Ralf Bernhard Schäfer

Effects of toxicants on freshwater ecosystems

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Table of Contents

1. Introduction **5**

2. Publication I **13**
Aquatic passive sampling of a short-term thiacloprid pulse with the Chemcatcher: Impact of biofouling and use of a diffusion-limiting membrane on the sampling rate.

3. Publication II **21**
Using silicone passive samplers to detect polycyclic aromatic hydrocarbons from wildfires in streams and potential acute effects for invertebrate communities.

4. Publication III **33**
The footprint of pesticide stress in communities - species traits reveal community effects of toxicants.

5. Publication IV **41**
A trait database of stream invertebrates for the ecological risk assessment of single and combined effects of salinity and pesticides in South-East Australia.

6. Publication V **51**
Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: Effect concentrations and recovery dynamics.

7. Publication VI **65**
Effects of pesticides monitored with three sampling methods in 24 sites on macroinvertebrates and microorganisms.

8. Publication VII **75**
Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems.

9. Publication VIII **85**
Occurrence of 331 organic pollutants in four rivers of North Germany between 1994 and 2004 and risk assessment for algae, invertebrates and fish.

10. Publication IX **95**
A similarity-index based method to estimate chemical concentration limits protective for ecological communities.

11. Publication X **105**
Is there an interaction of the effects of salinity and pesticides on the community structure of macroinvertebrates?

12. Publication XI **113**
Biodiversity, Ecosystem functions and services in Environmental Risk Assessment: Introduction to the Special Issue.

13. Publication XII **117**
Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services.

14. Synoptic Discussion and Conclusions **129**

15. Summary **143**

16. References **145**

1. Introduction

Structure of this habilitation thesis and included publications

This cumulative habilitation thesis consists of several peer-reviewed journal articles that have been published within the last five years. The research questions of all publications revolve around the effects of toxicants on freshwater ecosystems. This first chapter outlines the research context of the individual publications. The following chapters feature the different publications arranged according to topics. Publications I and II primarily deal with the passive sampling of organic toxicants in a mesocosm and field study. Publications III to VII mainly focus on trait-based approaches for the detection of effects of toxicants, with publications III and IV including a discussion of the general framework and publications V to VII addressing the application in mesocosm and field studies. Statistical data analysis approaches to examine effects of toxicants in freshwater ecosystems are the subject of publications VIII to X. Publications XI and XII concentrate on the effects of toxicants on biodiversity, ecosystem functions and services. For a brief overview on the interrelation between the publications, I refer the reader to Figure 1. The publications are discussed synoptically and general conclusions are drawn in the chapter following the publications. In addition, avenues for future research are delineated. The final chapter briefly summarises this habilitation thesis.

Toxicants in freshwater ecosystems

Human societies are altering natural systems on a global scale (Imhoff et al., 2004; Rockstrom et al., 2009; Tollefson and Gilbert, 2012). If current trends prevail this may lead to major losses in biodiversity and ecosystem functions that are crucial for human societies(Cardinale et al., 2012; Hooper et al., 2012; MEA, 2005). Species in freshwater ecosystems are among those facing the highest extinction risks (Heino et al., 2009; MEA, 2005; Pereira et al., 2010; Revenga et al., 2005). There are a multitude of stressors contributing to the ecological deterioration of freshwater ecosystems including contamination by toxicants, eutrophication, input of organic matter and habitat degradation (Vorosmarty et al., 2010; Woodward et al., 2012). The main groups of toxicants mentioned in the Millenium Ecosystem Assessment encompass pesticides and

heavy metals (MEA, 2005). Moreover, salinisation is listed as a major factor altering freshwater quality. Thus, salts can also be considered as toxicants (Kefford et al., 2002).

Most of the knowledge on the effects of toxicants on freshwater ecosystems comes from laboratory studies or studies conducted in semi-natural experimental systems (hereafter: mesocosms) (Beketov and Liess, 2012; Mayer-Pinto et al., 2010). For example, a literature analysis reported that only 0.6% of studies on the effects of pesticides on freshwater invertebrates were done in the field (Beketov and Liess, 2012). This follows the paradigm that insights into causal relationships can only convincingly be gained from randomised experiments (Shipley, 2004), e.g. in laboratory or controlled field settings. But since laboratory and mesocosm systems may differ from natural systems regarding characteristics such as species composition and sensitivity (Beketov et al., 2008), availability of recolonisation pools (Sundermann et al., 2011), species interactions and co-occurring stressors (Liess and Beketov, 2011), field studies are crucial for the validation of insights gained from artificial systems (Carpenter, 1996; Mayer-Pinto et al., 2010). In contrast to experimental settings, the establishment of a causal link between toxicant exposure and observed ecological patterns in field studies is often aggravated by the occurrence of potentially collinear confounding factors (Liess et al., 2008). In addition, toxicants such as pesticides are difficult to monitor under field conditions because they typically occur episodically in freshwater ecosystems. The episodic occurrence is due to (1) specific application periods and (2) entry paths, which are partly associated with strong precipitation events (Guo et al., 2004; Kreuger, 1998; Leu et al., 2004). Since even short-term pesticide exposures of a few hours may cause adverse ecological effects (Andersen et al., 2006; Hose et al., 2003; Schulz and Liess, 2000), field studies aiming at a realistic ecological risk assessment of pesticides require sampling methods that are suitable to capture short-term pulses (Mortimer et al., 2007).

Time-integrative passive sampling of toxicants

Passive samplers can be defined as devices with a receiving phase that passively accumulates substances from the sampled medium via free diffusion (International Organization for Standardization, 2011). Advantages of passive sampling over active sampling can include lower limit of detection, less matrix interference in chemical analysis and lower costs (Miege et al., 2012; Morin et al., 2012; Zabiegała et al., 2010). Disadvantages can include more intensive sample processing and the necessity of

laboratory studies to determine sampling rates in the case of continuous monitoring (Miege et al., 2012; Morin et al., 2012; Zabiegała et al., 2010). In the last two decades, several passive sampling techniques have been developed for the continuous (i.e. time-integrative) monitoring of chemicals over time frames from days to months (see reviews by Kot et al., 2000; Kot-Wasik et al., 2007; Seethapathy et al., 2007; Stuer-Lauridsen, 2005; Vrana et al., 2005). Recent studies have shown that integrative passive samplers are also suitable to detect short-term pulses of heavy metals and pesticides (Allan et al., 2010; Blom et al., 2002; Persson et al., 2001; Schäfer et al., 2008; Shaw and Mueller, 2009). Time-integrative passive sampling requires that the receiving phase remains in the kinetic uptake regime and does not reach thermodynamic equilibrium, which is determined by the passive sampler – medium partition coefficient (Booij et al., 2007). The sampling rate R_s for a substance s is approximately linear until the mass of s in the receiving phase M_s reaches half-saturation (Greenwood et al., 2007; Vrana et al., 2005). Within this linear uptake regime, the Time-Weighted Average (TWA) concentration C_{TWA} for s can be derived for the deployment time t according to:

$$C_{TWA} = \frac{M_s(t)}{R_s t} \qquad \text{Equation 1}$$

Thus, for the calculation of the C_{TWA} of a substance, R_s and the kinetic regime have to be known. Although the sampling rates for a substance can be determined in laboratory experiments (Gunold et al., 2008; Macleod et al., 2007; Tran et al., 2007), fluctuating environmental conditions in the field can strongly influence the exchange kinetics between the receiving phase and the sampled medium, consequently altering the sampling rate in the field. In this context, the first publication examines the impact of biofouling and the use of diffusion-limiting membranes on the sampling rate for a neonicotinoid insecticide pulse in a mesocosm experiment. Publication II describes the application of a novel method to assess the kinetic status of passive samplers in the field. Finally, publication VI includes a comparison of the performance of passive sampling with sediment and grab water sampling when used for the assessment of ecological risks from pesticides (Fig. 1).

Trait-based approaches for ecological risk assessment

Besides challenges to appropriately characterise the exposure of toxicants in the field,

there is an even greater challenge to identify effects of toxicants in ecosystems given that the effects may be masked by confounding environmental factors and natural variability. Two prominent approaches to link toxicants and community composition are the use of ecological indices and multivariate statistics (Newman and Clements, 2008). Many ecological indices have been developed in the last 100 years to assess the ecological status of freshwater ecosystems (Bonada et al., 2006). Most indices are calculated using taxonomic properties of the aquatic macroinvertebrate community such as the fraction of Ephemeroptera, Plecoptera and Trichoptera taxa (% EPT)(Plafkin et al., 1989), absence/presence of selected index taxa (e.g. Hilsenhoff, 1987), the ratio of the number of observed (O) taxa to the taxa which would be expected (E) if the system was in a reference state (O/E) as in RIVPACS or AUSRIVAS (Marchant et al., 1999; Marchant et al., 1997; Wright et al., 1993), species diversity or species richness (Bonada et al., 2006; Cairns and Pratt, 1993). Although several indices worked reliably in detecting general ecological degradation (Böhmer et al., 2004), most of the abovementioned indices as well as combinations, i.e. multimetric indices, do not allow for the establishment of unambiguous causal relationships with a specific stressor such as toxicants (Bonada et al., 2006; Culp et al., 2010; Menezes et al., 2010; Statzner and Beche, 2010).

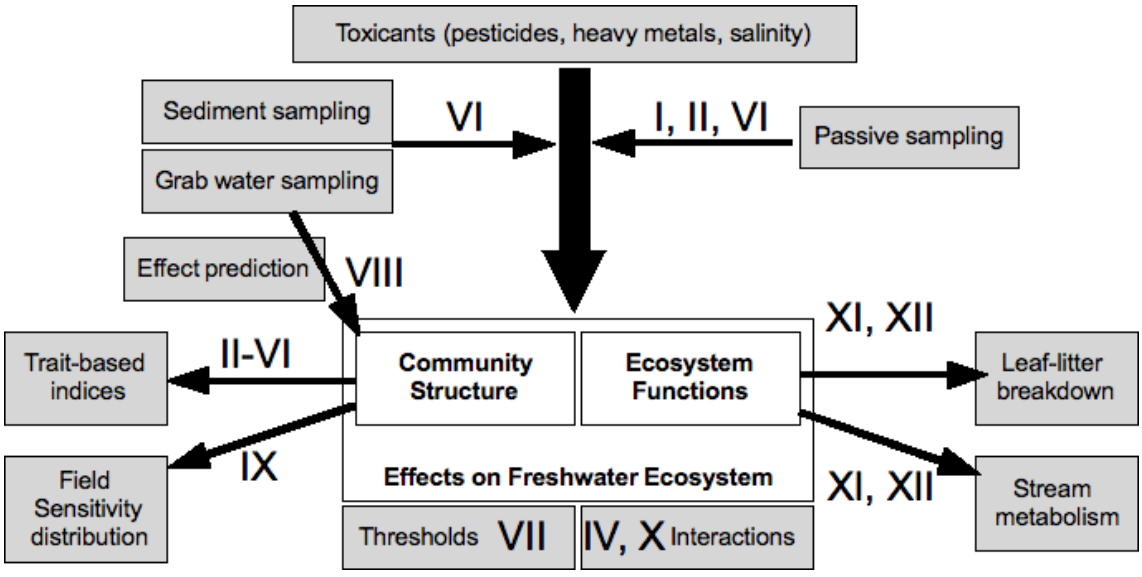

Figure 1: General context of the publications (roman numerals) included in this thesis.

The use of traits such as body size, generation time or reproduction mode has been advocated to link ecological communities and environmental factors including anthropogenic stressors (Heino et al., 2007; Keddy, 1992; McGill et al., 2006; Statzner

et al., 2001b; Townsend and Hildrew, 1994). Although the use of species traits has a long history in freshwater ecology (Statzner et al., 2001b), studies on the relationship between selected species traits and environmental factors as well as their potential for bioassessment have exponentially risen only in the last two decades (Statzner and Beche, 2010). Several studies demonstrated that traits of macroinvertebrates in least-impacted sites exhibit similar patterns over broad spatial scales (Doledec et al., 2011), (Pollard and Yuan, 2010), (Bonada et al., 2007; Schäfer et al., 2007; Statzner et al., 2001a; von der Ohe et al., 2007), enabling large-scale trait-based bioassessment. Moreover, traits have been suggested to facilitate the stressor-specific identification of effects under field conditions (Bonada et al., 2006; Menezes et al., 2010; Statzner and Beche, 2010).

A practical application of a trait-based index system represents the SPEcies At Risk (SPEAR) approach (Liess and von der Ohe, 2005). This approach uses a combination of physiological and biological traits of macroinvertebrate species to determine the most sensitive taxa in a community with respect to specific stressors (Liess and von der Ohe, 2005). SPEAR indices have been developed for pesticides (Liess and von der Ohe, 2005; von der Ohe et al., 2009) and organic toxicants (Beketov and Liess, 2008), where they generally demonstrated high specificity to the respective stressor in Central and North European as well as Siberian streams (Beketov and Liess, 2008; Liess and von der Ohe, 2005; Schäfer et al., 2007; von der Ohe et al., 2009; von der Ohe et al., 2007). In publication III, an overview on the challenges to relate toxicants and communities under field conditions as well as a summery of studies applying the SPEAR approach is given. Moreover, in this publication the specificity of the SPEAR index for pesticides (hereafter $SPEAR_{pesticides}$) and of several commonly used taxonomy-based indices is compared. Publication IV describes the compilation of a trait database for South-East Australian taxa and the development of a trait-based SPEAR index for salinisation, which is one of the most pressing environmental issues in large parts of Australia and other arid or semi-arid regions (Cañedo-Argüelles et al., 2012; Williams, 2001). In addition, a conceptual model for the development of further trait-based indices is suggested. The application of traits for the analysis of a mesocosm experiment on the effects of a neonicotinoid insecticide is described in publication V. This publication also discusses the relevance of considering species traits for the ecological risk assessment of mesocosm studies used in the authorisation of pesticides. The publications II and VI

report on the application of the SPEAR indices for pesticides and organic toxicants for the analysis of field studies in South-East Australian streams (Fig. 1). Thus, these studies also contribute to the question of transferability of approaches to other biogeographical regions, which is generally an "underappreciated aspect" (Wenger and Olden, 2012). Publication VII examines the threshold of pesticide effects for the structure of field communities employing dose-response modelling and the $SPEAR_{pesticides}$ index for pooled data from different continents (Fig. 1).

Statistical approaches to identify effects of toxicants

Statistical data analysis represents a crucial step in each scientific study. The primary focus of the publications related to this section is the statistical analysis of large datasets including the development of a novel method. In ecology, the recent decades have witnessed the birth of the sub-discipline of macroecology, which mainly relies on data analysis and modelling rather then experimental approaches as easily explained by the spatial scale of this sub-discipline (Blackburn, 2004; Kerr et al., 2007; Smith et al., 2008). Macroecological research aims at finding "general mechanisms operating at organism, population and ecosystem levels of organization" at large spatial or temporal scales (Smith et al., 2008). Studies on these scales are largely absent for the effects of toxicants on ecosystems (Beketov and Liess, 2012), with the exception of exposure modelling (Bach et al., 2001; Pistocchi et al., 2009; Schriever and Liess, 2007). This is partly because long-term or large-scale spatial data mainly originates from governmental monitoring programs. Although a wide range of toxicants are regularly monitored in the surface waters of many countries, these monitoring programs are rarely complemented by biomonitoring. Nevertheless, governmental data on toxicants allows for an ecological risk assessment based on existing laboratory toxicity data (e.g. De Zwart et al., 2006; Muschal, 2006; von der Ohe et al., 2011), though this can be hampered by the lack of ecotoxicological data for many chemical substances. For example, a study of von der Ohe et al. (2011) reported that for only 16% of 500 substances, selected for monitoring programmes in four European river basins due to their assumed ecotoxicological and toxicological relevance, complete ecotoxicological data for algae, invertebrates, and fish were available. However, if the toxicity for structurally similar chemical compounds is known, computer models can be employed to predict the toxicity of an unknown compound (Schüürmann et al., 2011; von der Ohe et al., 2005). Publication VIII describes a study where data on 331 organic toxicants

monitored in 4 large German rivers over 11 years were assessed regarding their ecotoxicological risk for algae, invertebrates and fish using laboratory and predicted toxicity data (Fig. 1). Thus, this publication clarifies to which extent toxicants can influence the ecological status of large rivers and identifies the ecotoxicologically most relevant substances.

A frequently used measure for the ecological risk assessment of toxicants represents the Potentially Affected Fraction (PAF) of species in a community (Posthuma and De Zwart, 2006; Posthuma et al., 2002). The PAF is mostly derived from Species Sensitivity Distributions (SSD) for laboratory toxicity data. SSDs have been criticised with respect to several aspects (Forbes and Calow, 2002; Kefford et al., 2005; Newman et al., 2000) including that the used laboratory toxicity data does not represent a sample of the species in the ecosystem for which conclusions are drawn, hence the estimated PAF can be incorrect (Forbes and Calow, 2002). However, if large toxicant exposure data sets with corresponding biomonitoring data are available, the PAF could be derived from the data directly (Kwok et al., 2008; Leung et al., 2005). This would enable an assessment of how many species are lost from the community with increasing toxicity, which in turn could ultimately lead to a reduction in biodiversity (McMahon et al., 2012). A novel similarity-index based method to derive the loss of species over different contamination categories is presented in publication IX. This method requires large (defined as a minimum of 100 samples but > 300 samples recommended) community data sets with associated toxicity data. A distinct feature of the method is that it pools data over spatial and/or temporal scales in order to remove community change due to natural variation and other environmental variables. Three case studies for pesticides, heavy metals and salinity illustrate the novel method. However, this method as well as the suggested stressor-specific indices described above aim at removing the effect of natural variability and of other environment stressors. But these effects can be of interest, for example when investigating the influence of toxicants in comparison to other stressors on community composition. To address such research questions, multivariate statistical methods are considered as appropriate (Zuur et al., 2007). The relative importance of pesticides and salinity for community composition is the subject of publication X, employing distance-based redundancy analysis (Legendre and Anderson, 1999; McArdle and Anderson, 2001). In addition, this study examines the question of a potential interaction effect between the two groups of toxicants. Similarly,

the potential interaction between pesticides and salinity is analysed in publication IV using a large governmental monitoring data set in concert with the modelling of pesticide exposure (Fig. 1).

Effects of toxicants on ecosystem functions

Toxicants can not only affect the structure of communities but alter ecosystem functions (Schäfer et al., 2007; Schäfer et al., 2011). The Millennium Ecosystem Assessment (MEA, 2005) has increased the awareness that ecosystem functions are a crucial prerequisite for the provisioning of ecosystem services to human societies such as drinking water, waste removal or food. Primary production and the decomposition of allochthonous organic matter such as leaves or plant matter from the riparian vegetation represent the most important ecosystem functions in stream ecosystems as they deliver energy for the freshwater food web (Tank et al., 2010; Wallace et al., 1997). Model-based calculations quantified the contribution of primary production and the decomposition of allochthonous organic matter (primarily leaves) to the total carbon budget of the first 100 km of a whole river system to 80 and 20%, respectively (Webster, 2007). An estimate for the local contribution of primary production to the energy budget is obtained when dividing primary production by the ecosystem respiration, both of which can be derived from the measurement of stream metabolism (Tank et al., 2010). A broad range of anthropogenic stressors have been identified that can adversely affect the primary production and decomposition of allochthonous organic matter (Gessner and Chauvet, 2002; Young et al., 2008). Impacts on these ecosystem functions may spread downstream across the whole river continuum (Delong and Brusven, 1994). Only few studies have examined the effects of toxicants on ecosystem functions, with an almost complete lack of field studies (Rasmussen et al., 2012). Publication XI briefly discusses the relationship of ecological risk assessment and biodiversity, ecosystem functions and ecosystem services with a special emphasis on toxicants and summarises several studies of a special issue on this topic (Fig. 1). Publication XII presents one of the few field studies on the effects of toxicants on the ecosystem functions of primary production, ecosystem respiration and allochthonous organic matter decomposition. Finally, publication VII elucidates the relationship between effects on the freshwater community and the decomposition of allochthonous organic matter and examines whether there is a threshold for the effects of pesticides on this ecosystem function.

2. Publication I

Schäfer, R. B., Paschke, A., Liess, M. 2008. Aquatic passive sampling of a short-term thiacloprid pulse with the Chemcatcher: Impact of biofouling and use of a diffusion-limiting membrane on the sampling rate. *Journal of Chromatography A*. 1203 (1): 1-6.

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Aquatic passive sampling of a short-term thiacloprid pulse with the Chemcatcher: Impact of biofouling and use of a diffusion-limiting membrane on the sampling rate

Ralf Bernhard Schäfer [a,*], Albrecht Paschke [b], Matthias Liess [a]

[a] Department of System Ecotoxicology, UFZ, Helmholtz Centre for Environmental Research, Permoserstrasse 15, 04318 Leipzig, Germany
[b] Department of Ecological Chemistry, UFZ, Helmholtz Centre for Environmental Research, Permoserstrasse 15, 04318 Leipzig, Germany

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ABSTRACT

We examined the performance of the Chemcatcher (University Portsmouth, UK) in two different configurations when used for the aquatic passive sampling of a 1-day pulse contamination with thiacloprid under field-relevant conditions. The configuration without diffusion-limiting membrane led to biofouling of the Empore disk receiving phase resulting in a fourfold reduction in analyte uptake compared to unfouled passive samplers. The sampling rate for the configuration with diffusion-limiting polyethersulfone membrane was also much lower than in a long-term exposure scenario, although no biofouling occurred. Both configurations of the Chemcatcher exhibited high variation in analyte uptake with up to 100% RSD. Short-term contamination events may be underestimated in passive sampling when the receiving phase is biofouled or a diffusion-limiting membrane is employed.

1. Introduction

Passive sampling represents a promising method for continuous monitoring for metals and organic pollutants in water bodies [1]. While several passive sampling devices are suitable for the sampling of non-polar organic pollutants [2,3], to date just two passive samplers have been developed for polar organic pollutants: the polar organic integrative sampler (POCIS) [4] and the Chemcatcher equipped with a polar receiving phase [5,6]. The receiving phase of both passive sampling devices is shielded with a diffusion-limiting membrane (optional for the Chemcatcher), which reduces the uptake rate of compounds into the receiving phase. The rationale for using such a membrane is to (1) decrease the sensitivity of the sampler to hydrodynamic changes in the surrounding medium [6], (2) prevent the development of a biofilm layer on the receiving phase ("biofouling"), which may influence uptake dynamics [7,8], and (3) extend the period in which receiving phase continues linear uptake (kinetic regime) during field monitoring. As long as a passive sampling device remains in the kinetic regime, time-weighted average (TWA) water concentrations can be derived from the contaminant concentrations in the receiving phase [3,9].

However, the diffusion-limiting membrane imposes a lag phase on the analyte uptake, which is determined by the time that the compounds need to diffuse through the membrane to the receiving phase. Due to this lag phase, uncertainties persist as to which extent short-term fluctuations in analyte concentrations are captured by both the POCIS and the Chemcatcher, although recent studies have shown that these passive samplers are in general suitable for the long-term monitoring of polar organic pollutants [5,8,10–12]. Thus, an episodic pulse contamination such as result from pesticide runoff could be missed including its peak concentration, though information on short-term maximum concentrations of toxicants are ecotoxicologically relevant.

To circumvent the lag phase in analyte uptake, passive samplers without diffusion-limiting membrane were proposed and successfully employed to detect episodic pulse exposures [6,13]. Despite the shortcoming that passive sampling delivers only TWA concentrations instead of peak water concentrations, the passive samplers TWA concentrations in a previous field study could be used to explain biological effects, presumably due to a strong correlation of the TWA concentrations with the peak water concentrations [13]. However, an unshielded receiving phase such as the Empore disk in the case of the Chemcatcher may be subject to biofouling even after short deployment times of several days, depending on the microbial water concentration, water temperature and stream current velocity [8,14].

* Corresponding author. Tel.: +49 341 235 2120; fax: +49 341 235 2401.
E-mail address: senator@ecotoxicology.de (R.B. Schäfer).

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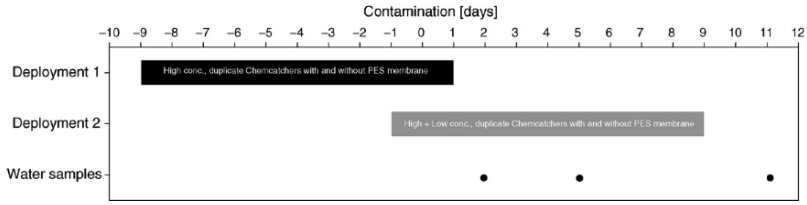

Fig. 1. Time schedule for the water samples and the deployment of Chemcatcher passive samplers into the streams. A contamination with 3.2 and 100 μg/L nominal concentration ("Low" and "High", respectively) was conducted at day 0 in two streams each.

In the present study we investigated the suitability of two Chemcatcher configurations for the detection of a 1-day contamination event: the Chemcatcher with a diffusion-limiting membrane that introduces a lag phase to the contaminant uptake, and the Chemcatcher without membrane, which may exhibit a sampling rate impaired by biofouling. To our knowledge, this is the first study that examines (1) the impact of biofouling on the sampling rate of polar passive samplers and (2) the suitability of polar passive samplers to monitor a 1-day pulse exposure. The neonicotinoid insecticide thiacloprid was chosen as study compound since it is very hydrophilic ($\log K_{ow}$ = 1.26, water solubility at 20 °C = 184 mg/L) and has rarely been studied [15]. Specifically, we exposed two configurations (with and without diffusion-limiting membrane) of Chemcatcher passive samplers in an artificial stream system to thiacloprid, with one set of each configuration subjected to potential biofouling prior to exposure.

2. Experimental

2.1. Chemicals and materials

Thiacloprid 3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene] cyanamide (CAS no. 111988-49-9) was obtained from Agrar-Handel and Transport (Schafstädt, Germany) as the commercial formulation Calypso (suspension concentrate) with 480 g/L of the active ingredient (Bayer CropScience Deutschland, Langerfeld, Germany). Triphenyl phosphate (TPP) at 500 μg/mL in acetone was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Solvents (HPLC-grade acetone, methanol, ethyl acetate, 2-propanol and acetonitrile) were obtained from Merck (Darmstadt, Germany). Empore SDB-XC disks were purchased from 3 M (St. Paul, MN, USA). Z-Bind polyethersulfone (PES) membranes (0.2 μm thickness) were obtained from Pall (Pensacola, FL, USA). Chromabond Easy cartridges (6 mL) were obtained from Macherey-Nagel (Düren, Germany). Solvent evaporation was done with a TurboVap 2 system (Zymark, Hopkington, MA, USA).

2.2. Description of the artificial streams and contamination

The four artificial outdoor streams (Supplementary Figures S1 and S2) had the following characteristics (±SD): 20 m length, 0.32 ± 0.03 m width at water surface, 0.25 ± 0.11 m depth, 160 ± 9 L/min discharge, 2% slope of stream-bed, water temperature 21.3 ± 0.7 °C and an approximate total volume of 1000 L. The stream bottom was lined with a polyvinyl chloride foil (thickness 0.8 mm) and covered with a 30–50 mm layer of a mixture of fine gravel and sand (particle size 0.2–3.7 mm) to simulate a natural streambed. Furthermore, water cress *Nasturtium officinale* was planted in the streams 1 year before the experiment. Each stream is a closed circulation system with water running by gravity, being collected downstream in a 200-L barrel and pumped back to the upstream reach through a 40-mm polyvinyl chloride tube (OASE, Hörstel, Germany) using an Atlantis 150 electric pump (OASE). For contamination, 1-L stock solutions of 3.2 and 100 mg/L thiacloprid in drinking water were prepared and poured into the barrels of each of two streams, respectively. Hence, the nominal concentrations were 3.2 and 100 μg/L, herein after referred to as "low" and "high". The low treatment represents a realistic field exposure situation whereas the high treatment can be regarded as a worst case scenario that was reported for the aerial application of pesticides [24].

2.3. Experimental design of the study

Duplicate Chemcatcher passive samplers (University Portsmouth, UK; commercially available at Alcontrol, Linkoping, Sweden) with and without PES membrane were exposed for 10 days at two deployment times in the two streams with high contamination (Fig. 1). The first set of Chemcatcher passive samplers was deployed 9 days before the contamination to induce biofouling before exposure. A second set of samplers was deployed on day 1 before contamination and served as reference for the compound uptake expressed by the substance-specific sampling rate R_S [16]. The two streams with low contamination were equipped with duplicate passive samplers of each configuration at the second deployment time (Fig. 1) to control for concentration-dependence of the results for the high-concentration streams.

Duplicate spot water samples (400 mL) were taken in the four streams according to Fig. 1 to characterise the thiacloprid concentration in the water phase over the course of the experiment and to allow for the computation of sampling rates for the passive sampler (see below).

2.4. Description and treatment of the Chemcatcher passive sampler

The sampler is described in detail by Kingston et al. [5]. Briefly, the Chemcatcher consists of a polytetrafluoroethylene (PTFE) body, in which an Empore disk (47 mm diameter; 15.9 cm^2 surface area) is placed as receiving phase (Figure S3, Supplementary material). In our study the Chemcatcher was equipped with an SDB-XC Empore disk. Depending on the configuration of the sampler, a diffusion-limiting PES membrane was placed above the receiving phase. Before use the SDB-XC Empore disk was conditioned with 10 mL acetone, 10 mL 2-propanol and 10 mL methanol. The PES membrane was conditioned by soaking in methanol for 12 h and then rinsed with ultrapure water. The preconditioned samplers were hung in the artificial streams with the open side directed towards the stream bottom.

