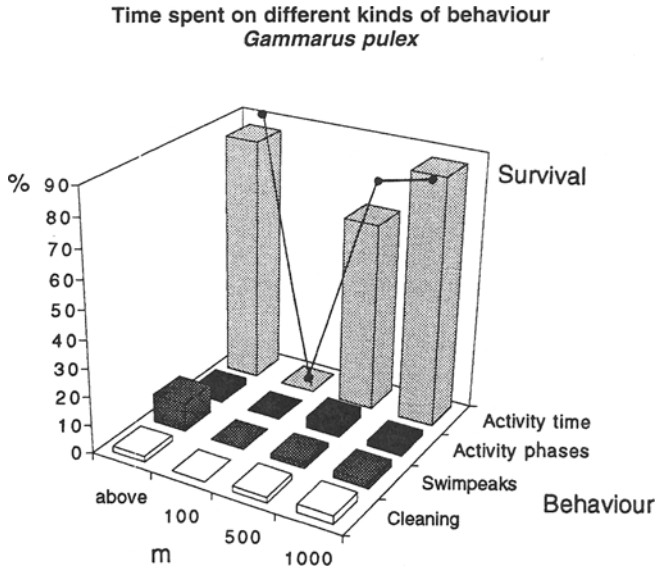

Fig. 4: Time spent on different types of behaviour of *G. pulex* in a pollution gradient after 24 h *in situ* exposure

5.3 Early warning responses of *Hydropsyche angustipennis*

In all types of water the organisms showed initially high locomotory acitivity (observed as "exploratory" behaviour), which decreased after about half an hour when the larvae found a place to settle. In general, the larvae were less locomotory active than *G. pulex* and no significant differences in activity due to water quality were found *(→ Fig. 5, p. 68)*. During the night, however, larvae exposed in water from below the effluent showed significantly more activity (p = 0.0001, t-test) than those exposed in reference water. Netspinning behaviour was not dependent on water quality. Time spent on ventilation increased with exposure time in all treatments (Df: 6, 2, F: 8.83, p: 0.0032) in concordance to decreased locomotion. Organisms exposed to water from below the effluent ventilated significantly shorter and with lower ventilation frequency than those exposed to water from above the factory (p = 0.001; Hz: p = 0.001) or reference water (p = 0.035; Hz: p = 0.025), whereas there were only slight differences between the reference water and water from above the factory (p = 0.04; Hz: p = 0.05).

6 Discussion

6.1 Gammarus pulex

Within one hour of exposure to water polluted with metals and persistent organic xenobiotics, *G. pulex* showed an early warning response consisting of less time spent on locomotion, fewer activity phases and decreasing number of "high swimming peaks" with increasing exposure time. In a previous experiment with similar design, except the use of smaller test chambers (2 x 1 x 1 cm^3), longer recording periods (280 s) and lower N (10, r = 2) of *G. pulex* from a winter population, the same results have been found. Or-

