Current Trends in
Ecology

Original Communication

GamTox© *in situ* test for monitoring streams below waste water treatment plants

A. Gerhardt[1], **K. Guthruf**[2], **K. Osswald**[1,3] **and B. Kuch**[4]

[1]LimCo International GmbH, Konstanz, Germany. [2]Amt für Wasser & Abfall Kanton Bern, Bern, Switzerland. [3]Universität Konstanz, Limnologisches Institut, Konstanz, Germany. [4]Universität Stuttgart, ISWA, Stuttgart, Germany

ABSTRACT

The EU Waterframework Directive aims to achieve a good ecological and chemical status of European surface waters until 2015 and includes monitoring and measures to maintain and improve water quality towards this ambitious aim. Uptil now, water authorities are not obligated to apply *in situ* ecotoxicity tests for risk assessment. GamTox *in situ* test was validated to detect and monitor the toxic potential of effluents from waste water treatment plants in two streams, with differences in land use, composition of micro-pollutants and with different species of gammarids as indicators. In both case studies (4 weeks) gammarids reacted already within 1-2 weeks with reduced survival and feeding when exposed in cages below the waste water effluent compared to upstream exposure. Survival appeared to be the more reliable endpoint, as feeding data were variable and gammarids might switch to other food sources which might not directly be related to a toxic effect but to differences in food supply at the exposure location. GamTox has shown potential to be applied for monitoring ecotoxicological water quality below waste water treatment plants.

KEYWORDS: micropollutants, *Gammarus*, waste water

INTRODUCTION

GamTox field test has been developed and successfully applied for monitoring toxic pulses of nutrients and pesticides in small streams in agricultural areas in Switzerland (InterReg IV A project 227) [1]. The test represents an active monitoring method: living gammarids in flow through acrylic glass tubes sealed with nylon net containing screw rings on both sides are placed in the stream with one elder leaf added as substrate and food source. The test has been operated up to 8 weeks with weekly visual controls, in order to gather both acute pollution pulses as well as chronic effects of long term exposure to low doses of chemicals. The aim of GamTox is to provide an easy, cost-effective ecotoxicological assessment and monitoring tool on the species and population levels, i.e. to help reaching the protection goals of European environmental law for water quality in surface waters.

Even though the test can be performed with different detritivorous invertebrates gammarids represent optimal test species as they are widely distributed in the Northern hemisphere, sensitive towards many pollutants, saproby class II indicators, dominant and abundant key-species in small stream ecosystems [2, 3].

Micropollutants are synthetic organic chemicals occurring in concentrations below µg/l [4] in surface waters. Main sources of micropollutants are pharmaceuticals, pesticides, biocides, endocrine disruptors, cosmetics and personal care products, household cleaners, etc. [5]. Next to several laboratory studies proving the toxicity of low doses of single micropollutants on selected aquatic test species effects on natural communities in the field have been demonstrated, *e.g.* for pesticides [6].

Up to now micropollutants cannot completely be eliminated in municipal waste water treatment plants (WWTPs) due to their low biological degradability and high persistence. Therefore several additional treatment steps such as ozonation, active coal filtration, membrane filtration, etc. are under discussion [7] and being tested compared to “natural” methods such as wetlands and the combination of secondary treatment with sand filtration [8, 9].

Both the Swiss environmental law and ordinance (GSchG, GSchV) and the EU Water Framework Directive (WFD, 2000/60/EG) demand the sustainable protection of aquatic biocoenoses and measures such as monitoring in order to achieve and maintain a good ecological and chemical water quality. In this context point pollution sources need to be monitored and reduced in the future. 33 priority substances from different substance classes have been selected for chemical monitoring. Several research projects such as RISKWa (Risk management of emerging compounds and pathogens in the water cycle; www.riskwa.de) have been initiated in Germany to develop new methods and concepts to improve water quality. Online biomonitoring of effluents from point pollution sources such as WWTPs might be a good automated and real-time based monitoring measure to be implemented in the EU Water Framework Directive. The Multispecies Freshwater Biomonitor© (MFB) [10, 11] records the vitality and behaviour of all kinds of invertebrate and fish species in unfiltered raw water, humid soil and sediment. The MFB is currently being used with gammarids (GamTox online) in a worldwide unique pilot project in a WWTP in Switzerland [12]. However, the responsibility for surface water quality monitoring in the receiving streams cannot be shouldered by the point polluters alone. Therefore, additional offline GamTox *in situ* tests provide an excellent cost-effective complement to monitor biological-ecotoxicological water quality in the receiving water bodies above and below point discharges.