After deployment, the Empore disk was carefully removed from the Chemcatcher body and dried under vacuum. Subsequently the disk was put inside a 25-mL glass vial (VWR International, Darm-

stadt, Germany) and extracted with 15 mL methanol–acetonitrile (1:1) for 10 min in an ultrasonic bath. The extract was gently evaporated to dryness under nitrogen and reconstituted with 300 μL of acetonitrile. Prior to analysis 5 μL of TPP standard solution were added in order to correct for volume differences between samples. The PES membranes were discarded after retrieval of the samplers.

2.5. Spot water sampling and treatment of water samples

Duplicate spot water samples were taken by hand with 400 mL amber glass bottles in non-vegetated up- and downstream sections of each channel, starting at the upstream section. Immediately after sampling, the water samples (400 mL) were taken through a preconditioned (6 mL methanol) Chromabond Easy cartridge at a speed of 5 mL/min. Then the columns were dried under vacuum for 30 min and subsequently eluted with 12 mL acetonitrile–ethyl acetate (1:1) under gravity flow. The eluate was handled as described above. Analytical recovery was 82% with 18% RSD ($n = 3$) for 400 mL of spiked drinking water samples (1 μg/L).

2.6. Chemical analysis

All extracts of disks and cartridges were analysed using an Agilent 1100 Series LC–MS system (Agilent Technologies Germany, Boeblingen, Germany) that includes a binary pump, vacuum degasser, autosampler, thermostated column compartment with column switching valve, multi-wavelength UV/vis detector and single-quadrupole MS system with an atmospheric-pressure ionisation source with electrospray ionisation (ESI) interface. Chromatographic separation was done at 25 °C on an Agilent Zorbax Eclipse XDB-C18 column (150 mm × 2.1 mm; 5 μm) under isocratic conditions with a mobile phase at a flow rate of 0.5 mL/min. The eluent contained 50% acetonitrile (ACN) and 50% aqueous buffer (with 2.5 mmol/L ammonium acetate). The sample volumes injected were 10 μL. ESI mass spectra were acquired in positive ion mode under the following conditions: drying gas (nitrogen) flow 10 L/min, drying gas temperature 350 °C, nebuliser pressure 50 psi, capillary voltage +4000 V and fragmentation voltage +190 V. Data acquisition was done in full scan mode (m/z values: 120–850) and TPP was quantified directly from its peak areas in the TIC. Thiacloprid was quantified by means of extracted ion chromatograms using external calibration on main m/z ion 253. Two calibration lines were established by linear regression based on series of injections of standard solutions in ACN (ranges: 10–1000 μg/L and 1–100 mg/L). The limit of detection (LOD) and the limit of quantification (LOQ) were derived from the lower calibration range. The calculation was done according to DIN 32645 (equivalent to ISO 11843) using a statistical probability of 95%; this yielded 15 μg/L as LOD and 55 μg/L as LOQ. Typical chromatograms are displayed in Fig. 2.

2.7. Data analysis

The sampling rate R_S in the dimension volume/day was calculated according to:

$$R_S = \frac{m_S}{c_{TWA} t} \quad (1)$$

where m_s is the accumulated mass in the receiving phase after exposure time t and c_{TWA} is the time-weighted average (TWA) concentration of the analyte in the water phase in the dimension mass/volume. c_{TWA} was calculated using Eq. (2):

$$c_{TWA} = \frac{1}{t} \int_0^t c_{thiac}(t)\, dt \quad (2)$$

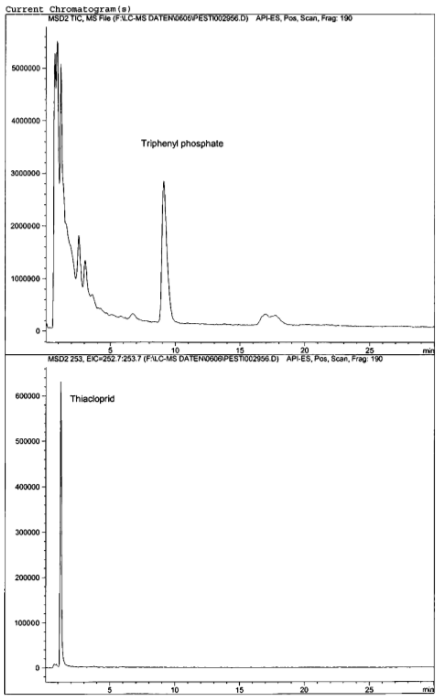

Fig. 2. Total ion chromatogram (upper) and extracted ion chromatogram at 252.7–253.7 m/z (lower).

where $c_{thiac}(t)$ is the concentration of thiacloprid in the streams at a given time t. We estimated $c_{thiac}(t)$ using non-linear regression:

$$c_{thiac}(t) = a e^{-bt} \quad (3)$$

where a and b are the fitted regression parameters (start values: $a = 95$, $b = 0.03$; function <nls> in the statistical software R, see below).

For the streams with low contamination no reliable regression curve and hence no c_{TWA} could be calculated due to high variation of the thiacloprid water concentrations (up to 120% RSD). For the high-contamination streams, the initial thiacloprid concentration was approximated to be the nominal concentration. However, assuming initial concentrations of 100 ± 50 μg/L resulted only in slightly different c_{TWA} values (±13%). Furthermore, using Eq. (3) to predict the initial concentration yielded an estimate value of 112.9 μg/L.

Integrating Eq. (2) for t and subsequent insertion into Eq. (1) (Supplementary material) yields Eq. (4):

$$R_S = \frac{m_S b}{a(1 - e^{-bt})} \quad (4)$$

The partial derivations for a, b and m_s of this equation (Supplementary material) were used to compute the standard error for the sampling rate according to the law of error propagation.

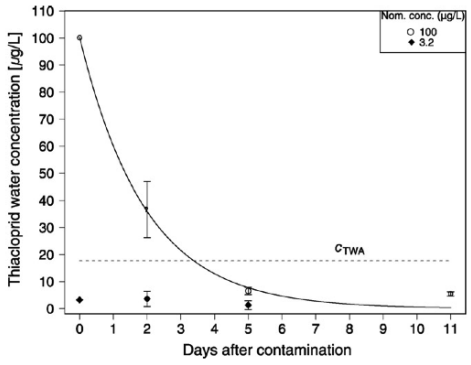

Fig. 3. Mean of thiacloprid concentrations with standard deviation ($n = 4$) from day 0 to day 11 in each of two streams with 100 and 3.2 µg/L nominal concentration, respectively. The initial concentration was not measured but approximated with the nominal concentration. The solid line gives the estimated degradation curve for the high concentration as described in Section 2 (regression parameters ± SD: $a = 100.2 \pm 11.7$; $b = 0.51 \pm 0.12$). The dashed line shows the computed time-weighted average concentration (c_{TWA}) for thiacloprid. No water samples were taken in the streams with 3.2 µg/L at day 11.

All statistical computations and graphics were created with the open-source statistical software package R (www.r-project.org) using version 2.62 (for Mac OS X, 10.4.11) [17].

3. Results and discussion

3.1. Time-weighted average concentrations in the streams

The thiacloprid concentrations in the water phase decreased quickly in the two streams of the high-concentration setup (Fig. 3). This decrease was faster than in another study where a half-life of 12–20 days was observed and microbial decomposition was found to be the primary degradation pathway [18]. The faster disappearance from the water phase in our study may be due to adsorption in the test system (organic debris, sand, gravel, tubing, foil). The respective TWA concentration (c_{TWA}) for thiacloprid was 17.7 µg/L for the 11-day period (Fig. 3).

In the streams with low contamination, the thiacloprid water concentrations also showed a reduction in time but varied widely, impeding the fitting of a degradation model.

The exposure situation in our study with a peak concentration followed by a decrease to a baseline exposure can be regarded as field-relevant, though the decrease would be more rapid in the field [19,20].

3.2. Impact of biofouling and the use of a polyethersulfone membrane on the sampling rate

After 10 days of deployment, the Empore disk receiving phase of the Chemcatcher passive samplers without membrane exhibited a biofilm layer, which covered almost the whole surface (Supplementary Figure S3). This biofilm layer could not be removed mechanically without damaging the Empore disk. The rate of biofouling in our experiment was presumably at the upper level of that in natural streams. Firstly, the temperature in the artificial streams was quite high compared to natural streams due to the low water volume, enhancing microbial growth. In addition, the effect of biofouling may decrease under high current velocities but the artificial streams exhibited flow velocities at the lower margin of natural streams (0.1 m/s) [21].

The sampling rate determined for the prefouled samplers without membrane (deployment set 1) was on average fourfold reduced compared to the respective reference samplers of deployment set 2 in the high-contamination streams (Table 1). A small part of the reduction may be attributed to inhomogeneous exposure conditions caused by incomplete dispersion of thiacloprid in the water phase directly after contamination, though the turnover rate in the system was quite high as 16% of the total water volume was pumped to the upstream part each minute (see Section 2 for details). The major part of the reduction in analyte uptake was most likely due to the development of a biofilm layer on the receiving phase. Previous studies of non-polar passive samplers such as semipermeable membrane devices (SPMDs) also showed a significant reduction in sampling rates due to biofouling. Ellis et al. reported a reduction of 26.1–38.6% in the uptake of phenantrene compared to unfouled SPMDs [22]. In another study, Richardson et al. demonstrated that the uptake of organochlorine pesticides was reduced to between 78.4 and 38.8% of the amount found in unfouled controls [23]. Furthermore, Richardson et al. found lower effects of biofouling for more hydrophilic compounds, which is in accordance with theoretical considerations. The biofilm can be regarded as an additional layer between the surrounding medium and the receiving phase, which has to be permeated before uptake in the receiving phase commences [16]. Hydrophilic compounds should permeate this layer faster because the biofilm may be modelled as a water layer with dispersed organic matter [16]. Nevertheless, we observed a strong reduction in the sampling rate of biofouled Empore disks for thiacloprid, as a very hydrophilic compound ($\log K_{ow} = 1.26$). Therefore, in the case of Empore disks biofouling could be associated with further processes that reduce the sampling rates. One possible explanation is that biofouling modifies the membrane surface and that this leads to a decrease of the analyte uptake into the receiving phase. Another explanation is that the biofilm hampers the extraction of analyte from the disks after exposure. It is also possible that the biofilm acts as a reactive zone where some decom-

Table 1
Average concentration per sampler ($n = 4$), ratio of average concentrations and sampling rates (R_s) for both deployment sets and each of the two nominal concentrations (nom. con.) in the four artificial streams

Configuration of the Chemcatcher	Nom. con. (µg/L)	Depl. set[a]	Average mass per sampler ± SD (ng)	Ratio con., No membrane/PES membrane	R_s ± SD (L/d)
No membrane	100	1	1550 ± 1364	10.5	0.019 ± 0.002
PES membrane	100	1	147 ± 147		0.002 ± 0.0002
No membrane	100	2	13746 ± 5223	2.0	0.071 ± 0.0048
PES membrane	100	2	6744 ± 3102		0.035 ± 0.002
No membrane	3.2	2	93 ± 29	1.7	–[b]
PES membrane	3.2	2	54 ± 22		–[b]

[a] Depl. = deployment. Deployment set 1 includes samplers that were biofouled before exposure while the deployment set 2 represents samplers that were deployed 1 day before contamination.

[b] Due to high variation of the water concentrations (up to 120% RSD) no reliable TWA concentration and thus no sampling rate was calculated.

position of thiacloprid appears, since microbial degradation is the primary degradation path [18]. However, this issue remains open for further investigations.

No biofouling occurred on samplers that were equipped with a PES membrane. Nevertheless, the samplers with PES membrane that were exposed to thiacloprid for 1 day only (deployment set 1) had a much lower sampling rate compared to those of deployment set 2 in the high-contamination streams (Table 1). Moreover, the ratio of the uptake rates between samplers with and without membrane was approximately 1:2 for the 9-day exposure to both concentrations (deployment 2) and decreased to 1:10 for the 1-day exposure (Table 1). Presumably, this was due to the lag time imposed by the diffusion-limiting membrane.

The amount of thiacloprid per sampler of deployment set 1 varied two- to threefold more than that of samplers of deployment set 2 (Table 1) with RSDs of 88 and 100%. The higher variation in the thiacloprid uptake rate of deployment set 1 may be explained partly by inhomogeneous exposure conditions at the beginning of the experiment (see above).

3.3. Suitability of passive sampling for the monitoring of a 1-day pulse contamination

In general, passive sampling is used to determine long-term environmental concentrations (TWA concentrations) of contaminants. To compute TWA concentrations after field deployments, compound-specific sampling rates (R_S) are needed (compare Eq. (1)) that can be predicted in laboratory calibration experiments [9]. The results of our experiment demonstrate that caution has to be taken when applying this approach to situations where contaminants appear in short-term episodic events, because the Chemcatcher in both configurations of deployment set 1 showed strongly reduced sampling rates compared to deployment set 2 (Table 1). Hence, the environmental concentrations would be underestimated in field monitoring studies if laboratory-derived sampling rates from continuous-exposure setups comparable to deployment set 2 were used for computation of TWA concentrations.

Moreover, both configurations of the Chemcatcher with SDB-XC receiving phase exhibited very high variation (88 and 100% RSD) in analyte uptake when used to characterise a 1-day pulse exposure and hence would not allow for precise quantification of thiacloprid water concentrations (Table 1). Future studies should elucidate if the variation is compound-specific and could be reduced by employing another receiving phase. Relatively high variation of 31–44% RSD was also observed for the 9-day sampling period of deployment set 2 corresponding to the variation in the uptake of various analytes for a short-term contamination event in a field study [13]. Nevertheless, the concentrations obtained in this field study could successfully be used to assess toxicity towards aquatic invertebrates according to the toxic unit concept [25].

In contrast to samplers with a diffusion-limiting membrane, where the decreased sampling rate due to the lag phase should be independent of deployment time, samplers without diffusion-limiting membrane should not suffer reduction in the sampling rate until a biofilm develops. Thus, samplers without diffusion-limiting membrane deployed shortly before an episodic contamination event will most likely not be impacted by biofouling. Indeed, no obvious reduction in the sampling rates of samplers without membrane was observed in a field study where a short-term contamination event occurred 4–5 days after deployment [13].

Furthermore, maximum exposure concentrations in the field are usually in the range of 0.1 to 10 μg/L and hence the concentrations in the samplers with PES membrane would be below the LOQ assuming similar sampling rates [24]. The sampling rates could presumably be increased by employing a more polar receiving phase such as the SDB-RPS Empore disk. This disk exhibited two- to threefold higher sampling rates for two pharmaceuticals with a $\log K_{OW}$ of 0.89 and 2.45 [26]. However, the sampling rate for a less hydrophilic pharmaceutical ($\log K_{OW} = 3.16$) was threefold lower compared to the sampling rates of the SDB-XC disk used in this study for two compounds with a comparable hydrophilicity ($\log K_{OW}$ of 2.9 and 3.2) [14,26].

Taking into consideration the 10-fold higher sampling rate of the Chemcatcher without membrane, this configuration seems more promising for the sampling of short-term episodic events.

4. Conclusions

The sampling rates of the Chemcatcher passive sampler equipped with a PES membrane or with a biofouled receiving phase may be reduced when sampling a short-term contamination event. This can lead to an underestimation of toxicant concentrations in the field when sampling rates of long-term laboratory calibrations without biofouling are used for the computation of TWA concentrations. In addition, the precise determination of toxicant concentrations can be hampered by high variation in analyte uptake.

However, in situations where (1) the occurrence time of an episodic event is predictable and the samplers can be deployed shortly before the event, (2) toxicant concentrations are assumed to be low or (3) biofouling is negligible due to low temperature or high current velocity, the Chemcatcher should be applied without diffusion-limiting membrane since the sampling rate is up to 10-fold higher.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2008.05.098.

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3. Publication II

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Using silicone passive samplers to detect polycyclic aromatic hydrocarbons from wildfires in streams and potential acute effects for invertebrate communities

Ralf Bernhard Schäfer [a,*], *Laurence Hearn* [b], *Ben J. Kefford* [a,c], *Jochen F. Mueller* [b], *Dayanthi Nugegoda* [a]

[a] School of Applied Sciences, RMIT University, PO Box 71, Bundoora, VIC 3083, Australia
[b] National Research Centre for Environmental Toxicology (Entox), University of Queensland, 39 Kessels Road, Coopers Plains, QLD 4108, Australia
[c] Centre for Environmental Sustainability, University of Technology Sydney, PO Box 123, Broadway, NSW 2007, Australia

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ABSTRACT

Silicone rubber passive samplers spiked with 4 deuterated performance reference compounds were deployed for 29–33 days to estimate the concentrations of 16 polycyclic aromatic hydrocarbons (PAHs) in 9 streams in Victoria, Australia, following a wildfire. Silicone rubber strips of 2 thicknesses were used to obtain information on the status of uptake of the chemicals of interest at retrieval. In addition, we monitored the stream macroinvertebrate community for potential effects of PAHs or other fire organics. All selected PAHs were detected in the passive samplers and the sampling rates ranged from 0.5 to 50 L/day significantly varying between sites but not compounds, presumably due to differences in current velocity. The estimated water concentrations were 0.1–10 ng/L for total PAHs with phenanthrene, pyrene and fluoranthene accounting for 91% of the total concentration. All PAHs were a factor of 1000 or more below the reported 48-h median lethal concentrations (48-h LC50) for *Daphnia magna*. Two sites located closest to the fires exhibited elevated concentrations compared to the other sites and the passive samplers in these sites remained in the integrative uptake regime for all compounds, suggesting precipitation-associated PAH input. No acute toxic effects of PAHs or other fire organics on the invertebrate community were detected using a biotic index for organic toxicants (SPEAR), whereas a non-specific biotic index (SIGNAL) decreased in two sites indicating impacts from changes in other environmental parameters. We conclude (1) that silicone-based passive samplers with two different area-to-volume ratios represent a promising tool for determining organic toxicants and (2) that PAHs from wildfires are unlikely to be a common main cause for fire-related ecological effects in streams adjacent to burnt regions.

1. Introduction

In several regions of the world such as South Europe, parts of America and Australia wildfires represent an integral part of the landscape and many species are tolerant of or even dependent on recurring wildfire events (Bradstock, 2008). Fires can have impacts on stream ecosystems. While the direct effects of fires on streams such as higher temperatures are in

* *Corresponding author*. Tel.: +61 (03) 9925 7105; fax: +61 (03) 9925 7110.
E-mail address: Ralf.Schaefer@rmit.edu.au (R.B. Schäfer).
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most cases rather negligible, subsequent indirect effects may be more severe (Minshall, 2003). Indirect effects include a decrease in dissolved oxygen, a decrease in allochthonous energy, an increase in nutrients and an increase in hydrodynamic stress resulting from the input of sediments and ash slurry, as well as channel and vegetational alterations (Minshall, 2003; Hall and Lombardozzi, 2008). Especially strong rain events following fires have been identified as a trigger for post-fire effects since they can wash considerable ash and sediments into the aquatic system. Declines of fish populations and changes in invertebrate communities have been attributed to the input of sediments and ash after fires (Earl and Blinn, 2003; Lyon and O'Connor, 2008). Since the input of ash and sediments simultaneously alters several water quality parameters (including turbidity, hydrological conditions and dissolved oxygen) it is in most cases unclear as to which factor or combination of factors is responsible for observed biological effects (Vieira et al., 2004). The potential contribution of polycyclic aromatic hydrocarbons (PAHs) and other fire organics that are released into the environment during fires resulting from the combustion of biomass (Hays et al., 2005; Yuan et al., 2008) has been little studied (Vila-Escale et al., 2007). These substances may enter freshwater systems adsorbed to ash, sediments or via dry deposition. To date, only two studies have been published on the input of PAHs in freshwater systems following wildfires, both conducted in Spain (Olivella et al., 2006; Vila-Escale et al., 2007).

Passive sampling has gained growing attention for the continuous sampling of pollutants in the environment and has been effective in catching episodic pulse exposures in streams (Schäfer et al., 2008b; Shaw and Mueller, 2009). Polydimethylsiloxane (PDMS) as a receiving phase represents a promising approach for the sampling of a wide-range of organic pollutants in terms of polarity including PAHs (Smedes, 2007; Bauer, 2008; ter Laak et al., 2008), whereas most existing receiving phases are relatively selective for compounds of a certain polarity. Several studies successfully employed PDMS fibres or rods as a receiving phase (Vrana et al., 2001; Wennrich et al., 2003; Ouyang et al., 2007). Due to the relatively small volume of the receiving phases used in these studies the samplers reach equilibrium with the sampling phase within a few days or use a membrane to slow-down the uptake rate, which may compromise the sampling of episodic events (Schäfer et al., 2008a). An alternative represents the use of large-volume PDMS rubber sheets without membrane, but it has only been applied rarely for passive sampling in the aquatic environment (Smedes, 2007; Bauer, 2008). For PDMS type samplers where the chemicals partition with the sampling phase, it is typically assumed that uptake (and release) follows first order kinetics. Thus a time integrated exposure concentration, the so-called time-weighted average (TWA) concentration, can be obtained provided that clearance of the chemical is low relative to the uptake i.e. sampling in the linear uptake phase. Depending on the volume of the sampler, the surface area exposed, the capacity of the sampler-to-water partition coefficient K_{sw} and factors that affect the kinetics, samplers will exit the linear uptake phase and enter the curvilinear uptake phase more or less rapidly i.e. from minutes to years (Vrana et al., 2005). For an adequate estimate of the water concentration of an analyte after field deployment it is crucial to know whether the passive sampler remained in the integrative uptake regime (i.e. linear uptake phase) or approached equilibrium within the deployment time (Vrana et al., 2005). Bartkow et al. (2004) employed two receiving phases with different thicknesses in air passive sampling for PAHs to determine the uptake regime (integrative or equilibrium) after deployment. Different thicknesses result in a different area-to-volume ratio of the receiving phases, which influences the equilibration times with the sampling phase. Consequently, the ratio of the analyte mass between receiving phases of different thicknesses changes until equilibrium is reached in both phases and allows for a derivation of the uptake regime (Bartkow et al., 2004). In water, this approach has been used only in one study employing polyethylene strips as receiving phase (Müller et al., 2001).

During the summer 2008/2009 Victoria, Australia was subject to the lowest precipitation (0 mm between 1.1.2009 and 28.2.2009) and highest temperatures on record in 120 years across several regions (BOM, 2009a,b). These conditions, on top of an extended drought, promoted the outbreak of several large forest fires in Victoria. The largest fire represented the Kilmore East–Murrurundi Complex (KEMC) fire to the north-east of Melbourne that burned approximately 260 000 ha of land (BOM, 2009c). We initiated an ad-hoc study that had the following objectives: (1) to determine the input of PAHs in streams during the fires and associated with rain events after the fires, (2) to assess the suitability of PDMS passive samplers with different thicknesses for the determination of the uptake regime (3) to assess potential impacts of PAHs or other fire organics released by the KEMC fire on invertebrates in streams adjacent and in the vicinity of the burned area.

2. Materials and methods

2.1. *Study design and rain events*

The study area was located within 200 km east of Melbourne, Victoria, Australia and comprised nine sampling sites in first, second and third-order streams (Fig. 1). The sites were selected (1) to represent a potential gradient in exposure from ash and smoke during the wildfires and potential runoff events and (2) based on safe accessibility after the outbreak of the fires (Fig. 1). The site code corresponded to the site code of the respective site in a larger study. No known industrial facilities were present in the catchments that could account for significant PAH discharges. Nevertheless, except for the Sites 14 and 15, which were located in forested areas, most of the sites were in the vicinity of highways or roads so that exhaust fumes represent a potential source of PAHs.

The passive samplers were deployed approximately 10 days after the outbreak of the fires (19th to 21st of February), which was in the middle of the period of the KEMC fire (9th February to 5th of March) but before the first rain event. The samplers were recovered between 29 and 33 days after deployment (22nd to 24th of March). The invertebrate fauna was monitored between the 15th and 20th of February (Supplementary material, Table S1). Two precipitation events

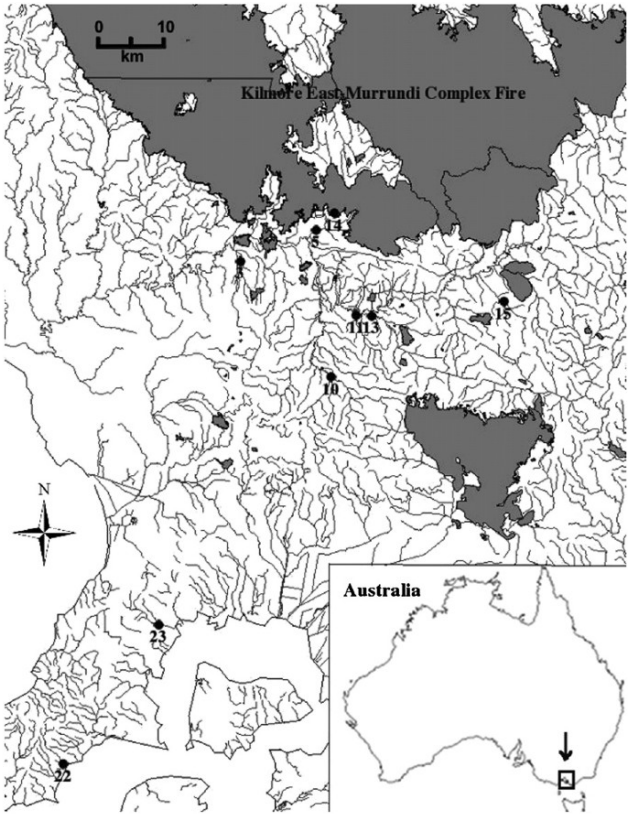

Fig. 1 – Location of the sampling sites (dots with numbers) in streams (black lines) in relation to the wildfires (grey area). The figure was generated using data on the hydrological network, wildfire areas and the base map for Victoria provided by the Department of Sustainability and Environment, Victoria. The exact positions of the sampling sites are given in Table S1 (Supplementary material).

of 15 and 25 mm within 24 h were recorded (4th to 15th of March, respectively) within the study period, which represented the first precipitation events after the outbreak of the KEMC fire (BOM, 2009a,c).

2.2. *Preparation, deployment time and extraction of the passive samplers*

Strips of 5 cm width, 62 cm length and two different thicknesses (0.5 and 1.5 mm, hereafter thin and thick) were cut from large-volume polydimethylsiloxane (PDMS) sheets (Purple pig, Notting Hill, Victoria, Australia), placed in a 0.5 L jar and twice pre-extracted before usage for 48 h with 500 mL of GC-grade n-hexane/acetone 3:1 (Ajax, Taren Point, NSW, Australia) to remove impurities. Rubber strips were then dried under nitrogen and returned to the jars for spiking with four deuterated compounds (purity of 98.5% or higher) acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12 (Accustandard, New Haven, CT, USA) and served as performance reference compounds (PRCs) (Huckins et al., 2002). The spiking was done by exposing the strips to 400 mL of a 20:80 (vol./vol.) solution of HPLC-grade MeOH: bi-distilled H_2O containing 100 μL of a stock solution (4 mg/100 mL) of the deuterated compounds in HPLC-grade MeOH (Merck, Darmstadt, Germany). The jars were shaken for 60 h as this time was sufficient in another study to reach equilibrium between the receiving phase and the standard in solution (Booij et al., 2002). A procedural blank and a method blank were processed concurrently and used to detect potential contamination of the samplers during the pre-extraction step and during

spiking. The spiking of PRCs allows for the in-situ determination of sampling rates, which is used in calculating water concentrations, and accounts for between-site variation in environmental parameters such as current velocity or temperature (Huckins et al., 2002).

Each passive sampler consisted of a thin and thick PDMS strip placed in a steel (1 cm) mesh. One sampler was fixed approximately 10–30 cm above the bottom of the stream at each site. A field blank was exposed to the air during deployment and retrieval of the samplers to account for airborne contamination during field handling. On recovery, the PDMS strips were cleaned with analytical-grade ethanol (Ajax, Taren Point, NSW, Australia) to eliminate biofilms. Samplers were then dried using paper tissue and stored at 4 °C in a glass jar. In the laboratory, all blanks and exposed samplers were thoroughly cleaned with bi-distilled water, subsequently rinsed with HPLC-grade acetone (Merck, Darmstadt, Germany) to remove traces of water and air dried for 10 min in a fume hood. Elution was conducted twice for 24 h each with 250 mL GC-grade n-hexane (Ajax, Taren Point, NSW, Australia). The eluate was gently blown down to 1 mL at 50 °C in a water bath in a nitrogen stream using Mini-vap evaporators (Sigma-Aldrich, Melbourne, Australia) and then transferred to a vial. Subsequently, 10 μL of triphenylphosphate (TPP) (100 ng/μL) (Merck, Darmstadt, Germany) was added as internal standard (IS).

2.3. Chemical analysis

The chemical analysis was conducted using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS, HP 5890 II GC coupled with VG AutoSpec); splitless injection; injector temperature 220 °C. The target PAHs (Table 1) were separated on a J & W Scientific DB-1701 column (30 m × 0.25 mm id., 0.25 μm film thickness) with ultra-high purity helium carrier gas; temperature program 110 °C for 2 min, 10 °C min^{-1} to 170 °C, 5 °C min^{-1} to 300 °C and held 6 min; total run-time 40 min. The mass spectrometer operating conditions were: ion source and transfer line temperatures 265 °C; ionization energy 38 eV; electron multiplier voltage set to produce a gain of 10^6. Resolution was maintained at 5000 (10% valley definition) throughout the sample sequence. Selective ion recording (SIR) experiments were performed in the electron impact (EI) mode monitoring the quantitative ion for each target analyte, including the recovery standard TPP, which was added to the samples prior to HRGC/HRMS analysis. Quantitation of PAHs was performed using an external standard calibration (ng/vial) with criteria for positive identification: (i) retention time within 1 s of the standard retention time (ii) signal to noise ratio > than 3:1 and (iii) signal > limit of reporting (LOR) referring to the average noise level in the field blanks. The limit of determination (LOD) was 0.5 ng/mL, the method detection limits (MDL) are given in Table 2.