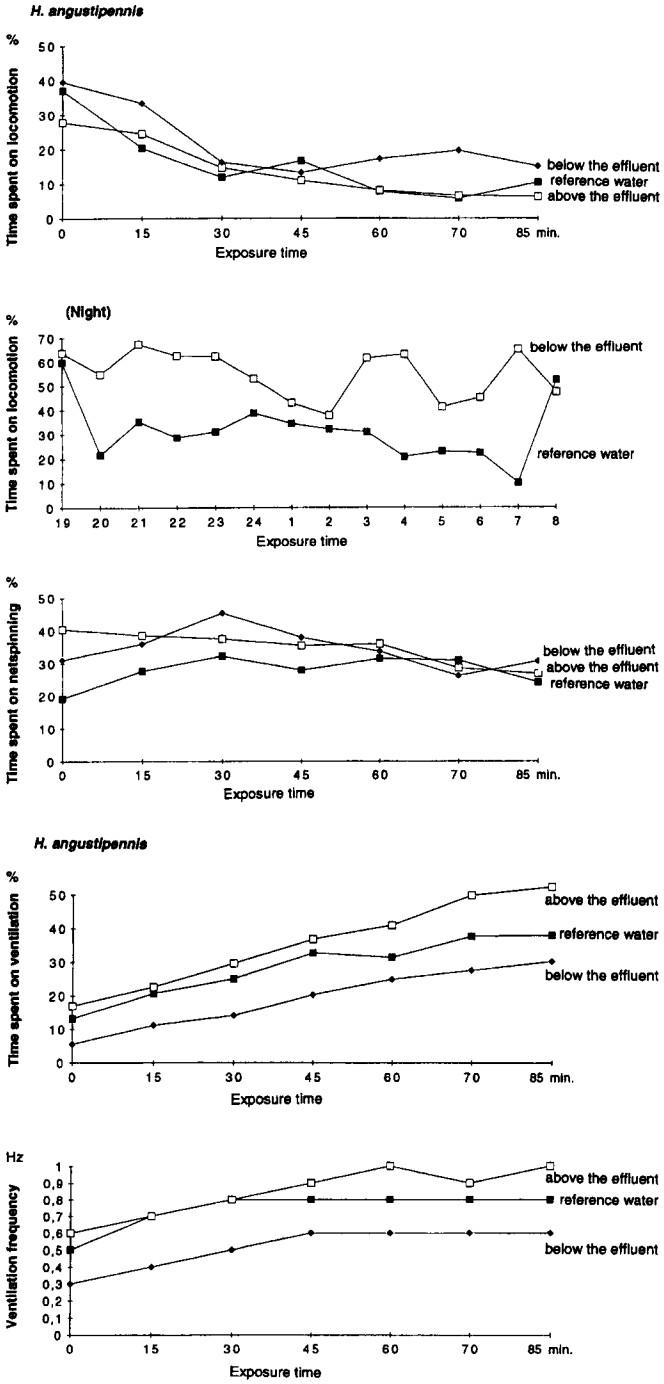

Fig. 5: Changes in different types of behaviour of *H. angustipennis* due to different water quality (reference, above, below effluent) and exposure time (85 min.):
a) Time spent on locomotion (day, night), b) Time spent on netspinning,c) Time spent on ventilation, d) Ventilation frequency

ganisms exposed for one hour to water from below the factory were less active and ventilated less with their pleopod gills than organisms exposed to reference water (GERHARDT unpubl.). Decreased locomotion, *i.e.* swimming, could result in increased downstream drift, away from the pollution source. This indicates *G. pulex* to be a sensitive, fast responding bioindicator in summer as well as in winter and behavioural parameters to be appropriate, reliable and reproducable biomarkers for aquatic pollution even though locomotory activity of *G. pulex* varies with season; *e.g.* in summer, there is an additional daytime activity peak of the mainly nocturnal species and a maximum of upstream movements (HULTIN 1971). *G. pulex* has earlier been shown to be a sensitive bioindicator with fast response times and low thresholds (60 min., ≥ 0.01 mg Pb/l: decreased locomotion; 30 min: ≤ 0.05 mg Cu/l: decreased locomotion, GERHARDT 1995b).

Feeding rate of *G. pulex* has been one of the most studied behaviours in ecotoxicology, however with contradictory results, *e.g.* decreased feeding rates due to metalliferous effluents (MALTBY & CRANE 1994) or ≥ 4 µg/l carbofuran (MATTHIESSEN *et al.* 1995), whereas no reliable responses were seen during exposure to Malathion 60 (CRANE *et al.* 1995) and different types of industrial effluents (CRANE & MALTBY 1991).

G. pulex has been shown to be a sensitive bioindicator with fast response times and low thresholds (permethrin (30 min: ≥ 0.5 mg/l, MUIRHEAD-THOMSON 1978) and oil pollution (≤ 200 mg/l: AUNAAS *et al.* 1991; UDALOVA *et al.* 1990). BORLAKAGLU & KICKUTH (1990) found that *G. pulex* reacted within 4 h to exposure to 1/20 of LC_{50} of chlorophenol with decreased movements. An escape behaviour was not observed by these authors, indicating a toxic effect of the phenolic compound on *G. pulex*, probably of membrane function (BORLAKAGLU & KICKUTH 1990).

Locomotory activity is a sensitive parameter in other freshwater species, *e.g. Daphnia pulex* was found to show erratic swimming ("escape") when being exposed to ≥ 1 µg/l carbaryl (DODSON *et al.* 1995). Reduced time spent on swimming of tadpoles of *Rana esculenta* was found after exposure to triphenyltin (SEMLITSCH *et al.* 1995). The tadpoles compensated the decreased locomotion with increased feeding at low exposure concentrations (0.5 µg/l), which however failed at high exposure concentrations (20 µg/l) due to physiological effects (SEMLITSCH *et al.* 1995). This indicates the advantage of monitoring different kinds of behavioural responses simultaneously.