HYPOTHESIS

GamTox sensitively detects effects of micropollutant mixtures below two waste water treatment plants in small receiving streams.

MATERIAL AND METHODS

Sites

Two small streams have been chosen with upstream sites where gammarids were collected for the exposures and downstream sites a few hundred meters below the effluents of the respective WWTPs. Transplantation experiments with gammarid species from the corresponding upstream locations were performed with healthy un-parasitized animals both above and below the WWTPs.

Site 1: WWPT Holzmühle along the stream Urtenen (Canton Bern, Switzerland) (Swiss coordinates: WWPT effluent: 607’658 / 211’743 = 7° 32’ 22’’ / 47° 03’ 24’’)

About 600 m upstream of the WWTP *G. fossarum* was collected in high numbers in a small tributary, Stepbach, and exposed both above and below the WWPT for a period of 4 weeks. Weekly cumulative water samples from autosamplers were analysed for pesticides by the Canton Bern. Both sites were only about 550 m apart from each other, being very similar concerning flow (ca. 0.1 cm/s), pH (7.7 - 8.4), oxygen saturation (> 90%), temperature (17 - 21°C) and had a low degree of shade. The stream section flows through agricultural area and has been ecologically improved by construction of meanders and plantation of aquatic macrophytes and bank vegetation. Regarding nutrient levels, Nitrate-N values were a bit higher below the WWTP than above (4 - 6.9 mg/L versus 3 - 4 mg/L), total Phosphor reached up to max 2.5 mg/L below the WWTP; DOC values were similar at both sites (around 4 mg/L) as well as Ammonium-N with a maximum of 0.1 (above) resp. 0.3 µg/l (below).

Site 2: WWTP University of Stuttgart, Bandtälesbrook (Germany) (48° 44’ 54’’ N / 9° 5’ 25’’ E)

The Bandtälesbrook flows through a small deciduous forest, a site ca. 300 m above and another site about 80 m below the effluent of the WWTP LFKW Stuttgart Büsnau (Lehr- und Forschungsklärwerk der Universität Stuttgart, Stuttgart, Germany) were selected for the study. *Gammarus pulex* from upstream was exposed both upstream and downstream of the WWTP effluent. At both sites gammarids occur naturally.

The following chemical parameters were analysed in the stream water above and below the WWTP each week: CSB (< 15 - 19 mg/L), Nitrite-N (< 0.02 mg/L), Nitrate-N (< 1mg/L), total Phosphor (< 0.1 mg/L).

GamTox test description

The test was performed as described in [1]. Only healthy animals were selected (> 7 mm), males and females randomly mixed. Ten animals were placed in a transparent plexiglass test tube (15 cm long, 5 cm diameter) together with one pre-conditioned alder leaf and closed with screw lids containing nylon gauze (0.5 mm mesh size) on both ends, thus the tubes were similar to the test chambers of the Multispecies Freshwater Biomonitor© [12]. Five replicate tubes were exposed and fixed with ropes in a plastic basket, attached on the stream bed with steel poles and additionally fastened on the banks with ropes. In weekly intervals each tube was controlled, by counting the animals, removing dead ones and estimating the surface of the leaf that has been skeletized by the gammarids in several classes (0, < 25%, < 50%, < 75%, < 100%). Photographs of the leaves were taken, too. Afterwards, the living animals were set back in the tube and a fresh leaf provided. The duration of the experiment was 4 weeks.

Statistical analysis

The variables survival (absolute numbers) and shredding activity (= feeding) (upper class limit of the %-classes) were analysed with non-parametric signed rank sum test (Wilcoxon) for two samples using SigmaStat/Plot 12.0 for each stream. As the 5 replicate tubes were individually marked, they were treated individually in the statistics, too.