2.4. Estimation of PAH concentrations in water

The mass of PAHs determined in the field blanks was subtracted from the field samples to correct for contamination during sample handling and processing. The method of calculation of the water concentrations was selected based on the kinetic regime of a compound (integrative or equilibrium) during field exposure. This was assessed using the ratio of the compound mass in the thick and thin samplers (Bartkow et al., 2004). During integrative uptake, the ratio of the mass in both samplers is approximately 1 and declines to the ratio of the

Table 1 – PAH target compounds and labelled standards with physicochemical properties.

Compound	Abbreviation	Number of rings	Quantitation ion	$\log_{10} K_{sw}$ [a]	$\log_{10} K_{ow}$ [b]	48-h LC50 ($\mu g\ L^{-1}$)[c]
Acenaphthene-d10	Ace-d10	3	164.1410	3.35	3.92	–
Phenanthrene-d10	Phe-d10	3	188.1410	3.61	–	–
Chrysene-d12	Chr-d12	4	240.1692	4.91	–	–
Perylene-d12	Per-d12	5	264.1692	5.38	–	–
Phenanthrene	Phe	3	178.0782	3.89	4.52	699
Fluoranthene	Flu	4	202.0782	4.38	5.2	11.38
Pyrene	Pyr	4	202.0782	4.44	5	4.33
Chrysene	Chr	4	228.0939	4.97	5.86	NT
Benzo (a) anthracene	B(a)A	4	228.0939	5.06	5.91	1.48
Perylene	Per	5	252.0939	5.44	6.25	–
Benzo (e) pyrene	B(e)P	5	252.0939	5.45	6.44	1.43
Benzo (k) fluoranthene	B(k)F	5	252.0939	5.51	6.11	–
Benzo (b) fluoranthene	B(b)F	5	252.0939	5.51	5.78	–
Benzo (a) pyrene	B(a)P	5	252.0939	5.52	6.35	1.62
Indeno (1,2,3cd) pyrene	I(c,d)P	6	276.0939	5.72	6.58	–
Benzo (ghi) perylene	B(g,h,i)P	6	276.0939	5.92	6.9	1.04

a taken from Smedes (2007), except for phenanthrene taken from Bauer (2008) and values in italics estimated with regression imputation ($\log K_{sw} = 0.85 \times \log K_{ow} + 0.12$; $r^2 = 0.95$).
b Sangster, J., 2009. LOGKOW. A databank of evaluated octanol–water partition coefficients (Log P). URL: http://logkow.cisti.nrc.ca/logkow/intro.html (accessed 27.10.09).
c 48-h median lethal concentration (48-h LC50) for Daphnia magna as given in Lampi et al. (2006). NT = Nontoxic at concentration levels below maximum water solubility.

Table 2 – Derived sampling rates (L/day) at the different sites for analytes (abbreviations see Table 1) which remained in integrative uptake regime during field deployment (see text for details).

Site	Phe	Flu	Pyr	B(a)A + Chr	Per	B(e)P	B(b)F + B(k)F	B(a)P	I(c,d)P	B(g,h,i)P
3					1.4	1.2	1.4	1.4	2.0	3.2
5	3.9	0.5	0.5	1.4	3.8	3.9	4.4	4.5	7.2	11
10				1.2	3.2	3.2	3.7	3.8	6.0	9.6
11				1.4	3.8	3.9	4.4	4.5	19	23
13					16	17	16	16	17	19
14	8.9	7.6	7.2	4.2	3.7	2.3	2.6	2.1	12	16
15					20	20	19	19	18	20
23					12	9.9	11	10	8.4	13

volumes of thick and thin samplers (mean: 0.29; standard deviation (sd): 0.03) when reaching equilibrium with the sampling phase. We assumed integrative uptake for a compound if two conditions were met: (1) mass ratio of thin to thick samplers >0.65 and (2) if the majority of compounds with a higher sampler-to-water partitioning coefficient (K_{sw}) value (Table 1) than the compound also remained in the integrative uptake regime, else equilibrium with the water phase was assumed. For integrative uptake, the TWA water concentration C_{TWA} was calculated according to:

$$C_{TWA} = \frac{m_s(t)}{K_{sw} V k_e t} \quad (1)$$

where $m_s(t)$ is the mass of compound s in the receiving phase after the deployment time t, V is the volume of the receiving phase and k_e is the exchange rate constant. The product of K_{sw}, k_e and V is also called the substance-specific sampling rate R_S. The values of k_e for the different compounds were derived from the relationship between K_{sw} and the k_e values of the four PRCs that were calculated according to:

$$k_e = \frac{-\ln\left(\frac{m_s(t)}{m_s(0)}\right)}{t} \quad (2)$$

where $m_s(0)$ is the initial mass of compound s in the receiving phase after spiking, which was determined using the method blanks. The relationship between K_{sw} and k_e was modelled using a four parametric log-logistic regression using maximum and minimum k_e values as upper and lower limit, respectively. Only the results for the PRCs of thick samplers were included as the PRCs of the thin samplers exhibited complete loss of most PRCs. In case of equilibrium, the water concentration C_{eq} was calculated according to:

$$C_{eq} = \frac{m_s(t)}{K_{sw} V} \quad (3)$$

2.5. Monitoring of physicochemical variables and macroinvertebrates and computation of biotic indices

D-Opto optical dissolved oxygen loggers (Zebra-Tech, Nelson, New Zealand) were deployed concurrently with the passive samplers at three sites (Fig. 1; Sites 5, 14 and 15) to measure a potential drop in dissolved oxygen from ash and sediment inputs (Minshall, 2003). Physicochemical parameters (temperature, pH, conductivity, dissolved oxygen and turbidity) were measured at the deployment and retrieval of the passive samplers (Hanna Instruments, Melbourne, Australia). Current velocity was estimated based on the time an object needed to travel 1 m stream distance.

The invertebrate fauna from edge/pool habitat was monitored using a rapid bioassessment method that comprised sweep sampling of representative habitats in the streams and field picking of the macroinvertebrates (Chessman, 1995; EPA, 2003). Since two family-level biotic indices were employed for data analysis, the taxa were identified to family-level or lower in the laboratory. We used the $SPEAR_{organic}$ biotic index to detect potential changes in the macroinvertebrate community composition due to toxic effects of PAHs or other fire organics as this index demonstrated high selectivity in its response towards organic toxicants in two other studies (Beketov and Liess, 2008; Schletterer et al., 2010). Secondly, an established Australian biotic index, SIGNAL scores, that responds to a variety of stressors was employed to identify general effects on the invertebrate community from fire-associated stressors (Chessman, 1995; EPA, 2003). Both indices are calculated by averaging sensitivity values of all taxa in a sample. For $SPEAR_{organic}$ and SIGNAL scores, the sensitivity value represents the calculated relative sensitivity of a taxon to organic toxicants ($S_{organic}$) (von der Ohe and Liess, 2004) and an assigned general pollution sensitivity grade (Chessman, 1995), respectively. The taxa found in the sampling, their sensitivity values and details on the calculation of $S_{organic}$ can be found in the Supporting material, Table S2. For both indices we examined changes from the beginning of the fires to the post-fire period including the first rainfall event by computing the ratio of the index values.

2.6. Data analysis

Although the thick and thin samplers do not represent a sample from the same statistical population in a strict sense, we calculated the relative range (RR) as dispersion measure for the estimated water concentrations:

$$RR(\%) = (\max(X) - \min(X)) \frac{}{\overline{X}} \quad (4)$$

where X are the observations for the respective compound at a certain site and $\overline{X}$ is the mean of X. The RR is a more conservative estimate of the sample dispersion compared to the relative standard deviation. All statistical computations and graphics (except Fig. 1 created with Quantum Gis, www.qgis.org) were created with the open-source software package R (www.r-project.org) using version 2.10.0 (R Development Core Team, 2009).

3. Results

3.1. Release of performance reference compounds from the passive samplers and sampling rates

The PRCs spiked into the PDMS passive samplers before exposure exhibited different release rates for the compounds and in the sites. Acenaphthene-d10 dissipated almost completely from the receiving phase in all sites relating to an exchange rate coefficient k_e of 0.15 d^{-1}, whereas perylene-d10 exhibited negligible dissipation into the water phase resulting in a very low k_e (Fig. 2). Phenanthrene-d10 showed the largest variation between sites in terms of release rate from the passive samplers ranging from complete loss to high retainment in the receiving phase. In all sites, PRCs exhibited an increasing release from the receiving phase with a decrease of the $\log_{10} K_{sw}$, which translates to a higher k_e with a lower log K_{sw} for a PRC (Fig. 2).

With a few exceptions, the sampling rates of compounds were relatively similar in a sampling site. The sampling rates between sites exhibited higher variation with Sites 13 and 15 having the highest sampling rates (Table 2).

3.2. Polycyclic aromatic hydrocarbons in the passive samplers and calculated water concentrations

All target analytes were quantified above the LOD in the PDMS passive samplers and except for Site 22 positive detections in thin samplers corresponded to a detection in thick samplers.

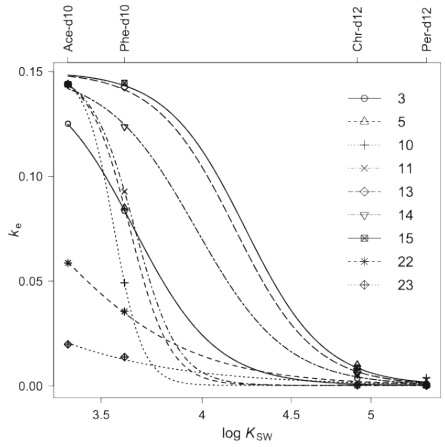

Fig. 2 – Relationship between the exchange rate constant k_e and the sampler-to-water partitioning coefficient log K_{SW} for the four performance reference compounds chrysene-d12 (Chr-d12), perylene-d12 (Per-d12), acenaphthene-d10 (Ace-d10) and phenanthrene-d10 (Phe-d10) in the passive samplers in the sampling sites. A four parametric log-logistic regression using maximum and minimum k_e values as upper and lower limit, respectively, was used to model the relationship.

Compounds with lower molecular mass and lower log K_{sw} values were more frequently detected (Tables 1 and 3). Phenanthrene, benzo (a) pyrene, perylene and the sum of chrysene and benzo (a) anthracene were found in all sites and phenanthrene, pyrene and fluoranthene accounted for 91% of total PAH water concentrations overall sites (for 89% of mass accumulated in samplers) with several orders of magnitude higher concentrations compared to other PAHs determined in this study (Table 3). Site 15 and 22 exhibited 5–60-fold lower concentrations in PAHs compared to the other sites.

Most compounds with a log $K_{sw} < 5$, which relates to an approximate $\log_{10} K_{ow} < 6$, reached equilibrium between the passive samplers' receiving phase and the water phase within exposure time. By contrast, for samplers deployed at Sites 5 and 14 (the closest sites to the fires) all of the compounds in the samplers remained in the integrative uptake regime indicated by a mass ratio of thin to thick samplers of approximately 1 (Fig. 3). These sites differed from the other sites especially with regard to the compounds fluoranthene, pyrene and the sum of benzo (a) anthracene and chrysene relating to PAH species with four or less aromatic rings (Tables 1 and 3). Site 5 exhibited the highest estimated PAH water concentrations of all sites and compounds with up to 20-fold higher concentrations for fluoranthene and pyrene and 5- to 50-fold higher concentration in the total PAHs than the other sites (Table 3).

The variation in the calculated water concentrations between the thin and thick passive samplers was relatively high ranging from 21% to 56% mean relative range for the different PAH species and reaching over 100% relative range for a few observations (Table 3).

3.3. Change of physicochemical parameters and biotic indices associated with the wildfires

Most physicochemical parameters exhibited only a slight change (<20%) from the beginning of the wildfires to the period after the fires. Dissolved oxygen exhibited the greatest change with up to 50% decrease at Sites 3 and 5 (Supplementary material, Table S1).

The continuous DO loggers showed a temporary decrease in Sites 5 and 14 that had a burnt catchment whereas Site 15 exhibited no response. The decrease was most pronounced in Site 5 where the daily minimum in %DO decreased from approximately 70–45% following the two rain events in march (Supplementary material, Fig. S1).

No decrease in the $SPEAR_{organic}$ was observed from pre- to post-rainfall period indicating no change in the macroinvertebrate community with respect to the proportion of sensitive species (Fig. 4). The biotic index SIGNAL scores exhibited a decrease in only two of the sampling sites (5 and 13) when compared to the period at the beginning of the wildfires (Fig. 4).

4. Discussion

4.1. Performance of the PDMS passive samplers when used to monitor organic toxicants

We used performance reference compounds (PRCs) as in-situ calibration to account for differences in the environmental

Table 3 – Time-weighted average (italics) and equilibrium water concentrations (pg/L) of PAHs with variation (±Relative range) and selected PAH ratios determined with passive samplers of two thicknesses in the sampling sites (numbers rounded to first two digits). Sites sorted according to distance to fires in increasing order. Compounds sorted according to molecular mass in increasing order.

Site	Phe	Flu	Pyr	B(a)A + Chr	Per	B(e)P	B(b)F + B(k)F	B(a)P	I(c,d)P	B(g,h,i)P	∑Total	Flu/ (Flu + Pyr)	I(c,d)P/(I(c,d)P + B(g,h,i)P)	Sum[d]
14	550	240	320	78	9.1	7.2	11	12	12	3.9	1200	0.43	0.75	1.18
	(±14%)	(±20%)	(±7.7%)	(±14%)	(±82%)	(±29%)	(±57%)	(±59%)	(±68%)	(±170%)				
3	630	180	130	8	24	9.1	38	48	13	9.3	1100	0.59	0.59	1.17
	(±43%)	(±56%)	(±18%)	(±26%)	(±105%)	(±25%)	(±6.8%)	(±4.2%)	(±36%)	(±34%)				
5	260	5300	3300	308	25	12	16	27	14	8.7	9300	0.62	0.61	1.22
	(±4.6%)	(±36%)	(±16%)	(±7.7%)	(±26%)	(±6.9%)	(±8.7%)	(±30%)	(±16%)	(±0.0%)				
11	280	57	89	310	21	18	28	48	22	15	890	0.39	0.58	0.97
	(±31%)	(±54%)	(±83%)	(±28%)	(±16%)	(±55%)	(±75%)	(±0.0%)	(±83%)	(±86%)				
13	1200	250	260	14	9.5	8.5	11	32	13	13	1900	0.48	0.5	0.99
	(±130%)	(±71%)	(±99%)	(±66%)	(±86%)	(±24%)	(±11%)	(±47%)	(±12%)	(±7.8%)				
10	460	55	80	130	6.9	9.7	22	67	17	13	850	0.41	0.57	0.98
	(±55%)	(±16%)	(±63%)	(±34%)	(±29%)	(±0.0%)	(±7.7%)	(±69%)	(±15%)	(±15%)				
15[a]	63	30	27	1.6	2.2	1.5	< 0.07	8.3	<0.04	<0.02	130	0.53	–[e]	–[e]
22[b]	160	<1	<0.8	0.6	<0.08	<0.08	0.08	0.09	0.06	0.04	160	–[e]	0.57	–[e]
	(±27%)													
23	780	126	120	4.8	6.4	5.3	8.2	4.9	7.7	6.1	1100	0.51	0.56	1.07
	(±27%)	(±12%)	(±17%)	(±54%)	(±47%)	(±5.5%)	(±6.9%)	(±170%)	(±10%)	(±6.9%)				
MDL[c]	3	1	0.8	0.2	0.08	0.08	0.07	0.06	0.04	0.02				

a No variation available since the thin sampler was lost.
b No variation given since estimated water concentrations were close to the LOD, resulting in observations <LOD in the thin samplers.
c Method detection limit calculated using limit of detection of 0.5 ng/mL. Note that the MDL varies with the kinetic regime and site, the MDL given here refers to equilibrium regime at Site 22.
d Sum of the two PAH ratios (Flu/(Flu + Pyr) + I(c,d)P/(I(c,d)P + B(g,h,i)P)).
e Not calculated due to observations <LOD.

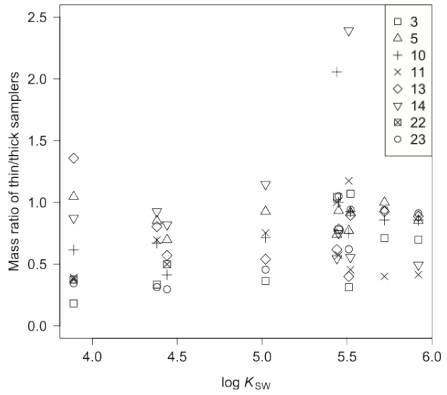

Fig. 3 – Relationship between the mass ratio of thin to thick samplers for the PAH analytes and the sampler-to-water partitioning coefficient log K_{sw} in the different sites. Site 15 is not displayed as the thin sampler was lost. Only ratios relating to concentrations above LOD in both samplers were included. Distance to burnt upstream sections was <1 km for Site 14, 5 km for Sites 3 and 5, 10 km for Sites 10 and 11, whereas all other sites had no burnt upstream catchment.

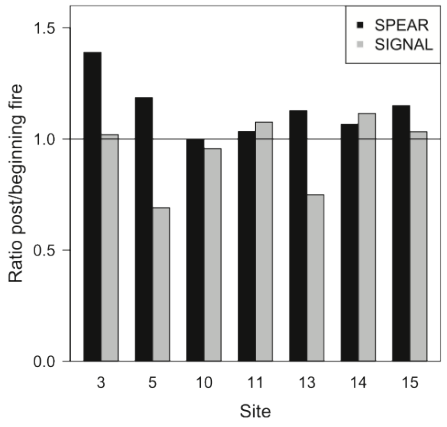

Fig. 4 – Ratio of two biotic indices ($SPEAR_{organic}$ and SIGNAL scores) from the post-fire to the beginning of the fire period in the different sampling sites. Sites 22 and 23 not displayed as flow ceased and therefore no biotic samples were available after the fires. Distance to burnt upstream sections was <1 km for Site 14, 5 km for Sites 3 and 5, 10 km for Sites 10 and 11, whereas all other sites had no burnt upstream catchment.

parameters between sites and to estimate water concentrations for compounds in the PDMS passive samplers that remained in the integrative uptake regime during deployment. PRCs are usually structurally related analogues of the target compounds that are spiked into the receiving phase before deployment and provide information on the dissipation rate. The release rates can be used to estimate uptake rates under the assumption of isotropic exchange (Huckins et al., 2002). Ideally, the selected PRCs would neither completely dissipate nor be fully retained in the receiving phase to allow for a precise estimate of the exchange rate coefficient and of the differences in the environmental conditions between sites. However, three of the four PRCs used in this study were almost completely released (acenaphthene-d10) or retained (perylene-d12, chrysene-d12) in all of the sites (Fig. 2). Only phenanthrene-d10 exhibited larger variation in the dissipation rate between sites resulting in a major influence on the modelling of the relationship between k_e and log K_{sw} (Fig. 2). The low dissipation rate resulting in high retainment of perylene-d12 and chrysene-d12 is in accordance with a study on the elimination of PRCs from various passive samplers that also reported almost complete retainment in the receiving phase for compounds with a log $K_{sw} > 4.5$ over a 28-day field trial (Allan et al., 2009). By contrast, the predictions of high retainment in the receiving phase for compounds with a log K_{sw} as low as 4 in half of the sites by the model for the relationship between k_e and log K_{sw} (Fig. 2) do not match with the study of Allan et al. (2009) where considerable dissipation was observed for such compounds. Furthermore, under the assumption of isotropic exchange low dissipation rates correspond to low uptake rates, which would result in remaining in the integrative uptake regime for compounds with a log K_{sw} between 4 and 5. This prediction of the model (Fig. 2) contrasts with the results obtained for the determination of the kinetic regime using passive samplers of two different thicknesses (Bartkow et al., 2004). Here, most compounds with a log $K_{sw} < 4.5$ reached equilibrium with the water phase during exposure (Fig. 3). Possible explanations for the discrepancy between the dissipation of PRCs and the assessment of the kinetic regime using passive samplers of two different thicknesses include (1) that the uptake was slightly higher than the release from the receiving phase as already observed in another study (Müller et al., 2001) and (2) that the modelled relationship underestimated the dissipation rates. From both explanations follows that k_e and consequently the sampling rates were underestimated (see Equation (1)). Nevertheless, the sampling rates derived using the predictions of k_e values ranged from 0.5 to 50 L/day (Table 2) and are in good agreement with PAH sampling rates of PDMS strips in a calibration study (Bauer, 2008). In this study, R_s was below 1 L/day under stagnant conditions, between 1 and 10 L/day under a flow of 5–17 cm/s and between 5 and 63 L/day under a flow of 32 cm/s. This matches generally with our observations as the R_s were between 0.5 and 18 L/day for streams with a flow ≤20 cm/s and the R_s values in the fastest flowing (approximately 25 cm/s) streams 13 and 15 were higher than the other streams reaching up to 50 L/day. However, the sampling rates determined in this study should be regarded as approximate estimate as they were derived from the modelled relationship

between k_e and log K_{sw} and the model was highly influenced by a single PRC.

For relatively polar compounds with a log $K_{sw} < 4$ the PRC-based model predicted a high exchange rate constant relating to equilibrium regime (Fig. 2) in the Sites 5 and 14, whereas the mass ratio of these compounds in thick and thin passive samplers indicated integrative uptake regime (Fig. 3). We suggest to explain this discrepancy with differences in the exposure profile. PRCs dissipate continuously from the receiving phase, whereas the uptake is discontinuous for exposure resulting from episodic events or for exposure commencing at an unknown point in time such as precipitation-driven input of PAHs associated with wildfires (Olivella et al., 2006; Vila-Escale et al., 2007). In this situation, PRC dissipation can correspond to equilibrium regime while the uptake of compounds with a similar log K_{sw} can remain integrative. In fact, the two sites were closest to the burnt catchment area and PAH exposure may have resulted from the input of ash slurry associated with rain events later during deployment. This explanation is supported by two further observations. Firstly, the DO loggers in the sites indicated at least a small drop in dissolved oxygen concentrations that is characteristic for the input of ash slurry after fires (Lyon and O'Connor, 2008) and was not observed in Site 15 without burnt catchment. Secondly, the concentrations of PAHs were elevated in both sites compared to sites with a similar surrounding (Supplementary material, Table S1).

The variation in the water concentrations determined with the thick and thin samplers was relatively high (Table 3) and exceeded the variation observed using the same approach in air passive sampling (Bartkow et al., 2004). In addition, the mass ratios for the compounds in the thick and thin samplers were relatively scattered between the two ratios relating to equilibrium (0.29) and integrative uptake (1) (Fig. 3). We attribute the variation to four sources: (1) general variation in field trials of aquatic passive samplers with low replication (Schäfer et al., 2008b), (2) variation resulting from matrix interference in chemical analysis, (3) concentrations close to the LOD for several compounds and (4) some compounds may have been in the curvilinear regime between integrative uptake and equilibrium. Future studies should consider employing samplers with three or more different thicknesses and/or replicate samplers to decrease variation and increase the robustness of the results.

4.2. PAH concentrations in the streams

The estimated water concentrations ranged from 0.1 to 9 ng/L in the streams of this study, with the highest total concentrations in Site 5 that had its catchment extensively burnt. Although the water concentrations for compounds in the integrative uptake regime may have been overestimated due to an underestimation of the sampling rates (see previous section), the concentrations found were in accordance with two other studies on PAH exposure from wildfires. A total of 5 ng/L of 18 PAHs were measured in grab water samples after the first rainfall event in a Spanish creek after wildfires (Vila-Escale et al., 2007). Similarly, in 4 Spanish streams in riverine remote areas 2, 6, 45 and 160 ng/L of 12 PAHs were detected one month after extensive wildfires in the catchment (Olivella et al., 2006). The concentrations found in our and the latter two studies were a factor of 4–200 lower than those reported for 9 European rivers reviewed in Olivella et al. (2006). Studies using passive samplers in freshwater systems found up to 500 ng/L in individual PAHs in areas with industrial agriculture in the US (Alvarez et al., 2008), 16–30 ng/L in a river draining industrialised agricultural and urban areas in the Netherlands (Allan et al., 2009) and 2.4–5.7 ng/L for 9 PAHs for an Australian urban stream (Müller et al., 1999).

The distribution by ring size showed a predominance of three- and four-ringed PAHs and was highest in the Sites 5 and 14 that were closest to the fires (Tables 1 and 3). Similarly, the two studies on Spanish streams found mainly low molecular size PAHs (phenanthrene and fluoranthene) that decreased with time after the wildfires (Olivella et al., 2006; Vila-Escale et al., 2007). However, studies in non-wildfire areas also reported a high ratio of three- and four-ringed PAHs (Müller et al., 1999; Alvarez et al., 2008) so that this distribution pattern cannot be used to identify wildfire-borne PAH contamination. Other indicators to identify the source of PAHs include the ratio of certain pairs of PAH compounds such as fluoranthene/(fluoranthene + pyrene) and methyl-substituted to non-substituted PAHs (Yunker et al., 2002) or the determination of C14. We calculated two ratios for pairs of PAHs (Flu/(Flu + Pyr) and I(c,d)P/(I(c,d)P + B(g,h,i)P), see Table 3), for which a ratio >0.50 has been attributed to the combustion of wood, grass and coal, and a ratio <0.5 to the combustion of liquid fossil fuels. Indeed, the sum of both ratios were >1 and significantly higher (Welch's *t*-test, $p < 0.01$, $n = 7$) for the three sites closest to the fires (14, 3 and 5) (Table 3). Nevertheless, care should be taken when using such ratios to infer the sources of PAHs as single ratios of these pairs were (1) >0.5 also for the sites most distant to the wildfires (22 and 23), where the PAHs exposure most likely originated from the combustion of liquid fossil fuels (Table 3, Supporting material Table S1) and (2) highly variable for Spanish streams exposed to wildfire emissions (Olivella et al., 2006; Vila-Escale et al., 2007) (own calculations, see Supporting material Table S3).

4.3. Effects on the invertebrate community

The determination of effects on ecological communities is often based on biotic indices and multiple indices have been established for aquatic invertebrates (Bonada et al., 2006). However, most indices are not capable of differentiating between causes for community change (Bonada et al., 2006; Liess et al., 2008). Biotic indices relying on ecological and/or physiological traits were recently introduced and have demonstrated their capability in selectively identifying effects of specific stressors (Beketov and Liess, 2008; Doledec and Statzner, 2008; Liess et al., 2008). In this study we used the $SPEAR_{organic}$ index (Beketov and Liess, 2008) to identify effects of PAHs or other fire organics on the invertebrate communities and the SIGNAL index (Chessman, 1995) to detect general changes in the community that may have resulted from the fires. The $SPEAR_{organic}$ index showed no change from the beginning of the fires to the post-fire period (Fig. 4) suggesting that organic toxicants including PAH input had no effects on

the communities. Our results are in accordance with a study on the acute toxicity of PAHs and pesticides monitored with passive samplers that found no effects of up to a factor of 100 higher than the PAH concentrations we estimated (Alvarez et al., 2008). Moreover, the concentrations detected in our study were approximately a factor of 1000 or more below the 48-h median lethal concentration (48-h LC50) of PAHs for *Daphnia magna* (Tables 1 and 3) and to our knowledge no field study has shown effects on invertebrate communities at this low toxicity (Schäfer et al., 2007; von der Ohe et al., 2009). Furthermore, widespread sublethal effects at such concentration levels are unlikely given that sublethal effects of aromatic hydrocarbons in aquatic communities were reported to occur a maximum of 24-times below the respective LC50 (Lange et al., 1998). Overall we suggest that the exposure to PAH and other organics related to the wildfire studied had no adverse short-term toxic effects on the macroinvertebrate community. Nevertheless, there may be long-term effects originating from PAHs and other fire organics adsorbed to sediments, which have not been the scope of this investigation (Maltby et al., 1995). In addition, in scenarios of more intensive rainfalls after fires the concentrations of PAHs and other fire organics may be higher and might reach levels of acute effects.

The SIGNAL index showed a decrease in ecologically sensitive species in two sampling sites (5 and 13) from the beginning of the fires to the post-fire period (Fig. 4). Site 5 was presumably subject to an input of ash slurry as it received discharge from several smaller streams from the burnt region (5 km downstream of burnt catchment) and the DO saturation dropped in association with rain events. This input may have affected the invertebrate community via the decrease in DO or a rise in sediments, though this remains open to speculation until a thorough investigation is conducted in these single sites.

5. Conclusions

Silicone-based passive samplers are suitable to monitor organic compounds of a wide-range of polarity and using passive samplers of two different thicknesses is superior to the PRC approach in determining the kinetic regime of a compound after field deployment. The estimated PAH water concentrations in streams in the vicinity of wildfires are of a similar order of magnitude or lower than those in streams in urban areas or in areas with industrialised agriculture. Acute toxicity from PAHs associated with wildfires is presumably not a key culprit for observed changes in aquatic communities following wildfires.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.watres.2010.05.044.