After 24 h of exposure *in situ* to polluted water below the factory, survival of *G. pulex* was dramatically affected and increased again with increasing distance from the factory, probably because the toxicants were diluted, precipitated or adsorbed to the sediment or detoxified. At 500 m and 1000 m below the factory the same behavioural alterations as in the shortterm exposure in the laboratory could be seen. The observed deviations from normal behaviour after 1 h of exposure may be of compensatory nature with little overt disability. After 24 h of exposure, however, compensatory responses fail with the result of increasing disability and death (DEPLEDGE 1989). This supports the results from the laboratory bioassay to be early warning responses to the toxicants in the effluent. *In situ* bioassays have been proven more ecologically relevant and sensitive, *e.g.* in finding population specific differences in behavioural responses than laboratory bioassays (MATLBY & CRANE 1994).

6.2 *Hydropsyche angustipennis*

This species reacted with increased locomotion to the pollution, however only during the night. This is reasonable as the older larval stages of *H. angustipennis* are predators and invertebrate predators are mostly nocturnal to avoid predation by fish. During the day, the larvae, living sheltered in their case made of stones and detritus, may have a strategy of "waiting" until the pollution has passed by, whereas during the night, their soft body parts are exposed to the pollution when they are hunting on the streambed. This increased exposure to pollutants may result in increased locomotion, a kind of "escape" behaviour. Metal contamination has been shown to change the competitive equilibrium between species in a guild. *e.g.* elimination of a keystone species. *H. pellucidula*, released the more resistant species from competitive pressure (PETERSEN 1986). *H. contubernalis* and *H. siltalai* exposed to Cd spent less time on fighting (intraspecific competition), probably in order to save energy for detoxification processes (VUORI 1994).

Another early warning response of *H. angustipennis* was decreased ventilation. This corresponds to earlier findings with *G. pulex* exposed to the same test water. *H. angustipennis* living "hemisessil" and producing a current through its case by undulation with the abdomen (ventilation) might try to avoid the toxicants and "wait" until they passed by, a response which has been discussed for other sessile organisms such as *Dreissena polymorpha* (BORCHERDING & VOLPERS, 1993; STUIJFZAND *et al.* 1995), the marine polychaeta *Nereis virens* after exposure to Hg (≥ 30 µg/l) (MIRON *et al.* 1994) or the filter feeding chironomid *Glyptotendipes pallens* exposed to Cd (≥ 1/100 of LC_{50}-144h) (HEINIS *et al.* 1990).

7 Conclusions

1. Alarm responses of aquatic organisms to complex effluents are species dependent due to different exposures in different microhabitats, way of living, *e.g.* sessil, hemisessil, free and functional feeding groups, *e.g.* predators, filter feeders, shredders and life history strategies.
2. Different species may react to different toxicants in a complex effluent; *e.g.* membrane toxicants, such as metal ions, should affect especially species with intense gill surfaces and soft body parts, such as small, frequently molting free-living insects, compared to crustaceans with their calcified bodies, or coleopterans with their sclerotised bodies.
   Therefore, different test species have to be used simultaneously in monitoring pollution effluents for a reliable risk assessment of ecosystem health.

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Monitoring Behavioural Responses to Metals in Gammarus pulex (L.) (Crustacea) with Impedance Conversion

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Abstract

An impedance conversion technique was used to study the behaviour of *Gammarus pulex* (L.) exposed to acutely toxic concentrations of Pb (0.01, 0.05, 0.1 and 0.5 mg Pb l^{-1}) and to field concentrations of Cu (≤ 0.05 mg Cu l^{-1}). Initial stress responses were studied during short-term exposure (1 h), and sublethal toxic effects were monitored during 7 (Pb) and 35 days (Cu), respectively.

Exposure to Pb caused 30 % mortality and resulted in a bioconcentration factor (BCF) of 2700 at 0.5 mg Pb l^{-1} after 168 h. Exposure to Cu polluted stream water caused no mortality within 35 days, and uptake was low (BCF 5.8).

Gammarus pulex reacted with initial stress responses to metal exposure within 30 min (Cu) or 1 h (Pb). The reactions consisted of increased ventilation and decreased locomotion.

Sublethal concentrations of Pb and Cu caused toxic effects on the behaviour of G. *pulex* after several days of exposure, consisting of increased ventilation and decreased locomotion.

Impedance conversion is an appropriate method for detecting stress responses to metals and can be used in "early warning" biomonitoring systems as well as for acute and chronic behavioural toxicity testing.