RESULTS

Pesticide analysis

Urtenen

The regular pesticide analysis of the water in the Urtenen collected from autosamplers throughout the whole exposure period provided a large data set, which has been condensed in Table 1, by taking maximal observed values of specific substances from the 4 weekly cumulative samples. Whereas most values were in the ng/l range and well below acute toxicity (> 1000) as reported in the literature, the neurotoxic substances Diazinon and Pirimicarb reached maximal values of <= 1000 x below acute toxicity levels reported for crustaceans in the literature (www.pesticideinfo.org). Considering the RAC (Regulatory Acceptable Concentrations) concept where a safety factor of 100 is applied to EC50-data, none of the substances except Diazinon, would be regarded as relevant [13]. The same authors also found out, that especially for neurotoxic neonicotinoid insecticides and insect growth regulators *Gammarus pulex* was more sensitive, *i.e.* protective than *Daphnia magna*. This shows that Diazinon is a serious stressor for gammarids in streams receiving this insecticide from both point and diffuse sources. The high toxicity of this group of neutrotoxic insecticides is supported in the literature, *e.g.* the LC50-96 h of Deltamethrin (neonicotinoid) was as low as 4.0 ng/l (*G. fossarum*) and 5.7 ng/l (*G. pulex*) testing juveniles, whereas adults were about 10 times less sensitive [14]. These values are reached below the WWTP. The toxic units (TU: concentration/LC50) TUs for Diazinon and Pirimicarb were 1 x 10^{-1} and 4 x 10^{-4} respectively, TU values with low exponents represent high toxicity. Carbamazepin reached a TU of 1 x 10^{-3} and Azoxystrobin a TU of 1.6 x 10^{-4}. Unfortunately for many chemicals no toxicity data as basis for TU calculations are available. Therefore, we can only say that the substances with a TU of an exponent below $^{-3}$ might contribute to the overall acute toxicity as based on literature data regarding acute mortality of Crustaceans. However, even the sum of chemicals in lower doses, which were somewhat higher below the WWTP compared to above, *e.g.* some pharmaceuticals and the pesticides Atrazine and Diuron, Terbutylazin and Metolachlor might also be of concern.

Bandtälesbrook

In this stream spot water samples were taken each week of exposure both above (A1, A2, A3, A4) and below (B2, B3, B4) the WWTP and chemical substances were analysed at ISWA (University of Stuttgart), selected according to local relevance (Table 2). Based on the few available toxicity data and the anticipated threshold of TU exponent “-3” to exert toxic effects, Benzophenon (TU 1 x 10^{-2})

Table 1. Chemical analysis of cumulative water samples in the Urtenen above (A) and below (B) the WWTP in different weeks of exposure (1-4), < DL: below detection limit (0.01 µg/l), nd: not determined.

Substances (µg/l)	**A1**	**A2**	**B2**	**A3**	**B3**	**A4**	**B4**
Pharmaceuticals							
Atenolol	< DL	< DL	0.288	< DL	0.224	< DL	0.174
Sulfamethoxazole	< DL	< DL	0.048	< DL	0.015	< DL	0.034
Metoprolol	< DL	< DL	0.209	< DL	0.154	< DL	0.139
Propranolol	< DL	< DL	0.056	< DL	0.039	< DL	0.034
Cotinine (nicotine metabolite)	0.012	0.026	0.026	0.045	0.076	0.024	0.024
Carbamazepine	< DL	< DL	0.219	< DL	0.154	< DL	0.173
Caffeine	< DL	0.136	0.034	0.334	0.804	0.148	0.056
Corrosion inhibitor							
Benzotriazole	0.024	0.088	nd	0.09	nd	0.088	nd
Pesticides							
2,6-Dichlorobenzamide	0.015	0.014	0.012	0.015	0.016	0.014	0.013
Metamitron	< DL	< DL	0.055	< DL	0.205	< DL	0.045
Chloridazon	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Carbendazim	< DL	< DL	0.023	< DL	0.045	< DL	0.016
Simazine	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Pirimicarb	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Atrazine	0.012	0.013	0.46	0.017	0.087	0.015	0.029
Diuron	< DL	< DL	0.05	0.011	0.205	< DL	0.059
Azoxystrobin	< DL	< DL	< DL	< DL	0.016	< DL	0.012
Terbuthylazine	< DL	< DL	0.038	0.027	0.1	0.016	0.028
Terbutryn	< DL	< DL	< DL	< DL	0.017	< DL	0.163
Metolachlor	< DL	0.012	0.034	0.026	0.147	0.016	0.036
Diazinon	< DL	< DL	0.041	< DL	0.124	< DL	0.022

was the most toxic substance, recorded in much higher levels below the WWTP than above. Benzophenon is a widely used substance in fragrances, plastics, in UV screens, pharmaceuticals and pesticides, aquatic ecotoxicity (LC50-24 h) for neonate *Daphnia magna* reported as 280 µg/L (www.pesticideinfo.org). According to the RAC concept (LC50/100) the sample below the WWTP reached toxic values. Moreover, the endocrine disruptor Bisphenol A was recorded up to 1 µg/l below the WWTP, values which are in the range of observed chronic toxic effects in brown trout (*Salmo trutta fario*: 1.75 - 2.4 µg/l reduced sperm vitality and delayed ovulation [15], snails *Marisa cornuarietis*: 0.1 - 1 µg/l caused oviduct malformations [16] and acute effects in insects *Chironomus riparius*: > 0.01 µg/l induced mouthpart deformations already after 2 days [17]). Considering this extremely low threshold value of acute effects, effects of BPA might occur at both sites.