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4. Publication III

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The footprint of pesticide stress in communities—Species traits reveal community effects of toxicants

Matthias Liess[a,*], *Ralf B. Schäfer*[a,b], *Carola A. Schriever*[a]

[a]*UFZ - Helmholtz Centre for Environmental Research, Department of System Ecotoxicology, Permoser Straße 15, 04318 Leipzig, Germany*
[b]*University Lüneburg, Institute for Ecology and Environmental Chemistry, Germany*

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ABSTRACT

The predictive power of the current risk-assessment framework for pesticides remains uncertain. This is because any extrapolation towards landscape-level effects encounters considerable uncertainties: (i) when proceeding from the level of individual single-species tests to populations and communities, biological interactions are not considered; (ii) from mesocosms to field communities, environmental factors and stressors that determine the effects of pesticides in the field are not considered; and (iii) most monitoring investigations are restricted spatially and do not consider recolonisation, and lack an adequate means of distinguishing confounding factors from natural variation. We advocate using species traits as community descriptors, to determine quantitative links between pesticide toxicity and community alterations. Recently, a trait-based indicator system was developed to identify SPEcies At Risk (SPEAR) of being affected by pesticides, with reference to life-history and physiological traits. This SPEAR system has now been successfully employed to link pesticide exposure and effects in Finland, France and Germany. The effect of pesticides on the structure of communities described with SPEAR was independent of the biogeographical region. We then extrapolated and visualised the anticipated risk for aquatic communities in small agricultural streams within Europe in a risk map. With this information we identified a potential risk from pesticide runoff in a high proportion of streams. By focusing on the ecological effect of selected environmental factors, trait-based approaches offer an increased realism for risk assessment of toxicants on the ecosystem level.

1. The challenge: to link pesticide exposure and effects on natural communities

1.1. *Standard laboratory tests—uncertainty in extrapolating effects of pesticides*

Prospective risk assessment of toxicants is traditionally based on the results of standard laboratory toxicity tests. These tests enable an explicit control of experimental conditions and hence allow for repetition (replicates). Thus different concentration levels can be applied to a set of replicates, so that a dose–response relationship can be established between the toxicant and the individuals investigated.

In order to translate laboratory responses to ecological effects in the ecosystem context, assessment factors are used by regulators to account for uncertainty when extrapolating from the laboratory-based assessment to the real-world situation in the field. Uncertainty arises from various parameters that influence the effect of pesticides in the field but are not considered in standard laboratory toxicity tests: i.e., (i) although the sensitivity of test organisms is relatively high compared to many autochthonous species (Wogram and Liess, 2001), there

* *Corresponding author*. Tel.: +49 341 235 15 78.
E-mail address: matthias.liess@ufz.de (M. Liess).

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will be species in the field that are more sensitive; (ii) recovery can occur at the suborganismal (Duquesne, 2006), organism (Duquesne et al., 2006) and population levels (Liess et al., 2006); (iii) interactions within and between species and populations can cause indirect effects (Liess, 2002; Fleeger et al., 2003; Beketov and Liess, 2006); (iv) environmental stressors like drying (Liess, 1998) or food limitation could alter individual sensitivity (Beketov and Liess, 2005; Pieters and Liess, 2006); and (v) exposure to subsequent pulses or multiple toxicants may amplify the effect of single pulses or compounds (Andersen et al., 2006; Zhao and Newman, 2006).

1.2. *Mesocosms—linking pesticide contamination and community response in aquatic communities*

To account for the shortcomings of standard laboratory tests described above, mesocosms have been employed in numerous investigations to reveal the community effects of pesticides. These systems also allow for the use of replicates with a similar community, so that a dose–response relationship can be obtained under controlled conditions. They have the advantage of accounting for various parameters that can determine the effect of pesticides in the field: (1) presence of various species with different sensitivities, (2) recovery and (3) biological interactions. For a review on the sensitivity of mesocosms and a comparison with the first-tier approach within European Union (EU) administration procedures see Van Wijngaarden et al. (2005).

Nevertheless, uncertainty remains regarding the extent to which the results of mesocosm studies can be extrapolated to the field situation. This is because the parameters that determine the effect of pesticides may differ considerably between mesocosms and the field situation. Therefore conditions in the mesocosms must be matched as closely as possible to those in the field community that needs to be protected. This refers especially to (i) the sensitivity and (ii) ecological traits (e.g. reproduction, mobility, emergence time) of investigated species, and (iii) their biological interactions. In addition (iv) environmental stressors and (v) the spatial connectivity between exposed and reference systems that enable re-invasion (Caquet et al., 2007) need to match the situation in the field. To address these issues a workshop on "Aquatic Mesocosms in Pesticide Registration in Europe: Recent Experiences (AMPERE)" was held in April 2007 under the auspices of SETAC Europe to identify where further work may be needed (http://www.systemecology.eu/AMPERE/Start.html).

1.3. *Field investigations—observing pesticide effects in the field*

Observation of pesticide effects in the field circumvents the problems that stem from artificial systems as described for standard laboratory tests and mesocosms. For several decades field experiments have been conducted on the effects of pesticides released into natural streams. The advantage of these experiments is a clear comparison of the situation before and after the event (pesticide input). Invertebrate community responses and alterations of ecosystem dynamics like detritus processing have been revealed (Yasuno et al., 1981; Wallace et al., 1982). Recently, some studies have also quantified agricultural pesticide exposure, adverse effects on aquatic life, and recovery of invertebrate communities in streams. Mortality of six mayfly species in an Australian river was linked to endosulfan contamination due to runoff (Leonard et al., 2001). Another investigation was able to establish a causal relationship between insecticide exposure and mortality of invertebrate species in streams by combining field observations with a bypass stream microcosm. In this investigation, several invertebrate species that declined in abundance due to pesticides were found to recover within a year (Liess and Schulz, 1999). A review on investigations quantifying the effects of agricultural pesticides in the field can be found in the report of the EU/SETAC workshop on "Effects of Pesticides in the Field" (EPIF) by Liess et al. (2005) (http://www.systemecology.eu/EPiF/Download.html). In several of the reviewed field studies effects of pesticides were identified. Direct and indirect effects have been observed, as well as recovery processes that often attenuate or compensate these effects. In addition it was stated that the risk associated with pesticide use could be predicted in a more realistic way if the risk-assessment strategies included additional processes that are relevant in determining pesticide effect at the landscape level (i.e., recovery through recolonisation or reproduction, biological interactions such as competition and predation, environmental stressors such as drying; potential consequences of indirect effects as well as chronic (long-term) and delayed effects should also be considered).

In addition, the review produced by the EPiF workshop pointed out that most existing studies lack sufficient numbers of sites in various streams of a wider geographical region to evaluate the frequency and distribution of potentially harmful effects of pesticides. Exceptions are (i) a study of twenty-nine Danish streams where the macroinvertebrate composition exhibited change along the gradient of sediment pesticide concentrations (Friberg et al., 2003); (ii) fish-abundance and abiotic monitoring data from Ohio, USA, which showed that the predicted impacted fractions of fish species were correlated with the observed fraction of species lost by the action of toxicant mixtures under field conditions, however with wide confidence limits (Posthuma and De Zwart, 2006); (iii) pesticide concentrations measured in 20 German streams were correlated with both a short-term and long-term change of community composition identified by a trait-based indicator (SPEAR) (Liess and Von der Ohe, 2005). The reasons for the paucity of large-scale studies can be found in the expenses associated with the sampling and analysis of pesticides and in the difficulty of linking exposure and effect at several sites characterized by a contrasting set of physico-chemical parameters determining community composition. As a result, it is often not possible to derive a dose–response relationship, since replicates comprising a similar community are not available. In addition, the occurrence of confounding factors makes it difficult to attribute observed effects to pesticides.

1.4. *Need for an improved risk assessment of pesticide effects in the field*

As outlined above, great uncertainty is currently associated with the risk assessment of pesticides — i.e., with upscaling the effect of pesticides from artificial systems to the field and attributing pesticide contamination to community alterations in the field. This is presently reflected in the use of large assessment factors of up to 100 to account for uncertainty in the risk-assessment procedure. To improve the current situation in risk assessment we need to enhance our ability

to detect effects of pesticides in the field and to establish quantitative links between pesticide concentrations and community alterations. Natural communities consist of various species that are present as a result of the respective set of abiotic and biotic factors at a location. Due to the variability of those factors in space and time, a great multiplicity of diverse communities is present. Hence a given toxicant will be acting on a set of different species at each location. However, species are adapted to their environment by a set of ecological traits. A possible way to reduce the complexity in describing natural communities is therefore to use species traits. The concept of using species traits as community descriptors and linking those to habitat factors was suggested almost two decades ago. Townsend and Hildrew (1994) proposed to link habitat parameters to traits like body size, generation time, reproductive tactics, body form, mobility and potential for regeneration. Recently, species traits were successfully used to reveal quantitative links between pesticide concentration and community alteration (Liess and Von der Ohe, 2005). For this purpose, traits were used that are known to reflect the effect of pesticides on those species. They include (i) physiological sensitivity, which determines the acute effect, (ii) generation time and migration ability, determining recovery ability, and (iii) life cycle characteristics, determining whether a species will be present in the water body during contamination. As shown below, this is an encouraging approach to stimulate further development of a more realistic risk-assessment framework including standard and higher-tier test systems, environmental monitoring, and the application of ecological knowledge in deriving relevant species traits.

2. Linking pesticide exposure to effects on field communities with reference to traits

2.1. *Framework of a trait-based indicator system for pesticides*

Site-specific combinations of environmental factors result in a unique composition of species at each site, making it difficult to identify the effect of individual environmental factors. Potential effects of pesticides are also masked by the variability in species composition. Furthermore, confounding factors can exert an effect on species that could be mistaken as a false positive response to pesticides; for example, the effect of hydrodynamic stress is often associated with runoff-induced pesticide contamination (Liess and Schulz, 1999). The use of species traits represents a promising approach to solve these problems. Regarding natural variability, it was reported that the proportion of modalities of biological traits was quite stable for least-impacted streams across Europe (Statzner et al., 2004). Furthermore, stressors influence certain trait modalities so as to allow interpretation and/or prediction of community change. The potential effects of a pesticide may thus not be masked when the community composition is described in terms of species traits. Ideally, pesticide-related species traits would be independent of other environmental parameters and only be influenced by the degree of pesticide stress.

Recently a trait-based indicator system was developed in which the community composition is described in terms of SPEcies At Risk (SPEAR) or not at risk (SPEnotAR) of being affected by pesticides. The following species' traits are components of SPEAR: (i) toxicological sensitivity to organic pollutants including pesticides (Wogram and Liess, 2001) (revised at http://www.systemecology.eu/SPEAR/Start.html), (ii) generation time, (iii) migration ability and (iv) emergence time (to indicate the presence of aquatic stages during the main period of agrochemical application) (Fig. 1). A detailed description of the method can be found in Liess and Von der Ohe (2005).

2.2. *Analysing field effects of pesticides using the SPEcies At Risk concept (SPEAR)*

The aim of the initial investigation was to find patterns in aquatic invertebrate community composition that are related to the effects of pesticides. Investigations were carried out in 20 streams in the central region of Germany. To reduce the site-specific variation of community descriptors due to environmental factors other than pesticides, species were classified and grouped according to their vulnerability to

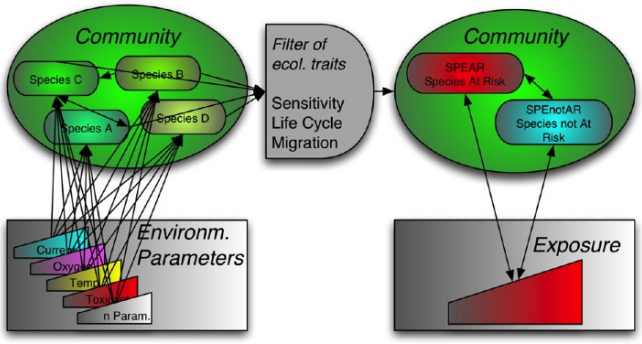

Fig. 1 – Diagram of a trait-based indicator system for pesticides.

pesticides as described above in SPEcies At Risk (SPEAR) and species not at risk (SPEnotAR). Results showed that measured pesticide concentrations of 1:10 of the acute 48-h median lethal concentration (LC50) for *Daphnia magna* led to a short- and long-term reduction of abundance and number of SPEAR and a corresponding increase in SPEnotAR. Measured peak concentrations in the water of less than 1:100 of the acute 48-h LC50 for *D. magna* correlated with a short-term reduction of sensitive species and a long-term change of community composition (Liess and Von der Ohe, 2005). However, it must be noted that aqueous phase pesticide toxicity may be underestimated due to a failure to detect the maximum peak of the event and due to losses during sample transportation and treatment. An extensive discussion of the relevance of other environmental factors can be found in Liess and Von der Ohe (2005). However, the observed temporal change of the community shows that effect and recovery as the consequence of a cyclic pulse contamination during the yearly use of insecticides in early summer should be interpreted on two different time scales:

1. A cyclic recovery within 1 year can be observed at sites affected by pesticides. Assuming a similar magnitude of pesticide stress each year during the application period, the respective community has reached an equilibrium such that recovery will be completed at the beginning of the new exposure period.
2. A long-term shift in invertebrate assemblages at sites influenced by pesticides compared to undisturbed sites. This alteration of community composition is in accordance with the concept of Pollution Induced Community Tolerance (PICT), which states that a toxicant acting as a selection pressure on a community causes a tolerance increase due to exclusion of sensitive species and/or individuals from the community (Blanck and Wangberg, 1988).

We believe that the concurrent occurrence of cyclic recovery and long-term effect "RecoveryEffect" as a consequence of a cyclic pulse contamination with pesticides each early summer is a general phenomenon and should be considered within the risk-assessment framework.

Besides the observed pesticide effects, the study showed that number and abundance of SPEAR in disturbed stream sections were increased significantly when undisturbed stream sections were present in the upstream reaches. Thus, undisturbed upstream reaches compensated for the negative effects of even high concentrations of pesticides, presumably by providing recolonisation pools. The results emphasise the potential of using species traits to reveal community effect of toxicants on the landscape level.

2.3. *Linking pesticide exposure to field effects within the geographical context*

The aim of the investigation following the initial proof of concept (above) was to examine whether the patterns in aquatic invertebrate community composition that are related to the effects of pesticides can be found as well in other biogeographical regions (defined according to Illies, 1978). For this purpose, we analyzed a total of 49 streams in three study regions of contrasting climate in Finland, France and Germany for measured pesticide exposure and invertebrate community composition (for methodological details see Liess and Von der Ohe, 2005; Schäfer et al., 2007). To link pesticide exposure and community composition we again applied the trait-based SPEcies At Risk (SPEAR) indicator system. In France, pesticide stress was associated with a decrease in the relative abundance and number of sensitive species in the communities. In order to account for the climatic differences among the investigated areas we omitted the climate-sensitive traits from the SPEAR framework (time of emergence of merolimnic insects and generation time). We also re-calculated this version of SPEAR for the German sites (Liess and Von der Ohe, 2005) to enable a comparison of all geographic regions investigated. The results are shown in Fig. 2. As in the initial study (Liess and Von der Ohe, 2005) a significant change of the community structure was detectable at a concentration range as low as 1/100 to 1/1000 of the acute 48h-LC50 for *D. magna* (ANOVA). With respect to the geographical differences among sites, it is apparent that the relationship between SPEAR and pesticide toxicity within the streams is the same for all the sites investigated in Finland, France and in Germany. This is

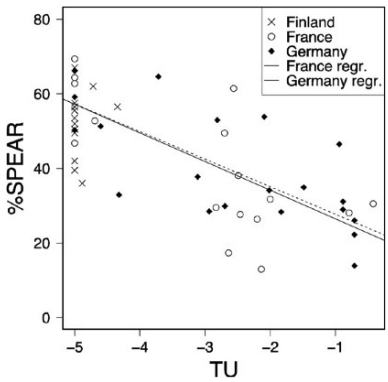

Fig. 2 – Relation between the benthic invertebrate community structure expressed as the ratio of abundance of SPEcies At Risk %SPEAR (ratio of abundance of sensitive species, determined using climate-invariant traits) and the toxic unit (*Daphnia magna*) of the sites in France ($n=16$), Germany ($n=20$) and Finland ($n=13$). Linear regression lines are significant with $P<0.01$, $r^2=0.53$ and 0.61 for French and German streams, respectively. The slopes and intercept are not significantly different (analysis of covariance, $P=0.85$). The parameter for the overall linear model including all sites from the three countries is: $r^2=0.56$, $P<0.01$. Note: Due to sparsity of data it remains open if the relationship is continuously linear in the range of TU-3 to TU-4. However, the present linear model represents the most parsimonious model and is therefore selected following Ockham's razor.

especially interesting as out of the total number of 290 taxa that were identified in Finland, France and Germany, only a subset of 20 species (7%) occurred in all of the countries. In regard to the number of taxa per country, the percentage of taxa that were found exclusively in one of the countries ranged from 40% to 47% to 74% for Finland, France and Germany, respectively.

This finding relates to a study comparing the toxicological sensitivity of invertebrates from different climatic regions (Maltby et al., 2005), where species from different geographical regions (palaearctic, nearctic, temperate, tropical) did not differ in their acute toxicity data for 16 insecticides and, hence, in the species sensitivity distributions.

In Finland and France, also the relationship between pesticide contamination and leaf litter processing was investigated. Pesticide contamination resulted in a significantly reduced leaf-breakdown rate for the French sites. Hence, alteration of the community structure due to pesticides may also translate to the functional level of aquatic ecosystems.

For the sites investigated in Finland no relevant pesticide contamination was measured. Nevertheless, the proportion of sensitive species (SPEAR) was comparable to that at similar sites (without pesticide contamination) in France and Germany. Moreover, for the data set comprising all three biogeographical regions (Baltic, Central, Atlantic), the presence of undisturbed upstream reaches partly compensated the effects of pesticide contamination (Schäfer et al., 2007). Our findings suggest that the trait-based SPEAR approach may also be suitable to detect effects of pesticides on the structure of invertebrate communities over large spatial scales.

2.4. *Response of other biological indices to pesticide exposure*

Several biotic metrics have been developed to describe the response of communities to environmental parameters and especially anthropogenic disturbance. In order to investigate the extent to which some of these indices (species number, BMWP scores, Saprobic index and %EPT) detect pesticide stress, a multivariate multiple linear regression analysis was performed with environmental variables as explanatory variables and community descriptors as response variables for the 49 sites investigated in Germany, France and Finland. Details on the environmental parameters measured can be found in the respective publications (Liess and Von der Ohe, 2005; Schäfer et al., 2007). In model-building, at each step we removed the variable with the lowest explanatory power until (i) the number of remaining explanatory variables was ≤6 and (ii) each variable significantly explained at least one response variable. Hierarchical partitioning was employed to determine the relative importance of the variables in the linear models that explained the respective community descriptors (Chevan and Sutherland, 1991).

The results show that SPEAR is the community descriptor most strongly related to toxic units (TU). All other biological indices listed in Table 1 respond to TU only to a minor extent. The strong response to TU is indicative of the high relevance of pesticides for the community structure in agricultural areas. In addition, the results reveal that SPEAR performs best in addressing pesticide toxicity and the importance of undisturbed upstream sections that are relevant for the recovery of the community. The other indices investigated are also affected by pesticide toxicity but are in addition influenced by at least one other environmental parameter (Table 1).

Table 1 – Results of multivariate multiple linear regression analysis with environmental variables as explanatory variables and community descriptors as response variables (n=49)

Biotic indices	SPEAR	Species number	BMWP-score	Saprobic index	% EPT
r^2 (all envir. var.)	70	49	61	55	72
Contribution of envir. var. (%)					
- TU	76	34	19	10	41
- Recovery section	23	32			16
- Velocity		35	55	51	10
- Temperature				39	29
- pH			26		4

Significant variables of the respective model ($P<0.05$ in t-test for significance of a single variable) are given with their percentage of explained variance determined in hierarchical partitioning. Community descriptors are SPEAR (SPEcies At Risk with toxicological sensitivity and migration potential as traits), Species number, BMWP score (Armitage et al., 1983), Saprobic Index (Friedrich, 1990), %EPT (Proportion of abundance of *Ephemeroptera*, *Plecoptera* and *Trichoptera*) (Wallace et al., 1996). Independent variables are TU (Toxic Units according to (Liess and Von der Ohe, 2005), Recovery sections (undisturbed stream sections upstream of the investigated sites (Liess and Von der Ohe, 2005), stream current velocity, mean temperature and pH at the investigated site.

3. Extrapolating pesticide effects to the continental scale

On the basis of the quantitative exposure–effect relationship derived with the SPEAR approach, we extrapolated the anticipated risk for aquatic communities in small agricultural streams to the European level. The extrapolation was based on modelling the runoff potential (RP) of stream sites, which was calculated in a spatially explicit manner from pesticide use, precipitation, topography, land use and soil characteristics in the near-stream environment. The underlying simplified OECD model for runoff complied with the limited availability and resolution of input data for models aiming at larger scales and was realized within a GIS application. A detailed description of the modelling approach validated with field data can be found in Schriever et al. (2007b). The RP was transformed, using a GIS application, to ecological risk at the landscape level by means of a runoff–response relationship between RP and invertebrate community composition given in a large-scale investigation that also took into account the influence of landscape-mediated recovery pools (Schriever et al., 2007a). The community composition was expressed as abundance of SPEAR species (using the traits toxicological sensitivity to organic pollutants and recolonisation potential) and species not at risk. A detailed description of the methods used can be found in Schriever and Liess (2007).

Raster maps for the EU (EU-15, before the enlargement in 2004) indicated that ecological risk from pesticide runoff is

potentially low for streams in 34% of the grid cells with non-irrigated arable land (mostly northern European countries, predicted effects at ≤20% of the streams per cell). In contrast, ecological risk was very high in 19% of the grid cells (central and southern European countries, predicted effects at >90% of the streams per cell). Fig. 3 illustrates the distribution of predicted ecological risk of runoff in the EU-15 countries. Details of results are described in Schriever and Liess (2007).

In a next step the estimates of ecological risk of runoff from this screening approach were compared with results of field investigations for selected regions in Finland, France and Germany (see above, location of sites marked in Fig. 3).

Predicted ecological risk was transformed to the expected number of stream sites per study area where community composition would be affected. This prediction was compared with the observed number of impacted sites. The 13 Finnish sites were located in grid cells with low predicted ecological risk for streams. The predicted median number of affected stream sites was 1.3 and corresponded well to the monitoring results, where no stream communities showed signs of pesticide effects. The 20 German streams were distributed across grid cells that belonged to 4 different classes of predicted ecological risk (low to very high). The resulting median number of affected stream sites was 9.3 and corresponded well to the results of the monitoring study, where the community composition was affected at 11 sites. The 16 French sites were located in grid cells with medium (5 sites) or high (11 sites) scores of ecological risk. The corresponding median estimate of affected stream sites was 9.9 sites and also corresponded well to the number of 9 stream sites that were observed to be affected. In summary, the match of predicted and observed numbers of affected sites suggested that this screening approach produced appropriate estimates of ecological risk resulting from pesticide runoff in the selected regions.

The screening approach presented here may be applied wherever data are available to specify parameters of the runoff

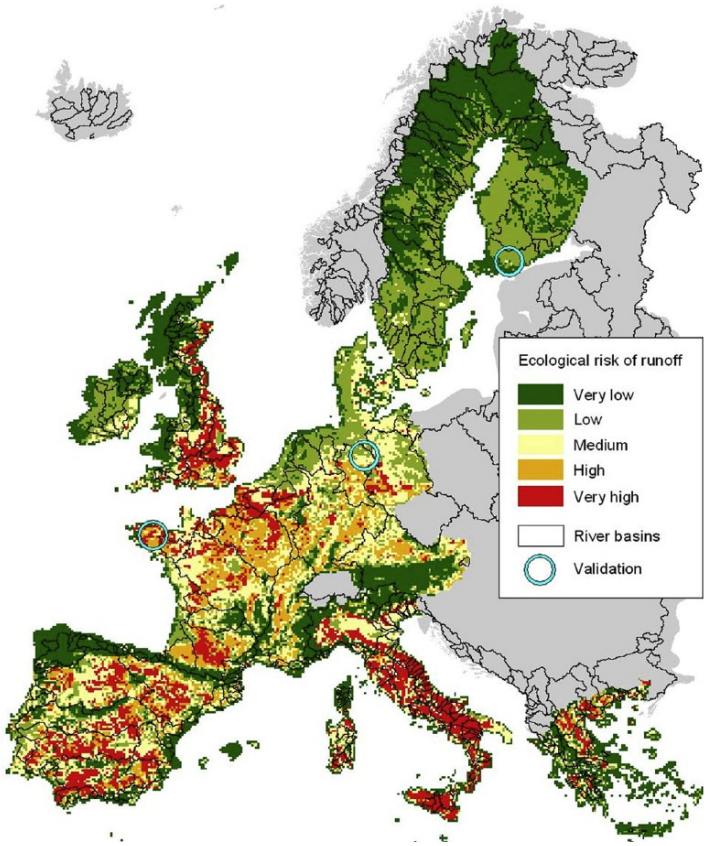

Fig. 3 – Distribution of predicted ecological risk of runoff in the EU-15 countries (10-km^2 raster).

model and to quantify landscape cover. The maps generated are relatively easy to interpret and facilitate communicating areas of concern, where a site-specific assessment would be necessary. Besides identifying areas of concern according to the current pesticide usage, the approach could be employed to establish scenarios to assess the performance of different strategies of exposure management or the effects of climate change.

4. Conclusions

By relating pesticide exposure to the distribution of species traits in communities, exposure can be linked to ecotoxicological effects on the ecosystem level. This approach (i) enables the use of monitoring investigation to identify the ecotoxicological effects of pesticides and (ii) facilitates the prediction of the ecotoxicological effects of pesticides on the ecosystem level. Hence, trait-based approaches offer an increased realism for the risk assessment of toxicants.

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5. Publication IV

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A trait database of stream invertebrates for the ecological risk assessment of single and combined effects of salinity and pesticides in South-East Australia

Ralf B. Schäfer [a,b,*], Ben J. Kefford [a,c], Leon Metzeling [d], Matthias Liess [e], Sinje Burgert [e], Richard Marchant [f], Vincent Pettigrove [g], Peter Goonan [h], Dayanthi Nugegoda [a]

[a] Biotechnology and Environmental Biology, School of Applied Sciences, RMIT University, PO Box 71, Bundoora, Victoria 3083, Australia
[b] Institute for Environmental Sciences, University Koblenz-Landau, Forststraße 7, 76829 Landau, Germany
[c] Centre for Environmental Sustainability, Department of Environmental Science, University of Technology Sydney, 15 Broadway, Ultimo, New South Wales 2007, Australia
[d] Environment Protection Authority Victoria, Ernest Jones Drive, Macleod, Victoria 3085, Australia
[e] UFZ-Helmholtz Centre for Environmental Research, Department System Ecotoxicology, Permoser Straße 15, 04318 Leipzig, Germany
[f] Department of Entomology, Museum of Victoria, PO Box 666, Victoria 3001, Australia
[g] CAPIM, Bio21 Institute, University of Melbourne, 30 Flemington Road, Parkville Vic 3010, Australia
[h] South Australia Environment Protection Authority, PO Box 2607, Adelaide 5001, South Australia, Australia

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ABSTRACT

We compiled a database on *a priori* selected traits for South-East Australian freshwater macroinvertebrate families and used this data for the development of a biotic indicator for the detection of the effects of salinisation on freshwater communities ($SPEAR_{salinity}$) and for the adaptation of the existing $SPEAR_{pesticides}$ index for South-East Australian taxa. The $SPEAR_{salinity}$ indicator showed a reasonably high relationship ($0.38 \leq r^2 \leq 0.5$) with salinity in terms of logarithmic electrical conductivity (log EC) using field biomonitoring data from 835 pools and riffle sites in Victoria and South Australia. Several other biotic indexes that were calculated for comparison purpose exhibited a lower relationship with log EC. In addition, $SPEAR_{salinity}$ was the only indicator that did not respond to other water quality variables and was therefore most selective. We used log EC data and modelled pesticide exposure for sites in Victoria in concert with $SPEAR_{salinity}$ and the existing $SPEAR_{pesticides}$ index to assess whether pesticides interacts with effects of salinity on invertebrate communities and vice versa. No interaction with pesticides was found for the effect of log EC on $SPEAR_{salinity}$, whereas EC interacted with the estimated pesticide exposure on the invertebrate communities. To foster the development of further trait-based ecological indicators, we suggest a conceptual model that predicts response traits based on the disturbance regime and disturbance mode of action of the stressor. Biotic indicators based on *a priori* selected traits represent a promising biomonitoring tool even for regions where ecological information is scarce.

1. Introduction

The sustainable management of freshwater resources relies on the continuous monitoring of their ecological status. Biotic indicators represent an important tool for the assessment of the ecological status of freshwater ecosystems. Most indicators are based on taxonomic properties of the aquatic macroinvertebrate community such as species richness, the fraction of Ephemeroptera, Plecoptera and Trichoptera taxa (% EPT) or the ratio of the number of observed (O) taxa to the taxa which would be expected (E) if the system was in a reference state (O/E) as in RIVPACS or AUSRIVAS (Marchant et al., 1997; Wright et al., 1993). Whereas several indicators are reliable in detecting ecological degradation (Böhmer et al., 2004), taxonomy-based indicators generally do not respond selectively to specific stressors and therefore they do not identify the stressor(s) responsible for an observed ecological impairment. This is because clear inference of causes of impairment based on the taxonomic composition of communities is difficult given (1) the variation of ecological communities in time and space and (2) combined effects of different stressors (Liess et al., 2008; Statzner and Beche, 2010). Biotic indicators based on biological (e.g. body size, generation time and mode of reproduction) and/or physiological traits (e.g. physiological sensitivity) have been advocated as a tool to identify stressor-specific effects and to disentangle effects of multiple stressors (Liess et al., 2008; Statzner and Beche, 2010). For example, the trait-based species at risk (SPEAR) approach has been used to link pesticide (Liess and von der Ohe, 2005; Schäfer et al., 2007) and organic toxicant (Beketov and Liess, 2008; von der Ohe et al., 2009) exposure to changes in the trait composition of invertebrate communities in streams of Europe

* Corresponding author at: Institute for Environmental Sciences, University Koblenz-Landau, Forststraße 7, 76829 Landau, Germany. Tel.: +49 6341 280 325; fax: +49 6341 280 326.
E-mail address: senator@ecotoxicology.de (R.B. Schäfer).

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and Siberia. These and further studies demonstrated that the link between the so-called $SPEAR_{pesticides}$ and $SPEAR_{organic}$ indicator and the respective stressors is selective as the indicators did not respond to other potentially confounding environmental gradients (Liess et al., 2008; von der Ohe et al., 2007).

The application of trait-based indicators in risk assessment and biomonitoring requires the compilation of trait information for taxa occurring in the region under consideration. Trait databases have been compiled for macroinvertebrates in Europe or North America e.g. (Schmidt-Kloiber et al., 2006; Vieira et al., 2006) but are not available for most regions of the Southern hemisphere and Asia. Compared to Europe and North America, ecological information in these regions is much scarcer and consequently a trait database would presumably contain less reliable ecological information on fewer traits and on a higher taxonomic level.

Salinisation of freshwater ecosystems is a global problem mainly affecting arid and semi-arid regions such as the Middle East, the Indian Subcontinent and South-East Australia. Anthropogenic drivers of salinisation include the land clearing of native vegetation and irrigation for agriculture both elevating saline discharges to freshwater streams and lakes (Williams, 1987). In many freshwater streams and rivers, anthropogenic salinisation has resulted in an increase of the electrical conductivity (EC) from below 500 µS/cm to several mS/cm (Williams, 1987). Although some variation is now acknowledged (Radke et al., 2002), most saline waters in southern Australia have ionic proportions similar to sea water (Bayly and Williams, 1973). The reason for this is that the salts originate from small masses of marine salts in rainfall, which over long time scales have concentrated in soils and groundwater (Herczeg et al., 2001).

The present study had the objectives (1) to develop a new trait-based indicator for the impact of salinisation on South-East Australian streams using the SPEAR approach ($SPEAR_{salinity}$) and evaluate its performance in comparison with other biotic indicators. Since pesticide exposure and anthropogenic salinisation of streams are both caused by agricultural land-use and can therefore co-occur, another aim (2) was to use $SPEAR_{salinity}$ and $SPEAR_{pesticides}$ to detect potential interaction in the effects of the salinity and pesticides. Finally, we wanted (3) to evaluate to which extent the limited availability of information on Australian invertebrate taxa constrains the implementation of trait-based approaches.

2. Methods

2.1. Selection of traits and compilation of the trait database

We compiled a family-level trait database for 172 taxa found in stream biomonitoring programs of the Environment Protection Authority (EPA) Victoria and the EPA South Australia (see next section). The database was generated at the taxonomic level of family as trait data is generally scarce and for most taxa only available at this level. For the development of the $SPEAR_{salinity}$ index, the traits were selected based on availability of trait information and *a priori* ecological hypotheses regarding which taxa would be most tolerant to salinisation of streams and availability of trait information. The resulting traits (and corresponding ecological hypotheses) were: (1) reproduction type (taxa with non-aquatic early life stages such as eggs and hatchlings are more tolerant (Kefford et al., 2004; Kefford et al., 2007)), (2) food source (carnivorous taxa are more tolerant due to an energy-rich diet (Piscart et al., 2006)), (3) respiration mode (taxa that do not respire in water reduce their permeability to ions and water and are thus less susceptible to increasing salinity) (4) physiological sensitivity to salinity (taxa with lower physiological sensitivity are more tolerant). For the adaptation of the $SPEAR_{pesticides}$ index for South-East Australian taxa, data on the traits generation time, dispersal capacity, time of emergence and physiological sensitivity to organic toxicants were compiled (see Liess and von der Ohe (2005) for details).

Trait information was collected consulting 85 peer-reviewed journal articles, books and identification keys (Table S1, Supplementary information). The physiological trait "sensitivity to organic toxicants" of the $SPEAR_{pesticides}$ (S_{org}) was based on laboratory data on organic toxicants for European and North American invertebrate taxa (von der Ohe and Liess, 2004) since this data is almost absent for Australasian taxa. For the trait "physiological sensitivity to salinity", laboratory determined median lethal concentrations following 72 h (72-h LC50) were available for most Australian families (Dunlop et al., 2008; Kefford et al., 2003; Kefford et al., 2005a; Kefford et al., 2006). Missing values in the trait database were filled (1) with expert knowledge from Victorian limnologists, (2) to a minor extent by consulting another database (Hawking et al., 2010) or (3) by interpolation from related families in the same order. The complete database is available as Supplementary information to this paper (Table S1).

2.2. Field data from stream biomonitoring programs in South-East Australia

For field testing of the developed and adapted trait indicators, we used biomonitoring data on macroinvertebrates from the Australian River Assessment System (AUSRIVAS) program from the adjoining states South Australia (SA EPA, 2002) and Victoria (Tiller and Metzeling, 1998). The data set comprised 482 sites in southern Victoria sampled between 2000 and 2008 and 408 sites in South Australia sampled between 1994 and 2001 (Fig. 1). Each site was sampled in spring and autumn and approximately 15% of the sites were sampled in multiple years. The sampling was conducted according to a rapid bioassessment method and included taking of a pool sample of representative habitats and of a kick sample where riffles were present (Chessman, 1995). In Victoria, live sorting of the pool and riffle samples occurred for a minimum of 30 min in the field (Chessman, 1995; EPA, 2003), whereas the South Australian method required preservation of the sample and sorting in the laboratory of a minimum of 10% of the sample (SA EPA, 2002). This may affect efficiency of detecting taxa (Marchant et al., 1997) and the data sets were therefore analysed separately. Indeed, the average taxa richness per sample differed between both regions with 22 for Victorian samples and 32 for South Australian samples, though we cannot quantify as to which extent the sampling method contributes to this difference in taxa richness per sample. The taxa were identified in the laboratory to family and species level for most taxa in Victoria and South Australia, respectively. Note that we refer to the data set containing the streams in Victoria with "Victoria", though the sampling sites were spatially confined to selected catchments of this state and thus are not representative for the complete state (Fig. 1). By contrast, most non-sampled areas in South Australia do not contain streams or rivers with regular flow.

For sites in Victorian streams, nine physicochemical variables were available that were measured concurrently with the macroinvertebrate sampling. These variables were salinity as indicated by electrical conductivity at 25 °C (EC), water temperature, pH, turbidity, alkalinity, dissolved oxygen, total phosphorus, NO_x (nitrate and nitrite) and total Kjeldahl nitrogen (Tiller and Metzeling, 1998) (see Table S2, Supplementary information for descriptive statistics). EC for the South Australian streams was also measured in situ (SA EPA, 2002).

2.3. Calculation of SPEAR and other biotic indicators

The SPEAR approach is based on the calculation of the fraction of the abundance of sensitive individuals in a community for a specific stressor (%SPEAR). The first step for this calculation is the binary classification of all taxa as "sensitive" or "tolerant" for each of the traits of the respective indicator. Taxa that are classified as "sensitive" for all traits belonging to the respective indicator are defined as SPEAR and

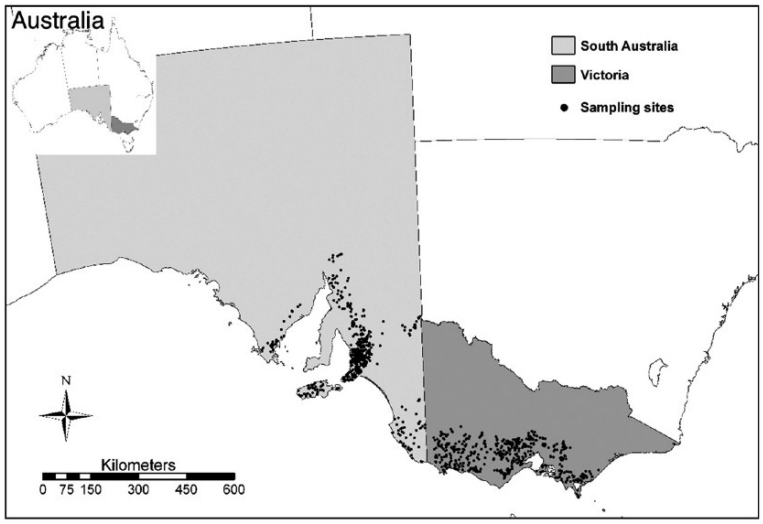

Fig. 1. Locations of the sampling sites in Victoria and South Australia, which were sampled between 2000 and 2008 and between 1994 and 2001, respectively. Ten and 45 sites in Victoria and South Australia were excluded from data analysis (see section "Data analysis") and are not displayed.

the fraction of SPEAR in a community (%SPEAR) of a sampling site is calculated as:

$$\%\mathrm{SPEAR} = \frac{\sum_{i}^{n} x_i y_i}{\sum_{i}^{n} x_i} \quad (1)$$

where x_i is the logarithm of the abundance + 1 of species i, n is the total number of species in the sample and y is 1 for a SPEAR taxon, else 0.

The classification criteria for the $SPEAR_{salinity}$ and $SPEAR_{pesticides}$ are displayed in Table 1 and were derived from the hypotheses outlined earlier and taken from Liess and von der Ohe (2005), respectively. Several versions of $SPEAR_{salinity}$ incorporating different trait combinations were analysed during development of the indicator. The final version of the $SPEAR_{salinity}$ indicator described in this paper relies only on physiological salinity sensitivity as this trait was sufficient to achieve a strong linear relationship with salinity. Further details and results for other versions of $SPEAR_{salinity}$ are given in the Supplementary information (Text S1, Table S3).

For comparison purpose, we calculated for the Victorian data set three commonly used biotic indicators (Species richness, Shannon–Wiener diversity and % of Ephemeroptera, Plectoptera and Trichoptera (EPT) taxa), two Australian-wide used indicators to detect general biotic impairment by pollution (SIGNAL (Chessman, 1995) with updated sensitivity scores as given in (Metzeling et al., 2003) and the ratio of observed/expected taxa (O/E) from an AUSRIVAS model that represents the Australian version of RIVPACs (Marchant et al., 1997)) and a specific indicator for the effects of salinity on macroinvertebrates called salinity index (SI) which is described in Horrigan et al. (2005).

2.4. Estimation of ecological risk of pesticides

Since no information on pesticide exposure was available for the sampling sites we used a geographic information system (GIS)-based runoff model to estimate pesticide exposure expressed as runoff potential and the associated ecological risk (ER) at a site (Schriever et al., 2007b). The model incorporated climatic, geographical and land-use information and has already been applied to identify potential hotspots of pesticide runoff in agricultural regions of North Germany (Schriever et al., 2007a) and the European Union (Schriever and Liess, 2007). The ER depended on the runoff potential as well as on the amount of potential recolonisation pools (forests or conservation area) in a model grid cell and was expressed in five categories "very low" to "very high". The ER categories of "medium" and "high" were merged to achieve a more balanced sample size per category and the category "very high" contained no samples. Further details on the adaptation of the GIS-based model for Victorian streams and the relationship with $SPEAR_{pesticides}$ can be found in (Burgert et al., 2010).

2.5. Data analysis

Pairwise correlations between trait indicators for the taxa in the database were calculated using the Pearson's correlation coefficient r and the phi correlation coefficient for binary data.

Prior to analysis of the field data, 42 sites in arid regions with less than 300 mm precipitation were removed from the South Australian data set as streams in these regions are usually ephemeral. In addition, samples with less than 10 reported taxa were removed from both data sets to exclude samples with a heavily degraded fauna or poor sampling performance, which lead to the exclusion of 10 sites in Victoria and 3 sites in South Australia. The decadic logarithm was used to transform physicochemical variables with a wide spread of values (maximum/minimum observation >1000) or a very left-skewed distribution (checked visually). Taxa abundances were log (x + 1) transformed.

The performance of a biotic indicator for detecting effects of salinity was evaluated based on the (1) strength of the relationship with log EC in a linear regression model and (2) selectivity to log EC, where ideal selectivity would translate to no explanatory power of physicochemical variables other than log EC in a linear multiple regression model.

Goodness of fit of linear regression models was assessed with the adjusted r^2 (r^2 for models with only one explanatory variable).

Table 1
Traits compiled for the SPEAR indicators with classification criteria.

Trait	Scale of trait and levels	Indicator	Criteria for species at risk (SPEAR)
Physiological sensitivity to salinity	Interval scale (0.1 to 160 mS/cm) or ordinal scale: "high"; "medium"; "medium–low", "low"	$SPEAR_{salinity}$	Taxa with <medium tolerance or majority of taxa in family with 72 h LC50<35 mS/cm[a]
Respiration mode[b]	Nominal scale: "air-breathing"; "cutaneous"; "gills"; "plastron"; "pneumostome"	$SPEAR_{salinity}$	All except air-breathing taxa
Reproduction type[b]	Nominal scale: "aquatic eggs"*; "eggs attached to substrate, plants or stones"*; "ovoviviparity"; "terrestrial eggs"; "moist places above water level"; "eggs in shallow water"*; "eggs inside plants/objects in water"*; "free eggs"*	$SPEAR_{salinity}$	All modalities indicated with*
Food source[b]	Nominal scale: "detritus"; "plants"; "prey"	$SPEAR_{salinity}$	All except taxa feeding on prey
Number of generations per year	Predominantly interval scale (0.5 to 12), few cases with ordinal scale ("many" or "several")	$SPEAR_{pesticides}$	Number of generations ≤2 and time to reproduction ≥0.5 years
Time to reproduction	Interval scale: 0.1 to 5 years	$SPEAR_{pesticides}$	
Dispersal capacity	Ordinal scale: "high"; "strong drifting or flying"; "some strong drifting or flying"; "low"	$SPEAR_{pesticides}$	"Low" or "some strong drifting or flying taxa"
Duration of life stages out of water[c]	Interval scale (1 to 12 weeks) or nominal scale: "live at the edge of water"; "live on water surface"; "semi-aquatic"; "most time in host"; "fully aquatic"; "short"; "few weeks"	$SPEAR_{pesticides}$	<8 weeks or "fully aquatic" or "short" or "few weeks"
Physiological sensitivity to organic toxicants (S_{org})	Ratio scale (−2.09 to 1)	$SPEAR_{pesticides}$	Taxa with S_{org} value ≥−0.36

[a] Median of the cumulative distribution function for insect taxa in (Kefford et al., 2003).
[b] Was not incorporated in the final version of the $SPEAR_{salinity}$ see Supplementary information (Text S1, Table S3).
[c] Replaces the trait "emergence time of merolimnic insects" in the original $SPEAR_{pesticides}$ (Liess and von der Ohe, 2005) as suggested by Beketov et al. (2009).

Analysis of covariance (ANCOVA) with t-test was applied to check for significant differences in slope or intercept for factors in the linear model. We aggregated the data over sampling period and sampling years since $SPEAR_{salinity}$ did not exhibit significant covariation over these factors (all $p>0.05$), also to avoid temporal pseudoreplication (Hurlbert, 1984). The aggregation was done using the minimum value for a biotic indicator and the maximum stressful value for a physicochemical variable of the raw samples. This aggregation method followed the rationale that semiannual point sampling of physicochemical parameters at a site is very likely to miss the highest stressful value of a pollutant (Richards and Baker, 1993), whereas corresponding biological effects may be expressed by the lowest indicator values. However, using the average for all variables as aggregation method did not change the results presented in this study. The data was analysed separately for pools and riffles since $SPEAR_{salinity}$ exhibited significant covariation over this factor ($p<0.001$ for Victoria and South-Australia).

To evaluate the selectivity of $SPEAR_{salinity}$ and the other biotic indicators, we examined the explanatory power of all physicochemical variables in the Victorian data set for the indicators, employing automatic model building starting with the null model (no explanatory variable included). The statistical procedure was backward and forward entering of physicochemical variables with Akaike's Information Criterion (AIC) as stepwise model selection criterion. Hierarchical partitioning was used to determine the independent explanatory power of the physicochemical variables (Chevan and Sutherland, 1991).

To assess whether pesticides and salinity exhibit interactions in their effects on the community level, we built two linear models: (1) a regression model with $SPEAR_{salinity}$ as response variable and log EC, the factor ER and their interaction term as explanatory variables and (2) a two-way analysis of variance (ANOVA) model with $SPEAR_{pesticides}$ as response variable and ER, classes of EC (EC<1000, 1000<EC<3000 and EC>3000) and their interaction term as explanatory variables. In the second model EC was transformed into a factor to detect which levels of salinity would interact with pesticide exposure. The selected class boundaries corresponded to notable changes in species richness in Victoria (Kefford et al., 2006). As ER was estimated using a pesticide exposure model for the land-use situation in 2006/2007, only biomonitoring data of the years 2004 to 2008 were included for the calculation of the SPEAR indicators. This represented a compromise between a higher sampling size by including more years of biomonitoring data and matching of the biomonitoring sampling time with the modelled exposure time. This analysis was only conducted for pool samples as the sample size for riffle samples was too low. All statistical computations and graphics were created with the open source software package R (www.r-project.org) using version 2.10.0 (for Mac OS X, 10.6.4) (R Development Core Team, 2010).

3. Results

3.1. Selected characteristics of the developed database and the traits

Information on traits and physiological sensitivity could be obtained for the majority of families in the database with only 2.4% of entries assigned as "unknown". Nevertheless, 17% of entries were interpolated from taxonomically related families and 12% of entries were provided by expert knowledge (Table S1, Supplementary information). This means that for each trait on an average for 31% of data entries (range 17% to 48%) no published data were available despite the use of family data. For the classified taxa in the database, the physiological traits (physiological sensitivity to pesticides (S_{org}) and salinity) were not significantly correlated ($\phi=0.08$; $p=0.28$; $n=172$), whereas $SPEAR_{pesticides}$ and $SPEAR_{salinity}$ exhibited a significant but low correlation ($\phi=0.23$; $p=0.002$; $n=172$).

3.2. Relationship between $SPEAR_{salinity}$ and log EC

For the Victorian data set, the $SPEAR_{salinity}$ index exhibited a close linear relationship with log EC in pools and riffles ($r^2=0.5$ and 0.44; $p<0.001$; $n=471$ and 145, respectively) (Fig. 2). For the South Australian data set, log EC explained 45% for pools ($p<0.001$; $n=353$) and 38% for riffles ($p<0.001$; $n=205$) of the variation in $SPEAR_{salinity}$ (Fig. 3).

3.3. Selectivity of $SPEAR_{salinity}$ and other biotic indicators to detect effects of salinity in Victoria

Log EC (and sampling habitat for $SPEAR_{salinity}$) was the only variable selected in automatic model building to explain variation in the $SPEAR_{salinity}$ indicator (Table 2), whereas all other (non-SPEAR) indicators responded significantly to physicochemical variables other than log EC. Only the $SPEAR_{salinity}$ indicator (Fig. 2), SIGNAL (Supplementary information Fig. S1), %EPT (Supplementary information Fig. S2) and the SI (Supplementary information Fig. S3) were explained with an $r^2>0.1$ by log EC, with $SPEAR_{salinity}$ exhibiting the strongest

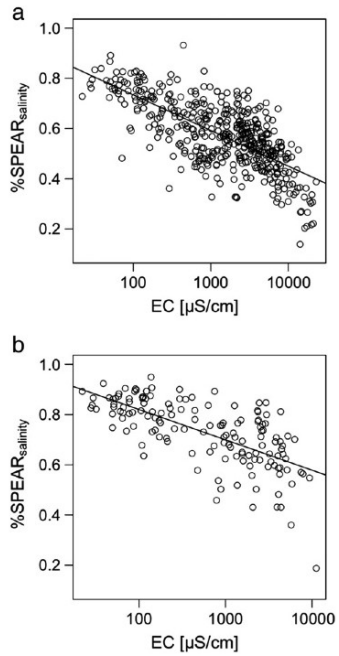

Fig. 2. Relation between the benthic invertebrate community structure expressed as % SPEAR$_{salinity}$ and the log EC in pools (a) and riffles (b) in streams of Victoria, Australia monitored from 2000 to 2008. Linear regression lines are significant with $p<0.001$ and $r^2=0.50$ and 0.45 with a sample size of 471 (pools) and 145 (riffles), respectively.

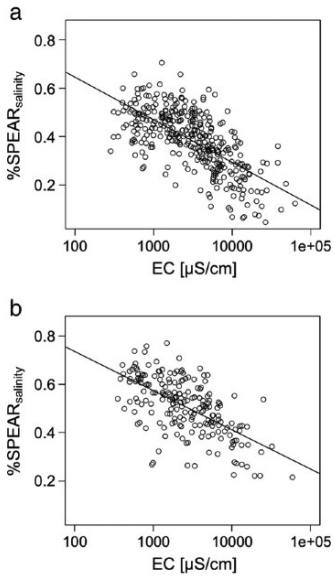

Fig. 3. Relation between the benthic invertebrate community structure expressed as % SPEAR$_{salinity}$ and the log EC in pools (a) and riffles (b) in streams of South Australia monitored from 1994 to 2001. Linear regression lines are significant with $p<0.001$ and $r^2=0.45$ and 0.38 with a sample size of 353 and 205 for pools and riffles, respectively.

relationship with log EC of all indicators (Table 2). Thus, only the SPEAR$_{salinity}$ indicator responded unequivocally to ecological effects of salinity against the background of variation in other physicochemical variables and had the strongest relationship with log EC.

3.4. Interaction in the effects of pesticides and salinity on the community level

Neither a significant main effect ($p=0.92$ and $p=0.94$ for factor level "low" and "medium/high", respectively; ANCOVA with t-test; $n=135$) nor a significant interaction effect with log EC ($p=0.99$ and 0.89) was detected for ER in the regression model for SPEAR$_{salinity}$. By contrast, the two-way ANOVA model for SPEAR$_{pesticides}$ exhibited a significant main effect of EC ($p<0.001$; F-test; $n=135$) and a significant interaction effect of EC with ER ($p=0.01$), which resulted from a different response of SPEAR$_{pesticides}$ to EC over levels of ER (Fig. 4). Whereas the SPEAR$_{pesticides}$ was relatively stable over the EC gradient in the "medium/high" category of ER, the ratio of pesticide-sensitive taxa dropped by approximately 50% in the classes with $EC>1000$ for "very low" and "low" estimated ecological risk of pesticides (Fig. 4).

4. Discussion

4.1. Performance of SPEAR$_{salinity}$ to identify effects of salinity relative to other indicators

We found a good linear relationship of SPEAR$_{salinity}$ (Figs. 2 and 3) with log EC for riffle and pool samples from streams of Victoria and South Australia ($0.38\le r^2\le 0.5$). Similarly, linear relationships were observed for SPEAR$_{pesticides}$ or SPEAR$_{organic}$ indicators (1) with toxicity of pesticides in 36 agricultural streams in two regions of France and Germany (Schäfer et al., 2007), (2) with concentrations of petrochemicals and surfactants in 8 sites in Siberia, Russia (Beketov and Liess, 2008) and (3) with toxicity of 194 organic toxicants in 28 sites in a Spanish catchment (von der Ohe et al., 2009). The amount of explained variance in these studies was generally higher: $0.48<r^2<0.87$. Several factors may explain this higher variability of SPEAR$_{salinity}$ compared to other SPEAR indicators. Firstly, the physiological sensitivity of invertebrates to organic toxicants (S_{org}), which represents the most important trait of both SPEAR$_{pesticides}$ and SPEAR$_{organic}$, ranges almost three logarithmic units from the most to the least sensitive taxa, whereas this range comprises only two logarithmic units for salinity (Table S1, Supplementary information). This means that the response to salinity is more uniform which can be explained by a less specific physiological mode of action compared to pesticides (Stenersen, 2004). Consequently the power to identify effects based on community trait changes is lower compared to organic toxicants. In addition, the physiological salinity sensitivity of almost half of the families in the trait database was classified on an ordinal scale due to a lack of data from laboratory toxicity experiments and most of the toxicological data for the remaining families are from rapid toxicity tests (Kefford et al., 2003; Kefford et al., 2005b), which yield less precise results than standard toxicity tests used for the calculation of the S_{org} (von der Ohe and Liess, 2004). Therefore, the physiological trait data for SPEAR$_{salinity}$ is less precise possibly leading to more misclassification of taxa relative to SPEAR$_{pesticides}$ and SPEAR$_{organic}$ relying on S_{org}. Moreover, the trait database for South-east Australia which was used for the computation of SPEAR$_{salinity}$ indicator was family level while the trait database used for the calculation of SPEAR indicator in Europe and Russia has a finer taxonomic resolution for many taxa (see http://www.systemecology.eu/SPEAR/index.php). However, no notable differences

Table 2
Environmental variables selected in automatic model building with goodness of fit measures and explanatory power for the biotic indicators as determined in hierarchical partitioning. Data set comprised physicochemical and macroinvertebrate observations for 472 Victorian streams. Two physicochemical variables (alkalinity and turbidity) are not displayed as they were not included in any model, which was due to multicollinearity in the case of alkalinity.

	$SPEAR_{salinity}$	Species richness	Shannon-Wiener diversity	SIGNAL	O/E	%EPT	SI
Dissolved oxygen [mg L^{-1}]		21%		23%			22%
EC at 25 °C [µS cm^{-1}][a]	100%	29%	69%	30%		40%	30%
NO_x–N [mg L^{-1}][a,b,c]		6%		11%	10%	15%	10%
Total Kjeldahl Nitrogen-N [mg L^{-1}][a,c]		9%		14%	31%	16%	14%
pH		14%	23%	15%	11%	18%	
Temperature [°C]		17%			48%		17%
Total phosphate-P [mg L^{-1}][a,d]		4%	8%	5%		5%	5%
Sampling method		1%	1%	3%		6%	3%
AIC	−2792	1912	−1179	−902	−1688	−2468	−463
r^2	0.51	0.19	0.05	0.48	0.09	0.55	0.53

[a] Variable was log-transformed.
[b] $NO_x = \Sigma(NO_2 + NO_3)$.
[c] Measured as nitrogen.
[d] Measured as phosphorus.

between species level and family level data for the $SPEAR_{pesticides}$ indicator were found (Beketov et al., 2009) and with few exceptions there is little variation in the acute lethal salinity tolerance within families compared to between families (Kefford et al., 2003; Kefford et al., 2006). Finally, the $SPEAR_{salinity}$ indicator developed in this study was applied on stream biomonitoring data from landscapes with very different land-use including agriculture, grazing, forestry, urban land and nature reserves. By contrast, the biomonitoring data used in former studies with $SPEAR_{pesticides}$ or $SPEAR_{organic}$ were limited to agricultural land-use or did not transcend parts of a single catchment. Whereas several studies showed that the trait patterns and response traits are relatively stable for non-impacted sites over larger biogeographical regions (Schäfer et al., 2007; Statzner et al., 2005; von der Ohe et al., 2007), further analyses should elucidate whether the dose–response relationship between disturbances and traits are stable over landscapes with different land-uses and regions. Variation over these spatial scales may account for some of the variation in the relationship between $SPEAR_{salinity}$ and log EC. Moreover, the ionic proportions are relatively similar to sea water in the southern Australian landscape (Bayly and Williams, 1973; Herczeg et al., 2001). But in regions with industrial discharges such as from saline mines the ionic proportions may differ greatly from sea water and this may in turn alter the relative and absolute toxicity to species. Therefore, a prerequisite before applying $SPEAR_{salinity}$ in such regions is the investigation whether the relative sensitivity ranking for species to sea water holds for other ionic proportions.

However, the trait-based indicator performed better than other indicators in terms of selectivity and strength of the relationship with log EC (Table 2). No strong response to the salinity gradient in terms of explained variance was found for Shannon–Wiener diversity, species richness and observed/expected taxa (O/E) scores. Other studies on the landscape or regional scale also reported that there is no simple linear relationship between species richness and conductivity below an EC of ≈10 mS/cm for streams (Horrigan et al., 2005; Kefford et al., 2006). The poor performance of the ratio of observed/expected taxa (O/E) scores, which were derived from an AUSRIVAS model (Marchant et al., 1997), is in accordance with the results of a previous study on a smaller, more homogeneous subset of our data (Metzeling et al., 2006). The lack of response of O/E scores to log EC may result from the incorporation of

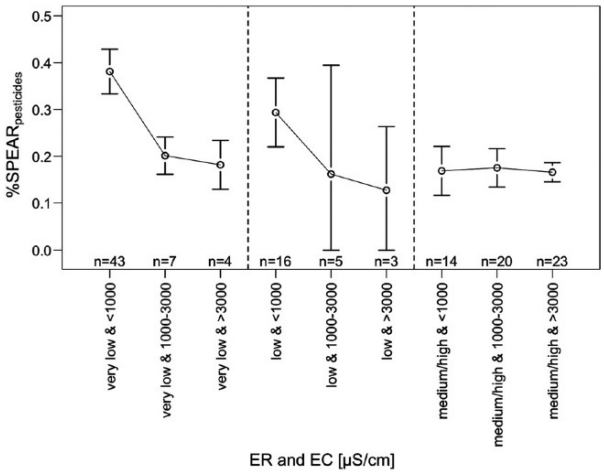

Fig. 4. Mean and 95%-confidence intervals of $SPEAR_{pesticides}$ for estimated Ecological Risk of pesticides and different levels of EC. Only biomonitoring data for pools in streams and rivers in Victoria for the years 2004 to 2008 were used to approximately match the timeframe of the runoff model that predicted the ER. n = sample size.

alkalinity as predictor variable in the AUSRIVAS model. Alkalinity was highly correlated with conductivity ($r = 0.87$; $p < 0.001$; $n = 606$) and elevated alkalinity is a natural feature of some streams in South-east Australia (Williams, 1980). This means that the expected taxa (taxa that occur with a probability >50%) in the AUSRIVAS model are determined from reference streams with a level of EC similar to the site from which the list of observed taxa originates. Hence, anthropogenic secondary salinisation of streams may be masked by streams with a naturally high level of salinity. By contrast, $SPEAR_{salinity}$ responds to an elevation of salinity irrespective of the origin. For freshwater conservation and management, the establishment of natural background levels for salinity at specific sites would be necessary to evaluate whether a reduced $SPEAR_{salinity}$ value refers to anthropogenic disturbance.