Moreover, the organic phosphor compound Tris-Phosphor, Diclofenac and ONTE were measured in higher levels below the WWTP than above. Compared to the analysis in the Urtenen, the

Table 2. Chemical analysis of cumulative water samples in the Bandtälesbrook above (A) and below (B) the WWTP in different weeks of exposure (1-4). < DL: below detection limit.

Substances, µg/L	**A1**	**A2**	**B2**	**A3**	**B3**	**A4**	**B4**	**DL**
Pharmaceuticals								
Diclofenac	< DL	< DL	0.763	< DL	0.538	< DL	1.481	< 0.01
Carbamazepin	< DL	< DL	0.648	< DL	0.396	< DL	0.600	< 0.01
Mirtazapin	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< 0.01
Lidocain	< DL	< DL	0.121	< DL	0.083	< DL	0.063	< 0.01
Diphenhydramin	< DL	< DL	0.006	< DL	0.004	< DL	0.001	< 0.01
Tramadol	< DL	< DL	0.191	< DL	0.137	< DL	0.080	< 0.01
Synthetic fragrances								
HHCB	0.009	0.009	1.616	0.008	1.310	0.011	0.802	< 0.002
HHCB-Lacton	0.063	0.023	2.315	0.028	1.731	0.088	2.694	< 0.002
AHTN	0.005	0.004	0.257	0.004	0.195	0.007	0.158	< 0.002
OTNE	< DL	< DL	6.090	< DL	4.851	< DL	4.145	< 0.010
Methyldihydrojasmonat	0.196	0.219	0.841	0.176	0.327	0.196	0.243	< 0.010
N,N'-Diethyltoluamid	0.033	0.032	1.078	0.035	0.686	0.057	0.943	< 0.005
Benzothiazol	0.400	0.328	1.254	0.524	0.888	1.094	2.160	< 0.010
Methylthiobenzothiazol	0.145	0.173	2.943	0.225	2.000	0.354	1.807	< 0.010
Organic phosphor compounds								
Tris-(butoxyethoxy)-phosphat	22.186	12.915	34.010	19.716	11.659	11.803	42.416	< 0.01
Triphenylphosphat	0.038	0.015	0.153	0.018	0.068	0.026	0.055	< 0.005
Tris-(chlorethyl)-phosphat	0.151	0.143	0.692	0.200	0.566	0.150	1.837	< 0.005
Tris-(chlorpropyl)-phosphat	0.065	0.042	1.338	0.064	1.030	0.108	1.190	< 0.005
Tris-(dichlorpropyl)-phosphat	0.018	0.010	0.190	0.010	0.216	0.016	0.155	< 0.005
Triphenylphosphinoxid	0.026	0.015	0.227	0.018	0.120	0.019	0.276	< 0.005
2-Hydroxybiphenyl	0.012	0.010	0.105	0.012	0.074	0.021	0.079	< 0.005
Bisphenol A	0.046	0.068	1.364	0.083	0.748	0.010	0.254	< 0.01
4-t-Octylphenol	0.014	0.025	0.373	0.024	0.288	0.015	0.089	< 0.005
4-Nonylphenole	0.078	0.105	0.832	0.174	0.585	0.174	0.622	< 0.005
Butylhydroxyanisol	0.035	0.019	1.019	0.064	0.949	0.062	0.688	< 0.005
Butylhydroxytoluol	0.012	0.004	0.162	0.018	0.145	0.009	0.173	< 0.005
Benzophenon	0.038	0.034	3.342	0.023	1.559	0.089	1.417	< 0.005
Octocrlyen	3.683	1.434	4.054	1.569	2.002	1.110	1.920	< 0.05
Terbutryn	0.066	0.003	0.030	0.003	0.039	< DL	0.087	< 0.002

values of micropollutants are generally higher at Büsnau. This might be partly related to the fact that these were spot samples, *i.e.* more variable in the course of time due to the influence of episodic pollution. It has to be noted, that in Urtenen some potential toxic substances such as BPA have not been measured as here the focus was on pesticides. Below both WWTPs both pharmaceuticals and

Nitrate were found in higher levels compared to the respective upstreams sites. Whereas in the Urtenen Diazinon plays an important role as chemical stressor, in Bandtälesbrook especially Benzophenon and BPA might contribute most to ecotoxicological effects.