The %EPT indicator, SIGNAL scores and the salinity index (SI) (Supplementary information Figs. S1–3) showed a good relationship with log EC, but these indicators responded also to other water quality variables whereas the trait-based $SPEAR_{salinity}$ indicator did not (Table 2). The %EPT indicator and SIGNAL scores aim at detecting general ecological impairment and it is thus not surprising that they do not respond selectively to log EC. By contrast, the SI was developed to identify effects of salinity on macroinvertebrate communities (Horrigan et al., 2005) and ideally should not respond to other water quality variables. However, Horrigan et al. (2005) reported a potential response of the SI to high levels of nutrients and turbidity. Our results confirm this response and SI additionally responded to changes in temperature, pH and alkalinity, although this may be partially attributed to intercorrelation with EC. Finally, the artificial neural network incorporated in the SI was initially calibrated for macroinvertebrate data from Queensland in North-east Australia and it may be that the field sensitivity of Victorian species is slightly different, though this is not supported by results from laboratory toxicity experiments (Dunlop et al., 2008). Overall, we suggest that using ecological reasoning to establish mechanistic models on biological and physiological trait responses to stressors represents a more promising approach for the identification of specific stressors than using statistical or taxonomy-based approaches.

4.2. Using traits to identify interactions of ecological risk

Biological and physiological traits represent a promising approach to disentangle effects and identify interactions of multiple stressors (Statzner and Beche, 2010). The unambiguous and selective identification of a single stressor is a prerequisite for the use of traits to assess the relative effects of multiple stressors. Single biological traits are unlikely to discriminate effects of multiple stressors since single traits usually respond to different stressors. For example, the trait small body size responded to cargo-ship traffic, heavy metal pollution and to differences between temperate and mediterranean streams (Bonada et al., 2007; Doledec and Statzner, 2008). This may be due to phylogenetic relationships between traits (Poff et al., 2006). Until unique biological traits for specific stressors have been identified and rigorously tested it remains difficult to analyse effects of multiple stressors using only biological traits.

By contrast, physiological traits alone had high discriminative power towards other stressors and environmental variables as demonstrated in this study, Beketov and Liess (2008) and in several studies where a physiological trait was combined with biological traits ($SPEAR_{pesticides}$) (Liess et al., 2008). In addition, the physiological traits salinity sensitivity and S_{org} in our study exhibited only negligible correlation ($\phi = 0.08$, $p = 0.28$, $n = 172$) for the taxa in the trait database. When used for the detection of interaction effects between estimated ecological risk from pesticide exposure (ER) and salinity, $SPEAR_{salinity}$ did not respond to different levels of ER. By contrast, $SPEAR_{pesticides}$ decreased for classes of EC>1000 in the ER categories "very low" and "low", but showed no response for "medium/high" ER (Fig. 4). Three hypotheses may explain this response pattern: (1) salinity influences the effect of pesticides, (2) the $SPEAR_{pesticides}$ indicator responds to salinity or (3) the ER underestimates the exposure to pesticides. Although several laboratory experiments reported an interaction of salinity with the effects of pesticides (Hall and Anderson, 1995; Heugens et al., 2001), this interaction would also be expected for the level of "medium/high" ER. Therefore, hypothesis (1) cannot completely explain the observed results. This holds also for hypothesis (2) as a response of $SPEAR_{pesticides}$ should also occur in the "medium/high" ER class. Nevertheless, $SPEAR_{pesticides}$ was slightly correlated with $SPEAR_{salinity}$ ($\phi = 0.23$; $p = 0.002$; $n = 172$) and this may lead to an albeit minor response of $SPEAR_{pesticides}$ to salinity. A finer taxonomic resolution of the trait database would presumably decrease the correlation of $SPEAR_{pesticides}$ and $SPEAR_{salinity}$. We suggest that hypothesis (3) is most likely to explain the observed response pattern of $SPEAR_{pesticides}$ to EC. This is because anthropogenic salinisation and pesticide exposure are both related to agricultural land use in southern Australia (Williams, 1987), therefore both stressors may co-occur. In addition, a field study on streams in three European regions found a significant correlation ($r = 0.54$; $p < 0.001$; $n = 49$) of pesticide toxicity in terms of toxic unit and EC (Schäfer et al., 2007). Hence, the sites in the ER categories "very low" and "low" with EC>1000 may in reality have higher pesticide exposure. This explanation is also supported by the observations that (1) the level of $SPEAR_{pesticides}$ in these categories ("very low" and "low") was similar to the "medium/high" ER category, (2) the variability was relatively high in the categories of lower ER (Fig. 4) and (3) the estimated pesticide exposure estimates risk for a 10 km^2 grid cell and cannot consider local factors that may contribute to higher risk at specific sites due to low resolution input data (see Burgert et al. 2010 for details). Further studies with measured pesticide concentrations and different levels of salinity are needed to clarify whether interaction effects between pesticides and salinity are relevant in the field.

4.3. The relationship between disturbances and traits

The physiological trait alone was sufficient to achieve a strong linear relationship of $SPEAR_{salinity}$ with and selectivity to log EC. The inclusion of additional biological traits did not lead to notable improvements (Supplementary material Text S1 and Table S3). Similarly, a study on eight contaminated streams in Siberia, Russia, found that the physiological trait "sensitivity to organic toxicants" (S_{org}) was sufficient to explain variation in the continuous exposure to petrochemicals and surfactants (Beketov and Liess, 2008). Except for isolated cases of saline water disposal, the exposure to salinity in the Victorian and South-Australian streams can also be characterised as continuous and relatively constant on a seasonal time scale (Metzeling et al., 2006). We suggest that the response of the trait composition of communities to a disturbance depends on the disturbance regime (pulse, press or ramp disturbance (Lake, 2000)) and the mode of action of the disturbance. Note that the temporal dimension of the disturbance has to be defined with regard to the biota under scrutiny. Salinity as in our study and the continuous exposure to organic toxicants represent a press disturbance for invertebrates i.e. a perturbation that temporally maintains a relatively constant level (Lake, 2000). The traits required to cope with a press disturbance are such that enable a species to tolerate a stressor, whereas biological traits linked to resistance (e.g. non-aquatic life stages) or resilience (e.g. low generation time, long-range dispersal) most likely play no, or only a minor role. Both salinity and organic toxicants act on the physiological level of organisms so that toleration of these stressors requires a low physiological sensitivity and this explains the paramount importance of physiological traits in our study and Beketov and Liess (2008). For the $SPEAR_{pesticides}$ indicator a combination of physiological and biological traits was most successful in terms of a high relationship with toxic exposure to pesticides (Liess et al., 2008). This can be explained by the fact that pesticides typically occur as a pulse disturbance (Leu et al., 2004), allowing for recovery after the perturbation, which is related to resilience traits, while the acute effect is caused by action on the

physiological level, which is related to the physiological sensitivity trait (Liess et al., 2008). The recovery of the community was associated with temporal changes in the trait composition in terms of $SPEAR_{pesticides}$ (Liess and von der Ohe, 2005), whereas the press disturbance corresponded to relative stability of $SPEAR_{salinity}$ over the seasons (no significant covariation for years or seasons, see Methods).

We propose a generalised conceptual framework for the identification of response traits in relation to the disturbance regime and the disturbance mode of action (Table 3). Generally, the effect of a disturbance that acts on the physiological level should be predicted from the physiological sensitivity of taxa, but resilience traits may also play a role depending on the disturbance regime as discussed for a pulsed disturbance caused by pesticides (Table 3). When the stressor acts on the whole organism, an effect of a pulse disturbance such as a flood may be predicted using both resistance and resilience traits (Townsend et al., 1997) (Table 3). For a press or ramp disturbance where the stressor acts on the whole organism we hypothesise that mainly biological resistance traits determine the effects. Indeed, a study on 58 sites in different catchments demonstrated a strong linear decline in the estimated probability for a moderate or high frequency of clinger taxa with increasing fine sediment concentration (Richards et al., 1997). In contrast to our prediction, also a resilience trait (number of generations per year) responded to fine sediment concentrations in terms of a negative correlation but this response was 1) not clearly linked to the stressor (fine sediments) given that in this study the trait exhibited also responses to other stressors and 2) mechanistically equivocal since taxa both with a high and a low number of generations per year decreased (Richards et al., 1997). We are aware that more investigations on the effects of single stressors on the trait composition of communities are required to test the hypotheses contained in this conceptual model and to potentially include further predictive factors such as the spatial dimension of the disturbance.

Some papers have suggested that physiological traits may be determined by biological traits (Baird and van den Brink, 2007; Rubach et al., 2010; Statzner and Beche, 2010). However, the results were either poor in terms of selectivity between multiple stressors (Doledec and Statzner, 2008) or the selectivity of the identified biological traits was not tested and the relationship between the biological traits and the physiological trait was not necessarily mechanistic (Baird and van den Brink, 2007; Rubach et al., 2010). However, we agree that biological traits may represent an interesting surrogate in case that a physiological trait for a stressor is unknown. Nevertheless, several techniques are available to estimate the sensitivity of taxa to stressors. One possibility represents the derivation of sensitivity thresholds for taxa from field data using methods such as the Threshold Indicator TAxa aNalysis (TITAN) (Baker and King, 2010). Another alternative is the use of rapid laboratory tests (Kefford et al., 2005a) and/or expert knowledge in conjunction with bayesian statistical methods to generate sensitivity data (Hickey et al., 2008) quickly and inexpensively. Indeed for indicators such as $SPEAR_{salinity}$ and $SPEAR_{pesticides}$ which use a binary classification of physiological sensitivity only coarse assessments are required. Thus the direct determination of physiological sensitivity for many taxa is more achievable than is generally acknowledged.

Table 3
Conceptual model for the relationship of response traits and disturbance.

		Disturbance mode of action	
		Stressor acts on the physiological level	Stressor acts on whole organism
Disturbance regime	Pulse	Physiological sensitivity trait and biological recovery and avoidance traits (e.g. response to pulsed pesticide exposure (Liess et al., 2008))	Biological resistance, recovery and avoidance traits (e.g. response to floods (Townsend et al., 1997))
	Press	Physiological sensitivity trait (e.g. response to salinity (this study))	Biological resistance trait (e.g. response to fine sediments (Richards et al., 1997))
	Ramp	Physiological sensitivity trait	Biological resistance traits

4.4. Application of trait-based approaches in ecological risk assessment

The availability of a trait database represents a crucial prerequisite for the application of trait-based approaches in the ecological risk assessment for freshwater ecosystems. Extensive trait databases are currently only available for Europe, North-America and New Zealand and predominantly for invertebrates (Schmidt-Kloiber et al., 2006; Vieira et al., 2006). The compilation of databases for other regions is constrained by data availability and funding. The database presented in this study was compiled on the family level as data for a lower taxonomic resolution was scarce. Still, approximately 30% of the data entries in the database could not be obtained from published sources and the situation is likely worse in other regions outside of Europe, North America, New Zealand and Australia. Despite limitations in taxonomic resolution and precision of some of the data, the derived trait-based indicator performed better than existing indicators to detect effects of salinity. In terms of labour, the compilation of this database with 7 biological and 2 physiological traits for 172 families took approximately 1.5 person years, whereas the compilation of a larger European biomonitoring database encompassing 14 traits and approximately 600 species involved 30 persons for approximately two years (Statzner and Beche, 2010). We assume that most of the European and North American databases required a similar effort as they usually describe 15–20 traits (Schmidt-Kloiber et al., 2006; Vieira et al., 2006). While larger databases may be desirable in all regions, we demonstrate that much smaller databases developed based on *a priori* hypotheses on the ecological effects of a stressor represent a useful starting point for including trait-based approaches in biomonitoring (Table 3). In the medium-term current databases should be harmonised in a global database and made publicly available as that would benefit biomonitoring application of environmental stressors and facilitate research in the ecological risk assessment of different stressors for freshwater ecosystems.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2011.01.053.

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Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: Effect concentrations and recovery dynamics

Mikhail A. Beketov[a,*], *Ralf Bernhard Schäfer*[a,b], *Andreas Marwitz*[a], *Albrecht Paschke*[c], *Matthias Liess*[a]

[a]*UFZ—Helmholtz Centre for Environmental Research, Department System Ecotoxicology, Permoserstrasse 15, 04318 Leipzig, Germany*
[b]*University Lüneburg, Institute for Ecology and Environmental Chemistry, Scharnhorststrasse 1, 21335 Lüneburg, Germany*
[c]*UFZ—Helmholtz Centre for Environmental Research, Department Ecological Chemistry, Permoserstrasse 15, 04318 Leipzig, Germany*

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ABSTRACT

In pesticide risk assessment, effect concentrations and dynamics of long-term community-level effects caused by pulse exposures remain to be investigated. This is because long-term experiments are exceptionally rare, and most of the previously investigated communities had low proportions of sensitive long-living species. The aim of the present study was to investigate the effect of a single pulse contamination with the insecticide thiacloprid on invertebrates. We employed mesocosms designed to realistically mimic communities in small streams within the agricultural landscape. Specifically, the objectives were to (i) compare the community Lowest-Observed-Effect Concentration (LOEC) with organism-level median lethal concentrations (LC50), and (ii) to assess recovery dynamics with special focus on short- and long-living taxa. The contamination resulted in long-term alteration of the overall invertebrate community structure (7 months, until the end of the experiment). Long-term community LOEC was 3.2 μg/L (Redundancy Analysis), slightly below the acute LC50s known for sensitive invertebrates relevant to the mesocosm community. However, one species (stonefly *Nemoura cinerea*) was affected at the lowest tested concentration, 70 times below the lowest known LC50. Concerning time to recovery from the effect, we found that the duration depends on the life-cycle characteristics of species, but not on the toxicant concentration: short-living (mulivoltine) species recovered after 10 weeks following contamination, whereas long-living (uni- and semivoltine) species did not recover until the end of the experiment (7 months). The present example shows that concentrations of pesticides at which majority of the species is affected can be predicted by acute organism-level toxicity tests with sensitive species. However, tests with longer observation periods, as well as consideration of environmental factors and inter-taxon variability in sensitivity are required to predict effects on all species comprising a community. Realistic prediction of community recovery dynamics requires consideration of the species' life-cycle traits.

* Corresponding author. UFZ—Helmholtz Centre for Environmental Research, Department System Ecotoxicology, Permoserstrasse 15, D-04318 Leipzig, Germany. Tel.: +49 341 2351498; fax: +49 341 2352401.
E-mail address: mikhail.beketov@ufz.de (M.A. Beketov).

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1. Introduction

One of the crucial aims of ecotoxicology is to assess and define the concentration levels at which contaminants cause effects on communities and ecosystems, and to investigate and predict recovery of these systems following toxicant stress (e.g. Campbell et al., 1999; Giddings et al., 2002). Among the contaminants in current use, modern non-persistent insecticides as well as other pesticides are relevant stressors for many aquatic and terrestrial organisms (Liess et al., 2005), and a great number and variety of studies have been conducted to derive and predict the effect concentrations for these toxicants and to understand the processes of recovery from the effects of these contaminants.

Concentration levels for ecological effects of pesticides and many other toxicants are typically derived from laboratory single-species tests. Results of such tests are used for predicting potential effects of toxicants on ecosystems either by applying safety factors (e.g. EEC, 1991) or using species-sensitivity distribution (SSD) methods (e.g. Posthuma et al., 2002). In addition to these predictive methods based on laboratory single-species tests, a wide array of more complex experimental systems is used for validation of the laboratory tests in semi-natural conditions. These model ecosystems, referred to as micro- and mesocosms, are used for risk assessment of pesticides and are known as higher-tier risk assessment testing systems (Campbell et al., 1999).

For pesticides, a recent review focused on comparison of the result from laboratory and mesocosm test systems revealed that effects of these toxicants on biological communities in mesocosms have rarely been observed at concentrations >10 times lower than the acute Median Effective Concentrations (EC50) obtained for the species known to be sensitive in laboratory conditions (*Daphnia magna*), and in most cases have been observed at much higher concentrations (Van Wijngaarden et al., 2005). On the other hand, several microcosm studies focused on chronic post-exposure effects of insecticides have shown that these toxicants can have a long-term influence on most sensitive endpoints even at concentrations up to 1000 times lower than the laboratory-generated acute EC50s (for *D. magna* or sensitive insect species) (Lozano et al., 1992; Liess and Schulz, 1996; Liess, 2002; Beketov and Liess, 2005). In addition, existing field monitoring studies indicate that pesticides may have adverse effects on freshwater invertebrates at concentrations more than 100 times below the laboratory-generated acute EC50s derived for *D. magna* (Liess and von der Ohe 2005; Schäfer et al., 2007).

Recovery of ecological systems after chemical stress caused by pesticides and other environmental toxicants currently receives increasing attention from scientists and regulators (Giddings et al., 2002; Barnthouse 2004; Caquet et al., 2007). Investigations of the recovery processes usually employ micro- and mesocosms. For pesticides, community recovery in mesocosms is frequently observed within a relatively short period after contamination. Thus, for non-persistent insecticides the majority of previously published studies have shown that recovery is already completed within two months after contamination (reviewed by Van Wijngaarden et al., 2005). However, a few long-term mesocosm experiments have revealed that even a single short-term exposure to pesticides may result in long-term and permanent elimination of long-living species if external recolonisation is hampered (Van den Brink et al., 1996; Caquet et al., 2007). Hence, the rapid recovery observed in many mesocosm systems that are predominantly inhabited by short-living organisms (e.g. plankton and short-living benthic insects) and open for external recolonisation (e.g. aerial entry of insects from neighbouring controls) may easily underestimate the recovery duration for communities that include long-living species and are relatively isolated from unimpaired ecosystems (Caquet et al., 2007; Hanson et al., 2007).

Thus for pesticides uncertainty remains regarding both effect concentrations and recovery patterns. In the authors' opinion one main reason for this uncertainty is the paucity of long-term mesocosm experiments employing ecologically realistic communities with a large proportion of long-living taxa and extensive field monitoring studies. Long-term experimental studies are particularly important for understanding effects on long-living species, as experimental observation periods covering significant part of species lifespans are needed to understand duration of effects and recovery patterns (e.g. for univoltine taxa desirable observation period is from >0.5 year to ≤1 year).

Long-term mesocosm experiments are rare. To the authors' knowledge only 5 out of 62 community-level studies on non-persistent insecticides published so far (70 papers) include post-contamination observation periods longer than half a year. These are studies by Brock et al. (1992), Fairchild and Eidt (1993), Van den Brink et al. (1996), Woin (1998), and Hanson et al. (2007) (for the studies reported as paper series, only the first papers are cited). All these investigations were performed with standing-water systems.

Although these long-term studies were not focused on understanding the importance of species' life-cycle traits for post-exposure recovery, two of them have shown that recovery of long-living (univoltine) species after pronounced toxic effect can take long time periods comparable to the species' lifespans (≥1 year) (Van den Brink et al., 1996; Woin 1998). However, the numerical proportion of the long-living species (with generation time ≥1 year) in the communities analysed in these two studies was low (about 10 and 24% of the analysed communities respectively; own calculations based on reported information). Besides, long-term effects on the entire community structure were either not found under ecologically realistic conditions (as stated by the authors) because relatively few long-living taxa were affected (Van den Brink et al., 1996) or this aspect was not analysed (Woin, 1998). Importantly, invertebrate communities in natural streams uncontaminated with pesticides usually include much greater proportions of long-living taxa. For example in Europe, the percentage of the taxa having generation time ≥1 year in uncontaminated streams in France and Finland varies from 60 to 80% and from 40 to 70% of the overall taxa richness respectively (own calculations with data from Schäfer et al., 2007). However, significance and patterns of the long-term effects caused by single pulse contamination with an insecticide remain to be investigated.

The aim of the present study was to investigate long-term effects of a single pulse contamination with the neonicotinoid insecticide thiacloprid on invertebrate communities of stream mesocosms, which were allowed to establish a community

having a relatively high proportion of long-living univoltine taxa (about 50% of the taxa richness at levels of taxonomic identification similar to those used by Schäfer et al. (2007)) for 16 months before contamination. In particular, the objectives were (i) to derive the community Lowest-Observed-Effect Concentration (LOEC) and compare it with laboratory-generated toxicity data, and (ii) to assess the long-term effect-and-recovery dynamics with special focus on short- and long-living taxa. The insecticide was applied as a single pulse to simulate contamination due to spray drift or surface water runoff, which represent a relevant input path for small streams in agricultural areas (Liess et al., 1999, Neumann et al., 2002).

2. Materials and methods

2.1. Description of artificial stream system

The mesocosm system used in the present study consisted of 16 artificial streams. Each stream has the following characteristics: length 20 m, width at water surface 0.32 m (±0.03), average depth 0.25 m (±0.11), discharge 160 L/min (±9), slope 2%; approximate total volume 1000 L (range in parentheses). Each stream is designed as a closed circulation system. In this system the water flows as follows: from the upstream to the downstream sections of the stream it is propelled by gravity, then it falls down into the 200-L reservoir installed below the downstream margin of the stream, and then it is pumped back to the upstream section through a plastic tube (40 mm in diameter) by an electric pump (260W, Atlantis 150, OASE, Hörstel, Germany). At the end of each stream a dam with polyester net filter (1 mm mesh) is installed to prevent loss of the animals to the 200-L reservoirs.

The stream channels are situated in the ground to a depth of about 0.4 m, and lined with water-tight nontoxic polyvinylchloride foil (0.8 mm, Czebra, Lauterecke, Germany) to prevent leakage of water to the surrounding environment. The bottom of the streams is covered with a mixture of fine gravel and sand (particle size 0.2–3.7 mm, layer of 30–50 mm). The streams are located as parallel lines with 0.8 m distance between the stream channels on the territory of UFZ—Helmholtz Centre for Environmental Research (Leipzig, Germany). An area of ground was retained between the streams to support riparian vegetation, so as to provide a refuge for emerged insects, reduce the amount of direct sunlight, and in general to create as much ecological realism as possible.

The system was constructed in summer 2003. In September 2003 and April 2004—two years before contamination—the streams were planted with watercress *Nasturtium officinale*. In order to introduce macroinvertebrates into the streams the sediments (sand, clay, and organic debris) collected with a surber sampler (500 μm mesh) in an uncontaminated small stream near Gross Bardau village (south of Grimma city, Eastern Germany, 51°10′56 N and 12°46′29 E) were added to the streams. The sediments and in addition macroinvertebrates were added several times during winter 2004–2005, and also in October 2005 in order to mimic natural influx of species by drift.

The main physico-chemical parameters of water were measured approximately every four months starting from June 2005 (Table 1). Concentrations of ammonium, nitrite, nitrate, phosphate, and total hardness were determined with Aquamerck colorimetric tests (Merck, Darmstadt, Germany). Conductivity, dissolved oxygen, and pH were measured with LF330, OXI 340, and Multi 340i electronic meters respectively (WTW, Weilheim, Germany). No significant differences between the groups of treatment and control streams were found concerning the physico-chemical parameters ($P>0.05$, analysed for every measuring date with multivariate analysis of variance (MANOVA)).

Table 1 – Main physico-chemical parameters of water in stream mesocosms

Parameter	Mean	Standard deviation
Ammonium (mg/L)	0.053	0.11
Nitrate (mg/L)	4.04	6.4
Nitrite (mg/L)	0.004	0.01
Phosphate (mg/L)	0.21	0.15
Hardness (mg Ca/L)	57.14	2.44
Dissolved oxygen (%)	95.5	15.4
pH	7.88	0.17
Conductivity (μS/cm)	496.21	85.27

Temperature was measured constantly (every 3 h) by μS-LOG540 data logger (Driessen + Kern, Bad Bramstedt, Germany) in two randomly selected streams. The maximum summer (April to September) and minimum winter (October to March) temperatures were 25.9 and 2.7 °C respectively. Mean summer and winter temperatures were 20.33 and 4.52 °C respectively. To compare temperature regimes in all streams DK501-PL data loggers (Driesen + Kern, Bad Bramstedt, Germany) were located in each stream for one month (02–30.03.2007) to measure temperature every 3 h. No significant differences between the groups of treatment and control streams were found for mean, maximum, and minimum temperatures ($P>0.05$, univariate analysis of variance (ANOVA)).

2.2. Thiacloprid application and monitoring

Thiacloprid (generic name (CA) [3-[(6-chloro-3-pyridinyl) methyl]-2-thiazolidinylidene] cyanamide, CAS number 111988-49-9) belongs to the group of neonicotinoid insecticides. Biological activity of neonicotinoids is based on their interference with the nicotinic acetylcholine receptors and, therefore, they exhibit specific activity against the insect nervous system (Tomizawa and Casida, 2005). Among neonicotinoids, thiacloprid is a new and promising insecticide active against various chewing and sucking pests (Elbert et al., 2001).

Thiacloprid was obtained from Agrar–Handel und Transport (Schafstädt, Germany) as the commercial formulation Calypso (suspension concentrate) with 480 g/L of the active ingredient (Bayer CropScience, Langerfeld, Germany). Nominal concentrations of the three treatment levels were 0.1, 3.2 and 100 μg/L in terms of active ingredient for the low, medium, and high treatments respectively. Throughout the paper we refer to these nominal concentrations.

Thiacloprid has high water solubility (water solubility and log octanol–water partition coefficient (log K_{OW}) of active ingredient at 20 °C is 185 mg/L and 1.26, respectively) (USEPA, 2003). One litre of stock solution was prepared for each

channel at the respective concentrations by diluting the toxicant formulation with distilled water. The stock solutions were poured into the water reservoirs installed below the streams (see above for the mesocosm system description) to dilute the toxicant and make the input gradual. This was done to simulate contamination due to spray drift and surface water runoff, which represent a relevant input path for small streams in agricultural areas (Liess et al., 1999; Neumann et al., 2002). The contamination was performed 18 May 2006.

Exposure to thiacloprid was monitored in the high- and medium-concentration streams using spot water samples taken 48 h, 120 h and 264 h after contamination (measurements at low concentration have not been performed due to technical difficulties). Two 200-ml samples were taken per each channel (in up- and downstream sections, non-vegetated areas) that resulted in 4 samples per each concentration (except the sampling of the channels treated at 100 μg/L performed 264 h after contamination, Table 2). The samples were solid-phase-extracted immediately after sampling using 6 ml Chromabond Easy columns (Macherey-Nagel, Düren, Germany) preconditioned with 6 ml methanol. The columns were eluted with 12 ml acetonitrile-ethylacetate (1:1 v/v) and gently evaporated to 300 μl under nitrogen. Analytical recovery was 82% with 18% standard deviation ($n=3$) for 200 ml of spiked water samples. All solvents used were of HPLC-grade and obtained from Merck KGaA (Darmstadt, Germany).

All analyses were conducted by high-performance liquid chromatography (Agilent 1100 series, Agilent Technologies Germany, Boeblingen, Germany) using a XDB-C18 column (150×2.1 i.d., Agilent Technologies) and an Agilent 1100 liquid chromatograph/mass selective detector (LC/MSD) for quantification. The limit of detection (LOD) for the concentration in the water phase was 0.03 μg/L. The measurements were performed by UFZ—Helmholtz Centre for Environmental Research (Leipzig, Germany).

2.3. *Complementary experiment on thiacloprid dynamics in the stream system*

In order to better investigate the temporal dynamic of thiacloprid exposure in the experimental streams a complementary experiment was conducted in May 2007. Set up of this experiment was identical to the main experiment described above concerning the nominal concentrations, and the methods of preparation of stock solutions, contamination, and water sampling (exception: 2-L samples were taken for concentrations ≤0.1 μg/L). Thiacloprid residues were monitored until the concentrations in the water phase decreased below limit of quantification (Table 3).

Table 2 – Residue analysis of thiacloprid

Time after contamination (h)	Mean measured concentrations ± standard deviation ($n=4$) [a] at different time-points after contamination (μg/L)		
	Nominal concentration (μg/L)		
	0.1	3.2	100
48	NM	3.55±2.80	36.64±10.37
120	NM	1.29±1.61 [b]	6.48±1.43
264	NM	NM	5.43±0.75 [b]

NM—not measured.
[a] two samples in each of the two channels.
[b] $n=2$.