Survival and feeding

Gammarids survived > 60 % at all sites up to 4 weeks of exposure, proving the test to be sufficiently robust and reliable also in polluted areas and able to show both acute and chronic effects. In the Urtenen, survival below the WWTP was significantly lower compared to upstream, starting already in the 2^{nd} week of exposure ($p < 0.001$) (Fig. 1a), thus showing (sub)acute effects. In this case we define acute effects to occur within 4 days (EC-96 h). Feeding activity (shredding % of leaf surface) did not differ significantly due to high variation, however there was a trend towards less feeding below the WWTP (Fig. 1b). In Bandtälesbrook survival of the gammarids did not differ significantly when exposed above and below the WWTP ($p = 0.08$) due to high variability, however a clear trend of less survival below the WWTP can be stated (Fig. 1c). Already after 1 week of exposure feeding/ shredding was significantly reduced below the WWTP ($p < 0.03$) (Fig. 1d), indicating (sub)acute effects.

DISCUSSION

Generally the pollution by micropollutants appeared to be lower in Urtenen than in Bandtälesbrook, however different substances were recorded according to their local relevance. Whereas in Urtenen the neurotoxic insecticide Diazinon was the dominant pesticide, in Bandtälesbrook next to the suspected endocrine disruptors Benzophenon and BPA also synthetic fragrances were important as potential toxicants for (sub)acute effects on survival.

Nitrate and pharmaceuticals might also affect gammarids in both streams at sites below the WWTPs. Liess *et al.* [18] found that the population density of gammarids decreased in a mixture of 3 mg NH_4/l, 0.9 mg NO_2/l and 18.5 mg NO_3/l. On the other hand, Stelzer & Joachim [19] did not find any significant differences in survival of *G. pseudolimnaeus* at concentrations up to 128 mg NO_3/l, however a linear regression showed a trend to slower growth at higher concentration levels. These authors stress that further studies are needed before a clear answer can be provided on the toxicity of Nitrate towards gammarids.

In Bandtälesbrook gammarids generally showed less shredding activity compared to the animals exposed in the Urtenen. Whereas shredding activity in the Urtenen agreed with earlier findings from similar exposures [1], feeding activity in Bandtälesbrook was extremely low. This might be due to seasonal effects, as the study in Bandtälesbrook was performed in early spring while the study in Urtenen was performed in late spring. Below the WWTP there was less feeding activity compared to above, which might be due to (1) toxic effects or (2) change in food source, *i.e.* gammarids might have switched to small chironomids as food source, which occur in high densities below the WWTP and entered the cages in spite of small mesh size at the site below the WWTP in Bandtälesbrook. Chironomids represent a qualitatively higher (protein content) food source and are taken by gammarids held in aquaria, too. However, gammarids do not grow better when fed on chironomids compared to alder leaves in the laboratory (unpublished data). In order to get insight in this hypothesis of food source shift, naturally occurring gammarids from above and below the WWTP were collected and their lipid content was determined. The population below the WWTP contained 4 times as much lipids as the population above the WWTP, indicating another preference in food selection. Below WWTPs chironomids are occurring in large abundances, hence represent both an easy and energy-rich food source. Such ecological factors might affect *in situ* feeding experiments as so-called "indirect effect". However, in this study both feeding rate and survival were affected by the WWTP effluent, hence strongly indicating these effects to be direct toxic effects. Maltby *et al.* [20] also reported decreases in feeding rate in *G. pulex* by up to 99 % exposed below point polluters. In both streams sustainable natural populations of gammarids were found below the WWTPs. In the Bandtälesbrook gammarids differed in both physiology (lipid content) and feeding behaviour from the population upstream the WWTP, however

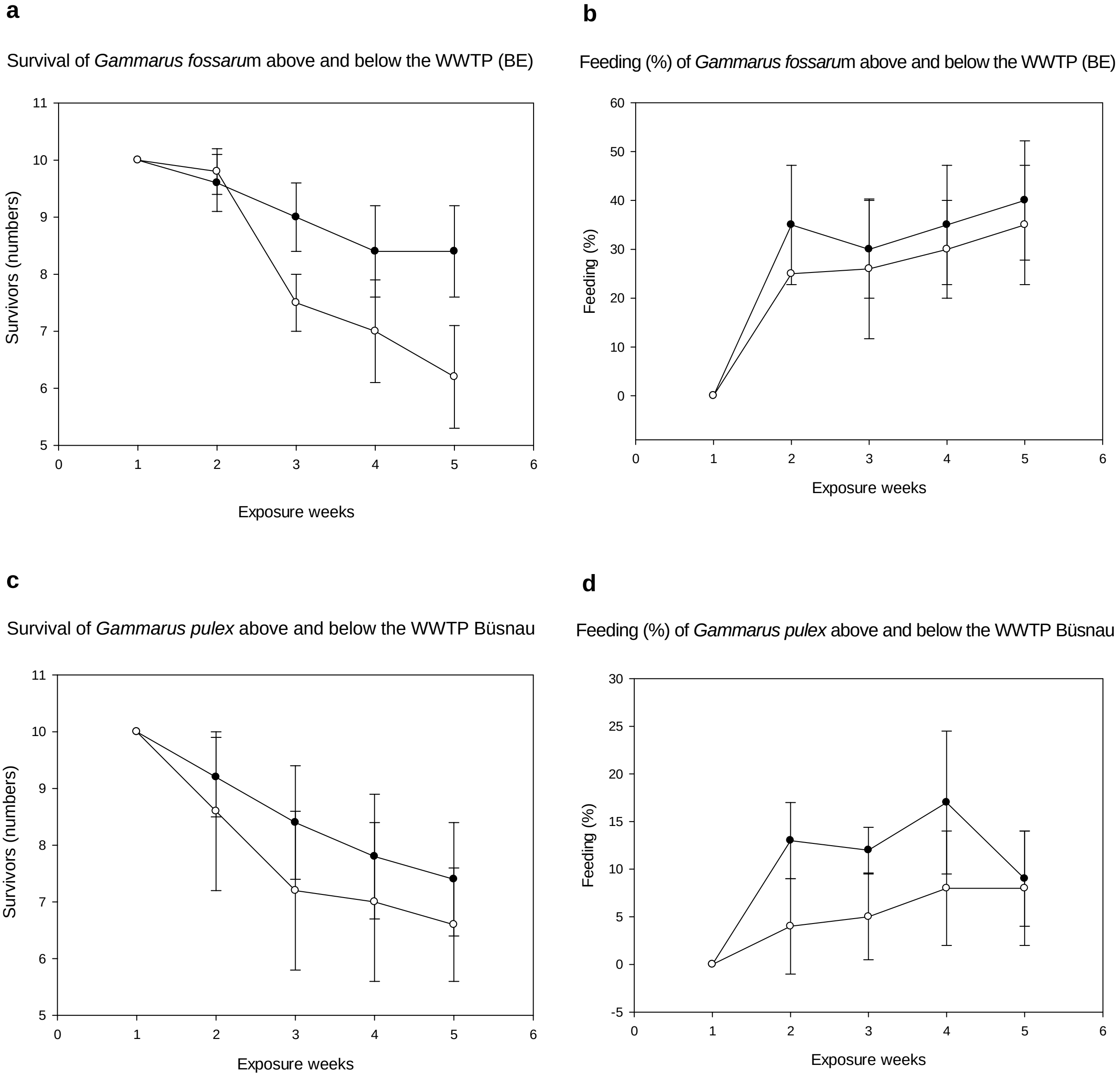

Fig. 1. Survival and feeding of gammarids above (●) and below (○) the WWTPs in Switzerland (BE) (1a and 1b) and Germany (Büsnau) (1c and 1d).

severe lethal effects of the chemicals in the WWTP effluent did not occur during the exposure period of 4 weeks. This indicates that the effluent did not contain any acutely toxic substances. As Benzophenon and BPA might be the most important toxicants during the exposure, being suspected endocrine disruptors, toxic effects related to survival and reproduction, e.g. population relevant effects, might occur only after even longer exposure.

In the Urtenen (sub)acute toxic effects of mainly the neurotoxic pesticides Diazinon and Pirimicarb on both survival and feeding were found, which supports the high sensitivity of gammarids towards neurotoxic substances [14, 2, 13].