Table 3 – Residue analysis in the complementary experiment on thiacloprid dynamic

Time after contamination (h)	Mean measured concentrations ± standard deviation ($n=4$) [a] at different time-points after contamination (μg/L)		
	Nominal concentration (μg L^{-1})		
	0.1	3.2	100
4	0.08±0.02	2.83±0.16	76.33±10.03 [b]
10	NM	NM	65.67±11.91 [b]
48	0.05±0.01	1.28±0.13	35.25±14.24
120	0.02±0.02	0.24±0.20	12.23±9.61
216	0.02±0.03 [c]	0.05±0.05	2.36±2.62
312	<0.01 [c]	<0.01	0.6±0.61
480	<0.01 [c]	<0.01	0.09±0.14
648	NM	NM	<0.01

NM—not measured.
[a] two samples in each of the two channels.
[b] $n=6$, three samples in each of the two channels.
[c] $n=2$, one sample in each of the two channels.

Measurements were performed with liquid chromatography (high-performance liquid chromatography system with Diodenarray Detector II Series 2000, binary pump, autosampler, column oven (30C), Perkin Elmer, Wellesley, MA, USA). The injection volume was 100 μl, dissolved in 25% acetonitril/water solution with gradient-grade pump program. The detection limit was 0.01 μg/L. The column LiChrospher 60, RP-select B, 5 μm (Merck, Darmstadt, Germany) was used for separation. The LOD for a sample was 0.01 μg/L.

2.4. *Invertebrate community sampling*

2.4.1. *Aquatic sampling*

Aquatic macroinvertebrates were sampled using a metal frame designed to cover a 15×15 cm area of the stream bottom. This frame, shaped like a short square pipe, has 20-cm-high walls and 15×15 cm openings at the bottom and top. During sampling all macrophytes were removed from the sampled area by hand and washed and checked for macroinvertebrates in a white plastic cuvet. Subsequently the water column was sieved and sediments were collected by small hand net (60×55 mm frame, 500 μm mesh) and examined for macroinvertebrates in the white cuvet. During each sampling four samples were taken from each experimental channel at up-, middle-up-, middle-down-, and downstream sections respectively. Each sample was taken from the bottom area including both macrophytes and non-vegetated substrate.

Except for the first sampling, made in September 2005 (34 weeks before contamination), the animals were identified in situ and put back in the stream. During the first sampling all the macroinvertebrates were preserved in 90% ethanol and identified in the laboratory. During subsequent samplings the same procedure was applied when required (e.g. new species

found that could not be identified in situ). Most of the Ephemeroptera, Odonata, Plecoptera, Trichoptera, Heteroptera, Coleoptera, Isopoda and Amphipoda were identified to the species level, while in all other taxonomic groups the identification level varied from species to family. Oligochaeta was identified at the class level only. The samplings were performed at the following time periods with respect to the contamination event: −34, −8, −4, −1, 1, 3, 10, 17, and 27 weeks.

2.4.2. *Emergence traps*

To assess the effect of the toxicant on emergence of merolimnic insects, 6 emergence traps were installed on each stream mesocosm. Each emergence trap was constructed as a pyramid-shaped net that covers approximately 0.165 m^2 of the stream surface area and is 0.76 m in height. The traps consist of a wood frame covered with net (0.7 mm mesh) and a plastic cone installed on the top of the trap. The cone is covered by a plastic 1-L bottle. The whole system was designed to collect all emerged insects in the plastic bottle installed on the top of the trap.

Emerged insects were counted and identified in situ three times per week from 17.05 to 30.09.2006, when insect emergence almost completely ceased. When necessary, insects were preserved in ethanol (representatives of Odonata were fixed with acetone and dried) and identified in the laboratory. Most of the Ephemeroptera, Odonata, Plecoptera, and Trichoptera were identified to the species level, while in Diptera the identification level varied from species to family (appendix table).

2.5. *Data analyses*

The experimental design includes 16 independent streams and four treatment levels (control, 0.1, 3.2, and 100 μg/L) with two replicates for each concentration level and ten for the control (regression experiment design, e.g. Van Wijngaarden et al., 1996). The relatively high number of control replicates was used to allow usage of the Monte Carlo permutation test following multivariate ordination techniques (described below). Data from separate samples taken from the same stream at a particular time-point was pooled to avoid pseudoreplication. Data from emergence traps collected three times per week were pooled for each week and then these weekly values were used to derive a value per month as the arithmetical mean. This was done to provide discrete data points comparable to those obtained in aquatic samples.

To give an overview of the thiacloprid effect on the macroinvertebrate communities the widely employed univariate parameters abundance and taxa richness were used. Abundance was measured as $\ln(x+1)$ transformed number of individuals per square meter. Taxa richness was measured as number of taxa (species or other lowest possible taxonomic category) per sample. Specific "regression" experimental design adopted for multivariate statistical methods (described below) makes difficult comparison of different treatment levels (checking of variance heterogeneity is impossible, tests suitable for such situation are not sensitive and type II error (possibility to find no difference between different populations) is highly probable). Therefore, abundance and taxa richness were plotted against time to only visually inspect for relationship (e.g. Van den Brink et al., 1996) (Fig. 1).

To test for significance of the toxicant's effect on particular species (two species only, explained below) we used ANOVA followed by Games–Howell and Tamhane post-hoc tests. These tests are robust with respect to the potential deviations from normality or variance homogeneity (Zar, 1996). In particular, Tamhane test exhibits good type I error rate (i.e. low probability to find difference between identical populations) and power properties. This conservative test was applied to confirm statistical significances taking into account low number of contaminated replicates.

The community response to the contamination was analysed using the Principal Response Curve (PRC) method and a set of Redundancy Analyses (RDA) performed for the different sampling time-periods. The PRC method is a multivariate technique specially developed for the analysis of data obtained in experimental community response studies. It is based on the RDA ordination technique, the constrained form of principal component analysis (Van den Brink and Ter Braak, 1999). Statistical significance of the PRC models, in terms of displayed treatment variance, was tested by Monte Carlo permutation tests performed for the entire time series in the RDAs from which the PRCs were obtained, using an *F*-type test statistic based on the eigenvalue of the components (Van den Brink and Ter Braak, 1999; Leps and Smilauer, 2003).

Prior to all the multivariate analyses species abundances were $\ln(10x+1)$ transformed, where x stands for the abundance value. This was done to down-weight high abundance values (for rationale see Leps and Smilauer, 2003). The PRC technique was used to analyse the entire process of community development before and after contamination. This statistical technique was applied for (i) the whole community and also (ii) separately for the short-living (multivoltine, life-cycle <1 year) and long-living (uni- and semivoltine, life-cycle ≥ 1 year) taxa, to understand the recovery dynamics of species having different life-cycle durations. Data from insect emergence traps was analysed separately from the aquatic data, by the PRC method only. In this analysis the entire assemblage of emerged insects was assessed.

The RDAs with toxicant concentration ($\ln(x+1)$ transformed) used as only one explanatory variable were applied in order to test the statistical significance of toxicant effects on the community structure at different toxicant concentrations and different time-points using the Monte Carlo permutation test, and therefore to infer the Lowest- and No-Observed-Effect Concentration (LOEC and NOEC respectively). The latter type of test was performed by testing every concentration level against the control. Community LOEC is defined here as the lowest toxicant concentration at which a significant difference from the control is detected for the community. Similarly, NOEC is defined as the highest concentration at which the effect is insignificant (Newman and Unger, 2003).

The applicability of the Monte Carlo permutation test to assess the significance of separate treatments in experimental community response studies is frequently restricted by a small amount of replicates, as few permutation possibilities cannot yield *P*-values lower than adopted α-level (discussed in Van den Brink and Ter Braak, 1999). In the present study the lowest amount of permutation possibilities available for the model with one concentration level (2 replicates) and control (10 replicates)

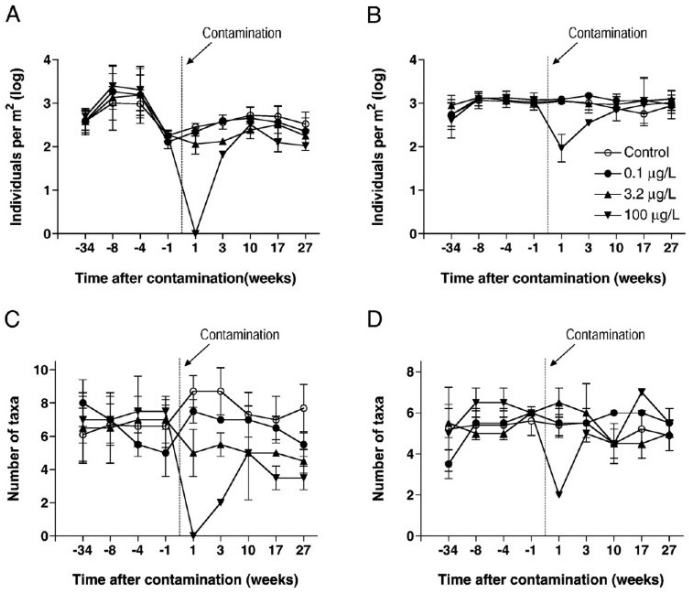

Fig. 1 – Dynamics of abundance and taxa richness of aquatic macroinvertabrates (log(*x* + 1)-transformed number of individuals per square meter and number of taxa respectively). Abundance of insects (A) and non-insects (B), taxa richness of insects (C) and non-insects (D).

was 66 (12!/[2!10!) and a corresponding lowest possible permutation-based *P*-value was 0.015 (1/66). Hence it was possible to use here the set of Monte Carlo permutation tests to test significance of effects of different concentrations and to infer community LOECs for particular time points.

All multivariate statistical analyses were made using the program CANOCO 4.5 for Windows (Wageningen, the Netherlands) according to the available guides (Ter Braak and Smilauer, 2002; Leps and Smilauer, 2003).

Recovery was considered to be achieved when statistical tests for the first time failed to detect a significant difference between contaminated and control mesocosms under condition that a significant effect was not detected later during the observation period.

3. Results

3.1. *Thiacloprid exposure dynamic*

In the main experiment thiacloprid concentrations were monitored during the eleven days after exposure (Table 2). As explained above, in order to better examine dynamic of the toxicant in water a complementary experiment was conducted. In this latter experiment thiacloprid was monitored until complete disappearance from the water phase (27 days, Table 3). In both experiments the measured concentrations of thiacloprid were within the range of nominal concentrations after contamination (Tables 2 and 3). The toxicant concentration in the streams was characterised by an initial rapid drop of the concentration followed by a lower decline in concentrations. Results of the measurements suggest that (i) there was no accumulation (at concentrations ≤ LOD) of thiacloprid in the water phase after 27 days following contamination (Table 3), and (ii) the exposure profile was of the pulse type (Tables 2 and 3) that is similar to pulse exposures observed in streams in the field with peak pesticide concentrations lasting for hours (Richards and Baker, 1993; Liess et al., 1999; Leu et al., 2004). Information about behaviour of thiacloprid in surface water is limited (Krohn, 2001), and detailed comparison of the observed exposure with other studies is problematic.

3.2. *Abundance and taxa richness*

A total of 35 macroinvertebrate taxa were identified for the mesocosm systems (appendix table). Only 21 out of these 35 taxa were found in more than two streams and on more than one occasion. Only these taxa were considered in the multivariate statistical analyses reported below. In terms of the numbers of taxa the richest taxonomic group was insects (26 taxa). The dominant species in all the streams were the isopod *Asellus aquaticus* and blackfly larvae *Simulium latigonium* (relative abundances were up to 70 and 75% respectively). The proportion of long-living taxa having no more than one generation per year was 54% of the overall taxa richness and

47% of the established taxa (21 species mentioned above, appendix table).

Abundance and taxa richness of insect and non-insect taxa were considered separately, as thiacloprid is much more toxic to insects than to other invertebrates, including crustaceans (Beketov and Liess, 2008a,b). The effect of thiacloprid on insect abundance was stronger than on non-insect macroinvertebrates (Fig. 1A and B). Total insect abundance recovered after 10 weeks following the contamination (Fig. 1A). In contrast to the abundance, no recovery was observed for insect taxa richness during the entire observational period at 3.2 and 100 μg/L. (Fig. 1C). Non-insect abundance and taxa richness only showed a transient reduction following contamination (Fig. 1B and D).

Abundance and taxa richness of emerged insects was suppressed at 100 and 3.2 μg/L and 100 μg/L respectively (Fig. 2A and B). Full recovery of these two parameters was observed after 4 and 8 weeks following the contamination respectively.

3.3. *Community structure and LOEC*

The diagram of the first PRC of the aquatic macroinvertebrates (Fig. 3) shows small variation in the pre-treatment period and clear concentration-dependent deviations from the control after the thiacloprid application. Statistical significance of the first PRC was confirmed by the permutation test ($P=0.01$). The second PRC was not statistically significant ($P>0.05$), and therefore is not considered here. Taxa indicated with a higher species scores (b_k), shown on the right side of the PRC diagram (e.g. *S. latigonium*, *Cloeon dipterum*, Fig. 3), decreased in abundance more severely at the higher toxicant levels. In contrast, taxa with negative scores (Oligochaeta and *Planorbis* sp.) increased at the higher toxicant levels. As in the PRC diagram constructed for the aquatic macroinvertebrates (Fig. 3), the first PRC of the emerged insects data set shows relatively small variation in the pre-treatment period and clear, but short-term (until 8 weeks after contamination) concentration-dependent deviations from the control after the contamination (not shown). The first PRC for emergence data set was statistically significant ($P=0.01$); the second was not ($P>0.05$).

Results of the Monte Carlo permutation tests subsequent to the RDAs performed for aquatic macroinvertebrates and emerged insects are summarised in Tables 4 and 5 respectively. Significances derived in the permutations for aquatic macroinvertebrates are also reported in the PRC diagram (Fig. 3).

Results of these permutation tests show that the effect of the toxicant on aquatic communities was significant at concentrations 3.2 and 100 μg/L after 1 and 3 weeks following contamination. During the following time (10–17 weeks after contamination) significance of the effect was only found for the whole model (i.e. permutation all concentrations and control series), which means that significant effect of toxicant cannot be attributed to any separate concentration (Table 4, Fig. 3). After 27 weeks following contamination, the effect of the toxicant again became significant at 3.2 and 100 μg/L (Table 4, Fig. 3).

These results suggest that aquatic macroinvertebrate community structure did not recover until the end of the observation period, as at concentration 3.2 μg/L a significant effect of the toxicant was detected 27 weeks after the contamination. The community LOEC for the latest observation period (27 weeks) is equal to 3.2 μg/L (Table 4).

For the assemblage of emerged insects a significant effect of the toxicant at the concentrations 3.2 and 100 μg/L was found after 1 week following contamination only. At four weeks after the contamination, the effect was significant at 100 μg/L only; and no significant differences were found during the entire subsequent observation period at any concentrations (Table 5).

3.4. *Effect dynamics of short- versus long-living taxa*

The species comprising the macroinvertebrate communities in the present experiment are characterised by contrasting life-cycle patterns such as seasonal dynamics and life-cycle duration. For example, two extremely contrasting dynamics of taxa having different life cycles, namely the abundance dynamics of short-living Chironomidae and the long-living stonefly *Nemoura cinerea*, are shown in Fig. 4. Representatives of the Chironomidae are known to be short-living and

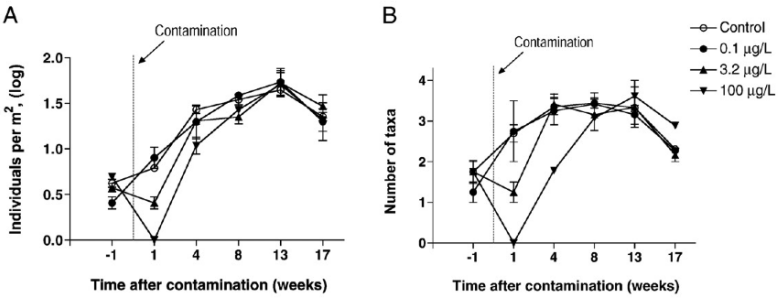

Fig. 2 – Dynamics of abundance (A) and taxa richness (B) of emerged insects (log(x+1)-transformed number of individuals per square meter and number of taxa respectively).

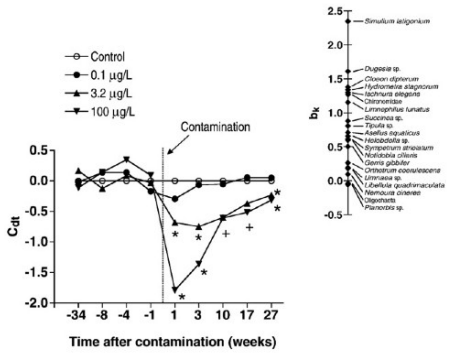

Fig. 3 – Principal Response Curves (PRC) indicating the effect of insecticide thiacloprid on macroinvertebrate community. The vertical axis represents the difference in community structure between treatments and the control expressed as regression coefficient (C_{dt}) of the PRC model. The species score (b_k) can be interpreted as a correlation of each species with the response given in the diagram (taxa indicated with a higher scores show a greater decrease in abundance at the higher toxicant levels). Asterisks indicate significant ($P<0.05$) effect of factor toxicant at particular concentrations tested by Monte Carlo permutation test followed RDA. Plus marks denote significance of the same factor in the whole model in case no particular concentrations yielded statistical significance.

multivoltine (Liess et al., 2008). It is clear from Fig. 4A that although abundance of this taxon was initially severely affected by the contamination, it fully recovered after 10 weeks following contamination. In contrast to Chironomidae, the stonefly *N. cinerea* has only one generation per year (Liess et al., 2008). In addition, the egg and larval development of this species is relatively slow, which makes it difficult to detect the larvae of this stonefly during some time (usually 1 to 3 months) after the flight period in April–May (Fialkowski, 1986; Brittain and Lillehammer, 1987). In the present investigation no larvae of *N. cinerea* were found during the period from 1 week before to 17 weeks after contamination (Fig. 4B). However, when the *N. cinerea* larvae became detectable (27 weeks after contamination) an apparent effect of the toxicant on this species was found (Fig. 4B). The same dynamics were recorded for the semivoltine mayfly *Ephemera vulgata*; however, this species was less abundant, and during the time period before contamination it was found as imago in two streams only (control and 0.1 µg/L, not shown).

This example suggests that (i) during the post-contamination period different species contribute differently to the observed overall community effect (Fig. 3, Tables 4 and 5), and (ii) recovery dynamics of species depend on their life-cycle traits.

To reveal differences in effect-and-recovery dynamics between short- and long-living organisms, the PRC analyses were done separately for assemblages of multivoltine and uni- as well as semivoltine macroinvertebrate taxa (Fig. 5A and B). These two diagrams show distinctly different post-contamination assemblages' responses. The short-living assemblage exhibits a strong initial effect and complete recovery after 10 weeks following contamination (Fig. 5A). In contrast, the long-living taxa's PRC demonstrates long-term effect and no recovery during the entire period of observation (27 weeks, Fig. 5B). The first PRCs for both of the assemblages were statistically significant ($P=0.002$), whereas the second PRCs were not ($P>0.05$).

As in the analyses of the entire community described above, the PRCs for short- and long-living assemblages were supplemented with a set of RDAs with Monte Carlo permutation tests in order to test statistical significance of factor toxicant at separate time-periods and concentrations. Significances derived in these permutation procedures are reported in the PRC diagrams (Fig. 5A and B) as described above for the analyses of the entire aquatic macroinvertebrate community.

Table 4 – Results of the Monte Carlo permutation tests followed the Redundancy Analyses for different sampling dates (data from aquatic samples)

Time after contamination (weeks)	P-values				LOEC (µg/L)	NOEC (µg/L)
	Complete model	Separate concentrations				
	All concentrations	0.1 µg/L	3.2 µg/L	100 µg/L		
−34	NS	NA	NA	NA	NA	NA
−8	NS	NA	NA	NA	NA	NA
−4	NS	NA	NA	NA	NA	NA
−1	NS	NA	NA	NA	NA	NA
1	0.002	NS	0.02	0.024	3.2	0.1
3	0.002	NS	0.032	0.024	3.2	0.1
10	0.009	NS	NS	NS	>0.1*	>0.1*
17	0.028	NS	NS	NS	>0.1*	>0.1*
27	0.008	NS	0.036	0.048	3.2	0.1

NS—not significant.
NA—not applicable.
*LOEC and NOEC cannot be precisely determined, as significant effect was only found for all concentrations together (complete model), but no significant effect was found for any of the separate concentrations.

Table 5 – Results of the Monte Carlo permutation tests followed the Redundancy Analyses for different sampling dates (data from insect emergence traps)

Time after contamination (weeks)	P-values				LOEC (μg/L)	NOEC (μg/L)
	Complete model	Separate concentrations				
	All concentrations	0.1 μg/L	3.2 μg/L	100 μg/L		
−1	NS	NA	NA	NA	NA	NA
1	0.002	NS	0.02	0.024	3.2	0.1
4	0.006	NS	NS	0.024	100	3.2
8	NS	NA	NA	NA	>100	≥100
13	NS	NA	NA	NA	>100	≥100
17	NS	NA	NA	NA	>100	≥100

NS—not significant.
NA—not applicable.

Remarkably, the deviation from control observed for the long-living species assemblage was associated not only with negatively affected sensitive insect species, but also with a strong positive and presumably indirect effect on the gastropod *Limnaea* sp. (Fig. 5B).

3.5. *Effect on the stonefly N. cinerea at concentration 0.1 μg/L*

As mentioned above, among the taxa affected by the toxicant there was one species, namely the stonefly *N. cinerea*, absent in all contaminated mesocosms, including the series with

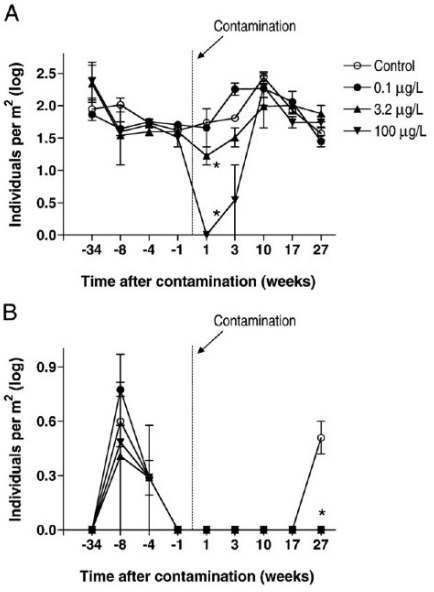

Fig. 4 – Abundance dynamic of two taxa having different life cycles and showing different effect-and-recovery dynamics: short-living multivoltine Chironomidae—strong immediate effect and fast recovery (A), univoltine stonefly *Nemoura cinerea* (B)—strong delayed effect and slow recovery. The latter species was hardly detectable during summer due to slow egg and larval development. Asterisks indicate significant ($P<0.05$, ANOVA, confirmed by both Games–Howell and Tamhane post-hoc tests) differences from the controls.

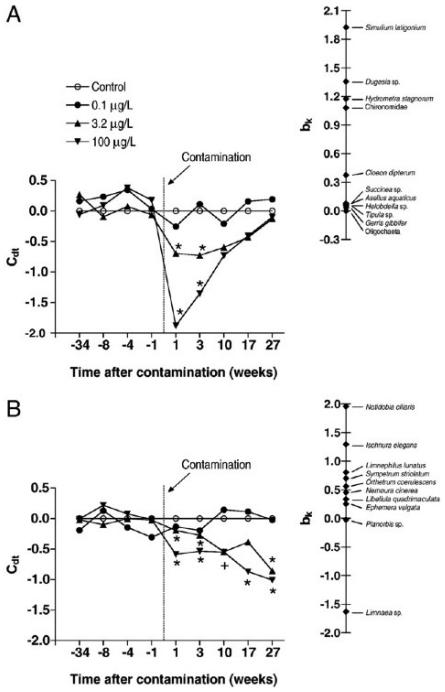

Fig. 5 – Principal Response Curves (PRC) indicating the effect of insecticide thiacloprid on short-living (A) and long-living (B) assemblages of macroinvertebrates (multivoltine and uni- and semivoltine taxa respectively). Explanations in Fig. 3.

lowest tested concentration 0.1 μg/L (Fig. 4B). As explained above, this species after the contamination was found in the late autumn sampling only (27 weeks after contamination). No individuals of this species were found during summer in any treatment including control, obviously because larvae and eggs were too small to be detected (Fialkowski, 1986; Brittain and Lillehammer, 1987). However, prior to the springtime contamination larvae of *N. cinerea* were detected in 9 of the 16 experimental streams, including the streams, which later were contaminated at concentration 0.1 μg/L. Hence this species existed in the streams, which were contaminated with 0.1 μg/L before the experimental contamination and disappeared from these streams after the contamination. In contrast, *N. cinerea* was well established in the control after the contamination period as detected during the autumn sampling. At this sampling period the larvae of *N. cinerea* were detected in 8 out of all 10 control streams with maximum abundances 22 individuals per square meter. Evidently the aquatic stages of this species were present in the water during the contamination period, because, having emerged about 5 weeks before contamination, the adults have certainly completed oviposition well before the contamination occurred. Effect of the toxicant on abundance of *N. cinerea* at concentration 0.1 μg/L was statistically significant ($P<0.05$, ANOVA, both Games–Howell and Tamhane post-hoc tests, Fig. 4B). All this suggests that the absence of *N. cinerea* in streams contaminated at 0.1 μg/L is caused by the toxicant, although the effect was observed only in the particular season and after the considerable time period following contamination. Importantly, this effect was confirmed with conservative Tamhane test that is robust with respect to the potential deviations from normality and variance homogeneity, and exhibits good type I error rates (i.e. low probability to find difference between identical populations) and power properties.

N. cinerea comprise approximately 5% of the total number of established macroinvertebrate species, i.e. taxa for which the toxicant effect could be assessed (21 taxa, the taxa found in more than two streams and for more than one time period, appendix table), and comprise on average 1% of the total abundance (maximum abundance is up to 3%, late autumn sampling, 27 weeks after contamination in the control).

Besides *N. cinerea*, there was one more species, namely the mayfly *E. vulgata*, present in the control streams only (27 weeks after contamination). However, this species was present in 4 out of all 10 control streams, and therefore probability of random non-occurrence of this species in the streams was too high (for one stream $P=0.6$, for two streams $P=0.36$) to test the effect significance with ANOVA and the post-hoc tests.

4. Discussion

4.1. *Comparison of concentrations causing effects in the mesocosm with organism-level toxicity data*

As mentioned, for pesticides there is some uncertainty regarding the levels of concentration that cause effects on aquatic non-target organisms in higher-tier test systems. Hence it is interesting to compare the concentrations causing effects in the present mesocosms with available laboratory-generated organism-level toxicity data.

Thiacloprid is known to be selectively toxic to insects (Beketov and Liess, 2008a,b). The lowest acute (observation time 96 h) LC50 known for pulse (24 h) exposure to thiacloprid is 7.04 (6.45–7.7) μg/L (95% confidence interval in parentheses, found for Trichoptera larvae *Notidobia ciliaris*, Beketov and Liess, 2008a). This LC50 is not significantly different from LC50s found in similar conditions for such sensitive aquatic insects as mosquito and blackfly larvae (7.1 and 7.79 μg/L for *Culex pipiens* and *S. latigonium* respectively, Beketov and Liess, 2008a). Hence the concentration level 7 μg/L is not an outlying value that is substantially lower than LC50s found for other sensitive species. Importantly, these LC50s were found for the short-term exposure (24 h) that is more comparable to the exposure observed in the mesocosms in the present study (rapid concentration decline, Tables 2 and 3) than to the continuous exposure profiles usually used in standard tests. In laboratory tests with continuous exposure (e.g. 96 h) a significantly slower concentration decline is expected than in the streams, as thiacloprid is resistant to degradation by photochemical reactions and hydrolysis, and is mainly eliminated by microbial metabolism (Krohn, 2001).

The lowest LOEC for entire community obtained by Monte Carlo permutation test in the present study is 3.2 μg/L (Tables 4 and 5). This value is approximately 2 times lower than the bottom 95% confidence interval of the lowest known acute LC50. This level is within the range of effective concentrations reported in the mesocosm studies with non-persistent insecticides as reviewed by Van Wijngaarden et al. (2005).

Although the community LOEC, found by Monte Carlo permutation test, was defined as 3.2 μg/L, among the taxa present in the mesocosms there was the stonefly *N. cinerea* that was absent in all contaminated streams including the series with the lowest tested concentration 0.1 μg/L, but were found in the control streams only (Fig. 4B). Hence, for this species only the long-term (27 weeks) LOEC in mesocosms is below 0.1 μg/L. This concentration level is 70 times lower than the lowest known laboratory-generated LC50 of thiacloprid (Beketov and Liess, 2008a). This level is lower than those reported in the mesocosm studies with non-persistent insecticides (Van Wijngaarden et al., 2005), but higher than those found by some microcosm and mesocosm studies focused on the chronic post-exposure effects in sensitive species (Lozano et al., 1992; Liess and Schulz, 1996; Liess, 2002; Beketov and Liess, 2005) and the field studies (Liess and von der Ohe, 2005; Schäfer et al., 2007).