Other field exposure studies with gammarids above and below WWTPs are rare. Englert *et al.* [21] found during laboratory and *in situ* exposures of several weeks duration that *G. fossarum* displayed decreases in feeding rate in waste water compared to stream water. This corresponded to reduced shredder abundances below WWTP. Ongoing studies seem to prove that feeding rate of *G. fossarum* was reduced in waste water, whereas it was high in wastewater treated by ozonation: comparing wastewater with low toxicant loads with and without ozonation, no differences in feeding rates were found [22]. Schirling *et al.* [23] studied *Gammarus fossarum* populations in a stream at both above and below wastewater discharges, finding effects on gonad maturity (larger late vitellogenic oocytes) and decreasing hsp90 levels, which indicated increased effects of endocrine active substances below the WWTP. Field biomonitoring studies revealed changes in macroinvertebrate species composition below WWTP effluents, *i.e.* replacement of sensitive (*e.g.* Ephemeroptera) by tolerant (*e.g.* Oligochaeta) species [24]. Caged *Gammarus fossarum* showed increased genotoxic effects when exposed below the WWTP effluents of three WWTPs in France [25]. Bloor & Banks [26] studied survival and feeding rate of *G. pulex* in mixed exposures together with *Asellus aquaticus* in both *in* and *ex situ* experiments considering pollution by landfill leakage. Both, survival and feeding showed a similar trend in both *in* and *ex situ* experiments, however higher responses were found in the *in situ* tests. This indicates that *in situ* tests are very valuable for the evaluation of point discharges in the field.

Although in the USA WET-testing (whole effluent testing) is a permit requirement, consisting of both *in vitro* tests and *ex vivo* tests, ecotoxicological WET testing in Europe is still evolving [27], whereby the emphasis lies on biochemical measures and *in vitro* receptor assays to rapidly detect bioavailable concentrations of mainly low-dose chemicals such as endocrine disruptors, exerting toxic effects at concentrations below analytical detection limits [28]. Although these "bioanalytical" tools are important, we still need *in vivo* ecotoxicity testing *in situ* in order to evaluate the ecological effects on species, population and community level, these levels being the basis for the protection goals and biodiversity strategies formulated by European environmental law.

CONCLUSIONS

In two different streams we could show that WWTP effluents negatively affect gammarids (survival and feeding rates) during 4 weeks of exposure with effects starting to be visible after 1-2 weeks already. Neurotoxic insecticides, endocrine disruptors, pharmaceuticals and synthetic fragrances contribute to these effects. As gammarids are important key species of stream ecosystems, WWTP effluents should be monitored and GamTox might be an ecologically relevant and efficient tool.

At some point pollution sources toxicants might need to be reduced in future in order to achieve the goals of the EU Water Framework Directive and the corresponding Swiss environmental laws to sustainably protect natural stream biota.

ACKNOWLEDGEMENTS

Support during the weekly controls was provided by S. Guthruf, A. Giovanoli, B. Popic, P. Espinoza, V. Maurer, M. Zeh, and U. Ochsenbein at the study sites in Canton Bern. The study was co-financed by the Canton Bern and the EU InterReg IV A project no. 227, funded by European Fund for Regional development (EFRE) and Swiss cantonal fund.

REFERENCES

1. Gerhardt, A., Koster, M., Lang, F. and Leib, V. 2012a, Journal of Environmental Protection, 3, 573.
2. Gerhardt, A. 2010, *Gammarus* spp.: Rückgang oder Fehlen von Bachflohkrebsen in Bächen: Literaturstudie. AquaPlus (Ed.) im Auftrag von Dept. Bau, Verkehr und Umwelt, Abt. f. Umwelt, Kanton Aargau, Schweiz.
3. Gerhardt, A. 2011, Development of a low-cost ecotoxicity test with *Gammarus* spp. for *in* and *ex situ* application. (In: Gerhardt, A., Bloor, M. and Lloyd-Mills C. (Eds.): *Gammarus* Important Taxon in Freshwater and Marine Changing Environments. International Journal of Zoology, Special issue, http://www.hindawi.com/journals/ijz/osi.html
4. IKSR. 2010, Strategie Mikroverunreinigungen-Strategie für die Siedlungs- und Industrieabwässer. Intern. Kommission zum Schutz des Rheins (Ed.), Nr. 181.

5. DWA. 2008, Anthropogene Spurenstoffe im Wasserkreislauf. Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall (Ed.), DWA, Hennef, Druckcenter Meckenheim, pp. 83.
6. Liess, M. and von der Ohe, P. C. 2005, Environ. Toxicol. Chem., 21(2), 172.
7. LBW. 2010, Antrag und Stellungnahme zur Entfernung von Spurenstoffen im Abwasser, Landtag baden Württemberg (Ed.), Nr. 14/6229.
8. Baynes, L., Green, C, Nicol, E., Beresford, N., Kanda, R., Henshaw, A., Churchley, J and Jobling, S. 2012, Environm. Science and Technology, 46(10), 5565.
9. Van der Oost, A., Wiliams, T. W., Foekema, E. M., Murk, T., Roex, E. W. M., Chipman, J. K. and Smedes, F. 2012, Integrative monitoring of wetlands WWTP effluent remediation in the WIPE project. SETAC World Congress, 20-24 May 2012, Berlin, Abstract.
10. Gerhardt, A., Clostermann, M., Fridlund, B. and Svensson, E. 1994, Environment International, 20(2), 209.
11. Gerhardt, A., Carlsson, A., Ressemann, C. and Stich, K. P. 1998, Environmental Science & Technology, 32(1), 150.
12. Gerhardt, A., Osswald, K., Kuch, B., Hofer, M. and Bühler, C. 2012b, Umwelt Perspektiven, 5, 18.
13. Brock, T. C. and Wijngaarden, R. P. 2012, Env. Sci., Pollut. Res. Int., 19(8), 3610.
14. Adam, O., Degiorgi, F., Crini, G. and Badot, P. M. 2010, Ecotox. Env. Safety, 73(6), 1402.
15. Lahnsteiner F., Berger B., Kletzl M. and Weismann T. 2005, Aquat. Toxicol., 75, 213.
16. Oehlmann J., Schulte-Oehlmann U., Bachmann J., Oetken M., Lutz I., Kloas W. and Ternes T. A. 2006, Environ. Health Perspectives, 114 (Suppl. 1), 127.
17. Watts M. M., Pascoe D. and Carroll K. 2003, Ecotox. Environ. Safety, 54, 207.
18. Liess, M., Schulz, R., Berenzen, N., Nanko-Dress, J. and Wogram, J. 2001, Pflanzenschutzmittelbelastung und Lebensgemeinschaften in Fliessgewässern mit landwirtschafltich genutztem Umland. Umweltbundesamt Berlin, Nr. 65.
19. Stelzer, R. S. and Joachim, B. L. 2010, Arch. Env. Contam. Tox., 58(3), 694.
20. Maltby, L., Clayton, S. A., Wood, R. M. and Mc Loughlin, N. 2002, Environ. Toxicol. Chem., 21, 361.
21. Englert, D., Bundschuh, M., Zubrod, J. P. and Schulz, R. 2012, Implications of municipal wastewater on macroinvertebrate communities structure and leaf litter breakdown. SETAC World Congress, 20-24 May 2012, Berlin, Abstract.
22. Bundschuh, M. and Schulz, R. 2012, Are the concentrations of micropollutants responsible for the reduction in wastewater toxicity for gammarids following application of ozone? SETAC World Congress, 20-24 May 2012, Berlin, Abstract.
23. Schirling, M. 2005, Effekte von Xenohormonen auf die limnischen Invertrabraten *Gammarus fossarum* (Crustacea, Amphipoda) und *Marisa cornuarietis* (Mollusca, Prosobranchia). Disssertation, Universität Tübingen, pp. 117.
24. Bernhard, S. 2010, Einfluss von Arzneistoffen auf aquatische Invertebraten. Dissertation Uni München (LMUM), Kap. 4 and 5.
25. Lacaze, E., Devaux, A., Mons, R., Bony, S., Garric, J., Geffard, A. and Geffard, O. 2011, Environmental Pollution, 159(6), 1682.
26. Bloor, M. and Banks, C. J. 2006, Environment International, 32(1), 22.
27. Marfil-Vega, R., Mills, M., Nakayama, S. F., Hornung, M. E., Gilbert, M. E., Crofton, K. E., Lazorchack, J., Paul, K., Tadele, K., Suzuki, G., Takigami, H. and Brouwer, B. 2012, Quantifying thyroid hormones and steroids in wastewater effluents by using a mode of action tool box, SETAC World Congress, 20-24 May 2012, Berlin, Abstract.
28. Scott, P. D., Coleman, H., Mcdonald, J., Lim, R., Nugegoda, D., Tremblay, L. A., Pettigrove, V., Chapman, H. F., Blackwell, S., Harper, B., Reitsema, T., Bartkow, M. and Leusch, F. D. 2012, Investigation of endocrine disruption in Australian environments. SETAC World Congress, 20-24 May 2012, Berlin, Abstract.