The mechanisms associated with toxicant effects at concentrations 70 times below the acute LC50 are currently unclear. One possible explanation may be that *N. cinerea* is more sensitive to thiacloprid than the sensitive insects tested in the laboratory conditions (larvae of caddisflies, mosquitoes, and blackflies, Beketov and Liess, 2008a), because stoneflies (Plecoptera) are known to be exceptionally sensitive to organic toxicants (Wogram and Liess, 2001). This hypothesis can be tested by future laboratory toxicity tests with larvae of *N. cinerea* or similar stonefly species, as currently no toxicity data exists for Plecoptera and thiacloprid. Other explanations may be that effect on *N. cinerea* at the concentration 0.1 μg/L might result from an interplay of many factors, such as the young age of the exposed individuals (Stark and Banken, 1999; Breitholtz et al., 2003; Pettigrove and Hoffmann, 2005 and references

therein), downstream drift initiated by the toxicant (for thiacloprid see Beketov and Liess, in press), additional stress due to food limitation (Beketov, 2004; Pieters et al., 2005), predation stress (Relyea, 2003; Beketov and Liess, 2006), and effects of abiotic factors (e.g. UV can significantly increase sensitivity to toxicants in field conditions as compared to the laboratory tests, Duquesne and Liess, 2003). Obviously, the relatively long post-exposure observation period is necessary to detect effects at such low concentrations, as effects at concentration levels more than 100 times lower than the acute EC50s were shown in the chronic (almost entire life cycle) microcosm experiments only (Liess and Schulz, 1996; Liess, 2002; Beketov and Liess, 2005). Further investigations are required for understanding the underlying mechanisms of toxic effects at such low concentrations.

For environmental risk assessment of toxicants the two hypotheses given above concerning the effect at the very low concentrations imply that (i) the range of species tested in laboratory toxicity tests should be representative to sufficiently consider the among-taxa variability in sensitivity, and (ii) the realistic environmental context including biotic and abiotic factors, which can exacerbate toxic effects, should be taken into account.

4.2. *Recovery dynamics: importance of species' life-cycle duration and seasonal dynamics*

As mentioned above, for pesticides there is some uncertainty not only regarding effect concentrations, but also concerning recovery duration of aquatic communities impaired by insecticides. As reviewed by Van Wijngaarden et al. (2005), most of the previously published mesocosm studies with non-persistent insecticides have shown that recovery is already completed within two months after contamination. However, a few long-term mesocosm experiments have revealed that even a single short-term exposure to pesticides may result in long-term and permanent elimination of long-living species (Van den Brink et al., 1996; Caquet et al., 2007).

The present study shows that a single pulse contamination at the concentration level close to the acute laboratory-generated LC50s can result in long-term alteration of community structure when long-living species are present in a high proportion (i.e. 50% of overall taxa richness) comparable with natural streams (e.g. 40–80%, own calculations with data from Schäfer et al., 2007). Importantly, the present results show that within the levels of effect concentrations, time for recovery of the affected organisms depends on the life-cycle duration, but not on the toxicant concentration. Thus, short-living (mulivoltine) species recovered already after 10 weeks following contamination, irrespective of the concentrations (Fig. 5A). In contrast, long-living taxa did not recover until the end of the observation period at the effective concentrations (for community structure 3.2 and 100 μg/L, Fig. 5B).

Obviously, long-living insects present in this mesocosm system may recover only after the flying period in the following year. This may be when imagoes emerged in uncontaminated streams will oviposit in the impaired streams. However, in river systems recolonisation of affected stream parts occurs not only through aerial dispersal, but also through the drift of aquatic stages of merolimnic insects and fully aquatic animals from unaffected upstream reaches. Several studies have shown that the presence of undisturbed upstream reaches significantly reduces pesticide effects on invertebrates and facilitates recovery of contaminated streams (Hatakeyama and Yokoyama, 1997; Liess and von der Ohe, 2005; Schäfer et al., 2007; Schriever and Liess, 2007; Schriever et al., 2007). All this suggests that prediction of ecosystem recovery after pesticide contamination should consider life-cycle traits of sensitive species and spatial isolation of the affected area from undisturbed ecosystems.

The endpoint insect emergence exhibited relatively rapid recovery in terms of taxonomic structure as compared to the aquatic communities. No significant effect of the toxicant on the emerged insects was found after 8 weeks following contamination (Table 4). This rapid recovery is obviously caused by strong prevalence of multivoltine taxa in the assemblage of emerged insects (Chironomidae and Simuliidae). These taxa have several emergence periods during the year and these periods are extended and overlapping.

The processes underlying the long-term community structure alteration observed in the present study included not only elimination of sensitive long-living species, but presumably also a positive indirect effect on the gastropod *Limnaea* sp. (Fig. 5). Such positive effects of pesticides on gastropods were observed previously in mesocosm (reviewed by Fleeger et al., 2003) and field studies (Liess and von der Ohe 2005). Mechanism of this effect may be explained by the reduced competition that results from elimination of more sensitive competitors (insects), as was also proposed in previous community-level studies (Fleeger et al., 2003).

5. Conclusion

We conclude that in mesocosms the long-term (7 month) LOEC calculated for the entire community by multivariate statistical methods can be found at concentrations in the range of the acute LC50 of sensitive species. However, it cannot be excluded that effect on a minority of species can occur at concentrations far below the laboratory-generated acute LC50.

Concerning the post-exposure recovery, we conclude that within the levels of effect concentrations, recovery of the affected organisms may be predominantly dependent on the life-cycle duration, and not on the toxicant concentration.

In environmental risk assessment realistic prediction of pesticide effects at the community level requires consideration of long-term effects. Prediction of recovery dynamics in communities impaired by pesticides should consider life-cycle duration of the species comprising the communities.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2008.07.001.

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7. Publication VI

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ARTICLE

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Effects of Pesticides Monitored with Three Sampling Methods in 24 Sites on Macroinvertebrates and Microorganisms

Ralf B. Schäfer,[*,†,‡] Vincent Pettigrove,[§,∥] Gavin Rose,[⊥] Graeme Allinson,[§,#] Adam Wightwick,[#] Peter C. von der Ohe,[▽] Jeff Shimeta,[†] Ralph Kühne,[○] and Ben J. Kefford[†,◆]

[†]Biotechnology and Environmental Biology, School of Applied Sciences, RMIT University, P.O. Box 71, Bundoora Vic 3083, Australia

[‡]Institute for Environmental Sciences, University Koblenz-Landau, Landau, Forststrasse 7, 76829 Landau, Germany

[§]CAPIM, Bio21 Institute, University of Melbourne, 30 Flemington Road, Parkville Vic 3010, Australia

[∥]Melbourne Water, Research and Technology, P.O. Box 4342, Melbourne Vic 3001, Australia

[⊥]Department of Primary Industries, Future Farming Systems Research, 621 Sneydes Road, Werribee Vic 3030, Australia

[#]Department of Primary Industries, Future Farming Systems Research, 2a Bellarine Highway, Queenscliff Vic 3225, Australia

[▽]Department of Effect-Directed Analysis, Helmholtz Centre for Environmental Research − UFZ, Permoser Strasse 15, 04318 Leipzig, Germany

[○]Department of Ecological Chemistry, Helmholtz Centre for Environmental Research − UFZ, Permoser Strasse 15, 04318 Leipzig, Germany

[◆]Centre for Environmental Sustainability, Department of Environmental Science, University of Technology Sydney, P.O. Box 123, Broadway NSW 2007, Australia

Supporting Information

ABSTRACT: Grab water samples, sediment samples, and 2,2,4-trimethylpentane passive samplers (TRIMPS) were used to determine the exposure to 97 pesticides in 24 southeast Australian stream sites over 5 months. Macroinvertebrate communities and selected microorganisms (bacteria, flagellates, ciliates, amoebas, nematodes, and gastrotrichs) were sampled to detect relationships with pesticide toxicity. Sediment samples had the highest estimated toxicities in terms of toxic units (TU) for *Daphnia magna* (TU_{DM}) and for *Selenastrum capricornutum* (TU_{SC}). The pesticide-selective $SPEAR_{pesticides}$ and the general SIGNAL index for macroinvertebrates exhibited negative linear relationships ($r^2 = 0.67$ and 0.36, respectively) with pesticide contamination in terms of log maximum TU_{DM} (log mTU_{DM}), suggesting macroinvertebrate community change due to pesticide exposure. Pesticide contamination was the only measured variable explaining variation in ecological quality. Variation in the densities of several microbial groups was best explained by environmental variables other than log TUs. The log mTU_{DM} values derived from sediment concentrations were most important to establish a link with effects on macroinvertebrates, whereas log mTU_{DM} of grab water samples had only minor contribution. Current-use insecticides and fungicides can affect macroinvertebrate communities and monitoring of sediment and continuous water sampling is needed to detect these effects.

INTRODUCTION

Pesticides represent an important stressor for freshwater ecosystems,[1] but the determination of effects faces challenges both on the exposure and on the effects side. For the former, the quantification of pesticide concentrations in streams is difficult because pesticide input usually occurs in pulses associated with rain events (surface runoff) so that grab water sampling at distinct time points may underestimate the exposure.[2] Passive sampling has been successfully employed for the monitoring of pulsed exposures and relies on the continuous integrative sampling of the water phase usually over days to weeks.[3] However, many pesticides are adsorbed to particles when they are washed from agricultural fields into adjacent water bodies,[4] and depending on the physicochemical properties of the compound, this in turn may result in delayed exposure of organisms through desorption from suspended solids or sediment particles or via ingestion of particles. Consequently, both the pulsed occurrence and the phase distribution between water and particles should be considered when monitoring pesticides and relating the exposure to effects on aquatic organisms.[4]

The unambiguous linking of ecological effects to a certain stressor is difficult due to spatial and temporal variability of natural communities, and the co-occurrence of other stressors that may be intercorrelated or may have interactions with the stressor under scrutiny.[5] For streams, freshwater macroinvertebrates are typically used to assess ecological quality, and biotic indicators relying on their traits may resolve the challenges outlined. The SPEcies At

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Risk indicator for pesticides ($SPEAR_{pesticides}$)[6] uses physiological (sensitivity to toxicants) and biological (generation time, dispersal capacity and length of life stages outside the aquatic habitat) traits of organisms to determine the fraction of the abundance of sensitive taxa in communities, and has been demonstrated (a) to be relatively constant over reference sites in different biogeographic regions,[7,8] and (b) to respond selectively to pesticide stress.[6,7] In contrast to macroinvertebrates, few field studies have investigated effects of pesticides on microbial communities.[9,10] Consequently, the relevance and magnitude of effects of pesticides on microorganisms such as bacteria and protozoa are largely unknown, despite having important functions in the nutrient cycling and as an energy source in the food web.[11]

The principal aim of this study was to investigate the relationship between pesticide toxicity and macroinvertebrate community composition and the density of selected groups of microorganisms (bacteria, flagellates, ciliates, amoebas, nematodes, and gastrotrichs) in streams of southern Victoria in southeast Australia. We monitored 24 sites over a period of five months for 97 pesticides and for the above-mentioned taxonomic groups. The pesticide monitoring encompassed the use of grab water samples, sediment samples, and passive sampling of water using low-density polyethylene (LDPE) bags filled with 2,2,4-trimethylpentane (TRIMPS).[12] Our research questions follow: (a) Is there a relationship between pesticide toxicity and the invertebrate community and selected microorganisms? (b) How suitable are the different sampling systems to establish relationships between pesticide toxicity and biotic end points?

EXPERIMENTAL SECTION

Sampling Sites and Study Design. The 24 sampling sites in streams and rivers were located within 150 km of Melbourne, Victoria, Australia (Supporting Information (SI) Figure S1). The sites were selected (a) to represent a potential gradient in exposure to pesticides and (b) to exhibit permanent flow. Most sites were in the Yarra River catchment, where the predominant agricultural land-use is horticulture, e.g., grapevine, fruit, and vegetable production. The main agricultural land-use in the catchments of the other sites is cereal and oilseed production. No known industrial facilities, mines, or wastewater treatment plants were present upstream of the sampling sites that could account for significant discharges of organic toxicants. Further information on the characteristics of the sampling sites is displayed in SI Table S1.

The field monitoring for pesticides, macroinvertebrate fauna, and selected microorganisms was conducted in 2008/2009 for five months over spring and summer (SI Figure S2), which represent the period of the most intensive pesticide application. We assume that most of the major runoff events were captured since only one precipitation event >5 mm in 24 h occurred from December to February [see ref 13], when the monitoring activity was reduced (SI Figure S2).

Pesticide Monitoring and Chemical Analysis. Grab water samples were taken in 1-L solvent-rinsed amber glass bottles and stored unfiltered at 4 °C before liquid−liquid extraction or solid phase extraction (see SI Table S2). Superficial sediments were sampled with a dip net, wet-sieved on site to 64 μm, and decanted into a 1-L solvent-rinsed jar after allowing for a 15-min settle period. The jars were stored at 4 °C during transport and in the laboratory, where the supernatant water was decanted after 72 h of storage. Afterward the sediment was dried at 40 °C, finely ground, and stored at room temperature until extraction and analysis. Sediment samples (10 g) were extracted with 100 mL of 35% (v/v) water/acetone, which was subsequently mixed with Na_2SO_4 and extracted with dichloromethane (see SI Table S2 for details).

The TRIMPS passive samplers consisted of prefabricated LDPE membrane (Scubs Brand) bags (30 mm × 100 mm × 0.03 mm width/length/thickness) that were prerinsed overnight in 2,2,4-trimethylpentane. Afterward, 2,2,4-trimethylpentane (10 mL) was added to each bag, the air was extruded, and the bags were sealed with plastic dialysis clips. The samplers were transported to the site at 4 °C in distilled water and deployed by retention of the bag inside a steel mesh envelope (mesh size 12 mm) that was placed in an outer wire or plastic cage (all sides 15 cm, mesh size 10−12 mm). Duplicate cages were fixed approximately 10 cm above the stream bottom and separated by some meters at each location. The reported values are the mean of the two duplicate determinations where both bags were retrieved. The bags were recovered after approximately 28 days, and subsequently, the solvent volume from each bag was recorded and transferred to a 10 mL crimp cap vial with approximately 500 mg of anhydrous Na_2SO_4. The vial was stored at 4 °C during transport and then frozen at −20 °C in the laboratory, until the 2,2,4-trimethylpentane solvent was directly used in analysis.

The final extracts of all sampling methods were used in 6 different analysis programs: organochlorines and synthetic pyrethroids with gas chromatography-electron capture detector (GC-ECD), organophosphates with GC-pulse flame photometric detector (GC-PFPD), selected fungicides with GC-nitrogen phosphorus detector (GC-NPD), screening and triazines with liquid chromatography-tandem mass spectrometry (LC-MS/MS). See SI Table S3 for the compounds determined with each program and SI Table S4 for details on instrument parameters. The limit of quantification (LOQ) was determined as the lowest concentration of a compound that can be reliably quantified (95% confidence interval) in the matrix in question. The results were not corrected for recovery, which was determined by spiking randomly selected samples of each analytical batch of water and sediment samples (typically the 24 sites) with each reported pesticide.

Estimation of Dissolved Water Concentrations of Toxicants. To avoid overestimation of the toxicity from whole-water concentrations and to calculate the pore water concentrations for sediment samples, we estimated the bioavailable dissolved water concentration C_d (in μg/L) for toxicants in water and sediment samples using a reformulation of the equilibrium partitioning approach[14]

$$C_d = \frac{C_{tot}}{(f_{OC}K_{OC} + 1)}$$

where C_{tot} is the total concentration in the whole water sample in μg/L or the sediment sample in μg/kg, K_{OC} is the soil organic carbon−water partitioning coefficient in L/kg (see SI Table S3), and f_{OC} is the fraction of organic carbon that was approximated with the total organic carbon content (TOC) for water samples and the organic carbon (OC) content for sediment samples.

For TRIMPS, we assumed integrative uptake of pesticides with a log $K_{OW} > 3.5$[12] (see ref 3 for theoretical background of

kinetic regimes). The time-weighted average water concentration C_{TWA} for integrative uptake was calculated as

$$\log(C_{TWA}(t)) = -r\log(t) - b + \log(C_s(t))$$

where r is the substance-specific uptake rate constant, b is the intercept and $C_s(t)$ is the concentration of the respective compound in the TRIMPS after the deployment time t (see SI Text S1 for details on the derivation of the equation). Values for r and b were from Leonard et al.[12] For pesticides where no values were reported in the literature, r was predicted using a regression between log K_{OW} and r ($r = 0.138 \log K_{OW} + 0.41$; $n = 9$; $r^2 = 0.67$; $p = 0.008$) and b was set to 1 as derived for $C_s(t) = C_{TWA} = 0$ at the start of deployment.

For pesticides with a log $K_{OW} < 3.5$ equilibrium with the water phase was assumed[12] and the equilibrium water concentration C_{eq} was calculated according to

$$C_{eq}(t) = \frac{C_s(t)}{K_{SW}}$$

where K_{SW} is the dimensionless sampler−water partitioning coefficient. Since K_{SW} values for the majority of pesticides with a log $K_{OW} < 3.5$ detected in TRIMPS were not available, we approximated the K_{SW} values using the octane−water partitioning coefficient (see SI Table S5).

Estimation of Toxicity. The toxicity for macroinvertebrates and microorganisms of the estimated water concentrations was predicted using toxic units (TU)

$$TU = \frac{C_d}{EC50}$$

where C_d is as defined above (equivalent to C_{eq} and C_{TWA} for TRIMPS) and EC50 is the median acute effect concentration for the standard test species. *Daphnia magna* was used as standard test species for macroinvertebrates. Since for microorganisms no standard test species are available, both *Daphnia magna* and *Selenastrum capricornutum* were used as surrogates to detect potential effects on microorganisms (TU_{DM} and TU_{SC}, respectively). The laboratory-derived acute toxicity data (48-h EC50 for *D. magna* and 48-h to 96-h EC50 for *S. capricornutum*) used in the calculation is given in SI Table S3. Where no experimental toxicity data was available for *D. magna* and *S. capricornutum* (for 9 and 41 of the 97 pesticides, respectively; SI Table S3), the baseline toxicity was estimated from the octanol/water partitioning coefficient employing existing QSAR models.[15,16] To aggregate the TUs of all detected compounds in a site per sampling, the maximum TU (mTU) and sum of TUs (sTU) were calculated, which relate to the minimal and maximal expected toxicity of C_d, C_{eq}, and C_{TWA}, respectively.[7] We only report mTUs as both TUs were highly intercorrelated ($r \geq 0.97$ for all pairs of mTUs and sTUs) and the relationship with biotic end points was similar or slightly higher in terms of explained variance when compared with sTUs, which is consistent with the results of previous studies.[6,7]

Environmental Variables, Macroinvertebrate Monitoring, and Indicator Calculation. Temperature, pH, conductivity, dissolved oxygen, ammonium, nitrite, nitrate, phosphate, and turbidity were measured in the field. In addition, 25 habitat and landscape variables (see Supplementary Table S1) were recorded by visual inspection or using maps following EPA Victoria protocol.[17] See SI Table S1 for the mean values of all variables.

The macroinvertebrate sampling was conducted according to the rapid bioassessment method of the EPA Victoria[17] and included taking a pool sample (with a 250 μm mesh size net) of representative habitats and a kick sample (with a 500 μm mesh size net) where riffles were present.[18] The macroinvertebrates in the pool and riffle samples were each picked in the field for a minimum of 30 person-minutes per sample.[18] Since two family level biotic indices were employed for data analysis, the taxa were identified to family level or lower in the laboratory (SI Table S6).

We calculated the $SPEAR_{pesticides}$ indicator for detecting effects of pesticides and for comparison purposes the SIGNAL index,[18] which is commonly used in Victoria to detect general ecological impairment. See SI Table S6 for details on both indicators.

Monitoring of Selected Microorganisms. Two dried leaves (48-h at 60 °C) of *Eucalyptus camaldulensis* (0.16 ± 0.08 g SD), a regionally common riparian tree, were deployed in each of fine mesh bags (mesh size, 50 μm; nylon cylinder length, 15 cm) as natural substratum for the colonization by microorganisms. Duplicate bags were fixed 10 cm above the stream bottom so that they touched the bottom and were exposed for approximately 5 weeks at each site (SI Figure S2). On retrieval, the bags were kept submerged in stream water using a bucket, and single leaves were transferred gently but quickly with minimal exposure to air into 50-mL sample tubes containing 30 mL of prefiltered (Millipore Stericup 0.22 μm filter with glass fiber prefilter) stream water with 1% glutaraldehyde (Merck). The samples were stored in the dark at 4 °C during transport and in the laboratory until analysis.

We determined the density of selected microorganisms, i.e., bacteria, flagellates, ciliates, amoebas, nematodes, and gastrotrichs, per unit of leaf mass, where two leaves were each used for analysis of bacteria and the other microbes. To remove bacteria for analysis, the detergent Triton X-100 (Sigma-Aldrich) was added to the preserved samples to 0.01%, and they were sonicated on ice for three 1-min bursts at 25% intensity on a Branson Ultrasonics S-250D probe. Other microbes were removed by agitating the preserved samples on a vortex mixer at medium strength for 10 s. They were then separated from debris by density-gradient centrifugation, following Shimeta and Sisson[19] except that reverse-osmosis purified water was used. All organisms were stained and mounted for epifluorescence microscopy according to Shimeta et al.,[20] and they were counted on a Leica DM2500 microscope. Leaves were dried at 60 °C and weighed to determine their masses. See SI Table S1 for mean densities of microorganisms.

Data Analysis. Analysis of variance (ANOVA) with *a priori* treatment contrasts was used to identify significant differences in log TUs between sampling methods and sampling periods, where minimum log TUs were set to −5 to reduce the influence of differences in the LOQ. The relationships between environmental variables and biotic end points (densities of microbial groups, $SPEAR_{pesticides}$, and SIGNAL) were examined using linear regression models. Before analysis, the data was aggregated per site using the mean, as the sampling periods of the different abiotic and biotic monitoring methods differed (SI Figure S2). The mTUs of different sampling methods were aggregated by selecting the maximum mTU per site. Furthermore, a variable cluster analysis was conducted to identify pairs of abiotic variables with high intercorrelation (Pearson's $r > 0.7$), where the variable with lower relevance for explaining aquatic community composition was removed based on expert judgment. Manual and automatic model building employing r^2 and Akaike Information Criterion

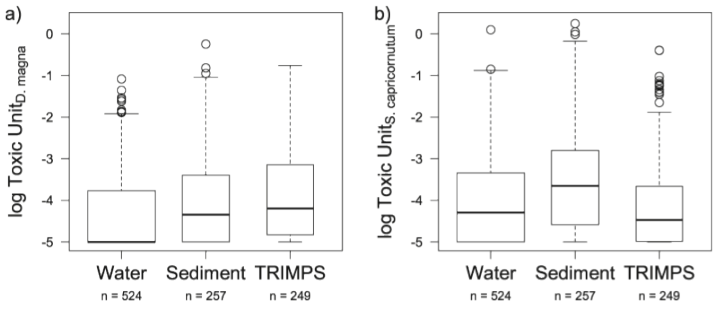

Figure 1. Box-and-whisker plot of the log toxic units (TU) for (a) *D. magna* and (b) *S. capricornutum* calculated for each pesticide detection and grouped by the three sampling methods. All log TUs < −5 were set to −5 to reduce influence of differences in the LOQ. *n* gives the sum of all pesticide detections of each sampling method for all sites and for the complete monitoring period.

(AIC) as goodness of fit measures and the *t* test for significance of individual variables were used to identify the best-fit linear regression model. Statistical models were checked for error assumptions (constant variance, noncorrelation, and normality of residuals) and unusual observations (leverage, outliers).[21] To evaluate the relevance of the different sampling methods for detecting effects on biota, the maximum log mTU per site was derived by aggregating different combinations of sampling methods and examining their relationship with $SPEAR_{pesticides}$. All graphics and computations were undertaken using the free software package R (www.r-project.org; version 2.11) for Mac OS X (10.6.4).

RESULTS

Pesticide Monitoring and Estimated Toxicity. Of the 97 pesticides investigated, 48, 27, and 34 compounds were detected in the grab water samples, sediment samples, and TRIMPS passive samplers, respectively (SI Table 3). Moreover, grab water samples yielded approximately twice the number of total detections > LOQ compared to sediment samples and TRIMPS passive samplers (Figure 1). The median K_{OC} was 970, 4230, and 13 083 for the pesticides detected in water samples, sediment samples, and TRIMPS, respectively (SI Table 3).

The insecticides carbaryl, chloropyrifos, methiocarb, pirimicarb, permethrin, and diazinon as well as the fungicides trifloxystrobin, chlorotholanil, pyrimethanil, and iprodione reached concentrations equivalent to a log TU > −2 for *D. magna* (log TU_{DM}) (Table 1). Pesticides with estimated log TUs > −2 for *S. capricornutum* (log TU_{SC}) encompassed the herbicides simazine, prometryn, linuron, propyzamide, hexazinone, and desethylatrazine as well as the fungicides oxadixyl, myclobutanil, and the 4 fungicides previously mentioned (Table 1). Pyraclostrobin, propargite, and propyzamide for TU_{DM}, and atrazine and indoxacarb for TU_{SC}, most frequently exceeded the related log TU of −3 but were not found at concentrations higher than a log TU of −2 (SI Table S3). Trifloxystrobin and pirimicarb were the most toxic pesticides for invertebrates at 14 of the 24 sites as indicated by the mTU_{DM}, whereas simazine and trifloxystrobin exhibited the highest toxicities at 14 sites in terms of mTU_{SC} (SI Table S1). Both the log TU_{DM} and log TU_{SC} for all detections were significantly different between sampling methods (ANOVA, $p < 0.001$), where the log TU_{DM} of grab water samples and both the log TU_{SC} of grab water samples and TRIMPS were significantly lower in treatment contrasts (all $p < 0.001$) than the related TUs of sediment samples (Figure 1 a,b). The log TU_{DM} values of the sampling methods were not significantly different between the sampled months ($p = 0.75$) (SI Figure S3a), whereas this was the case for the log TU_{SC} ($p = 0.03$) with log TU_{SC} in November and following months being significantly lower (all $p < 0.05$) than in September (SI Figure S3b).

Relationship between Estimated Toxicity and Biotic End Points. The $SPEAR_{pesticides}$ (Figure 2a) and SIGNAL indicator (Figure 2b) showed negative linear relationships with estimated pesticide toxicity for macroinvertebrates (log mTU_{DM}) that was derived from aggregating TU_{DM}s over sampling methods and sampling dates for each site. This relationship was stronger for $SPEAR_{pesticides}$. Neither indicators responded to other environmental variables (Table 2).

The microbial densities were not linearly related to the estimated toxicity in terms of log mTU_{DM} or log mTU_{SC} (all $p > 0.28$), but some groups exhibited linear relationships with the environmental variables temperature, conductivity, or turbidity (Table 2). A high intercorrelation ($r > 0.7$) between groups of microorganisms was only observed for gastrotrichs and amoebas ($r = 0.85$, $p < 0.001$).

Including grab water samples in the calculation of log mTU_{DM} yielded only a minor improvement of the relationship between log mTU_{DM} and $SPEAR_{pesticides}$ when compared to the log mTU_{DM} relying on sediment samples in conjunction with TRIMPS (SI Table S7). Log mTU_{DM} based on grab water sampling and TRIMPS passive sampling alone or in conjunction had a poor relationship with $SPEAR_{pesticides}$ (SI Table S7).

DISCUSSION

Estimated Toxicity of Pesticides. Half of the 97 pesticides investigated at the 24 sites were detected above the LOQ within the 5-month monitoring period, of which 18 compounds reached levels above a log TU_{DM} or log TU_{SC} of −2. This threshold is commonly regarded as protective in pesticide regulation, and no notable effects (>6 weeks compared to control) on primary producers, invertebrates, and fish have been observed in mesocosm studies below this level of toxicity.[1] In the Supporting Information (Table S3) we report compounds that exceeded a log TU of −3 as some field studies have reported change in the macroinvertebrate community up to concentrations related to a

Table 1. Maximum Concentrations (max conc), the Related Toxic Units for *D. magna* (TU_{DM}) and *S. capricornutum* (TU_{SC}), and Percent of All Samples *n* Exceeding log TU_{DM} or log TU_{SC} of −2 for Pesticides with At Least One log TU > −2 in the Different Sampling Methods with Complete Table Available as SI Table S3

		water samples (n = 144)					sediment samples (n = 144)					TRIMPS samples (n = 96)				
compd	class[a]	max conc (μg/L)	log TU_{DM}	% log TU_{DM} > −2	log TU_{SC}	% log TU_{SC} > −2	max conc (μg/kg)	log TU_{DM}	% log TU_{DM} > −2	log TU_{SC}	% log TU_{SC} > −2	max conc (μg/L)[b]	log TU_{DM}	% log TU_{DM} > −2	log TU_{SC}	% log TU_{SC} > −2
carbaryl	I	0.039	−2.4	0	−5.7	0	2	−1.4	1	−4.7	0	0.3	−3.8	0	−7.1	0
chlorothalonil	F						460	−1.3	3	−1.5	3					
chlorpyrifos	I	0.04	−1.5	1	−4.2	0	42	−1.0	4	−3.7	0	110	−1.3	5	−4.0	0
desethylatrazine	H	1.3	−3.2	0	−1.9	1										
diazinon	I						17	−1.2	1	−5.1	0	17	−1.1	2	−4.9	0
hexazinone	H	0.96	−5.5	0	−0.9	4	123	−4.4	0	0.2	3					
iprodione	F	3	−1.9	1	−2.8	0	170	−0.8	1	−1.7	1	120	−1.0	3	−1.9	1
linuron	H	0.6	−3.0	0	−2.1	0	18	−2.6	0	−1.7	1	19	−2.1	0	−1.2	2
methiocarb	I	1.2	−2.3	0	−5.9	0						153	−1.2	2	−4.8	0
myclobutanil	F	2.9	−3.7	0	−2.6	0	120	−4.1	0	−4.9	0	26	−3	0	−1.9	1
oxadixyl	F	0.4	−6.1	0	−3.4	0	2	−6.1	0	−3.3	0	0.2	−3.2	0	−0.4	1
permethrin	I						80	−1.9	1	−2.8	0					
pirimicarb	I	1.4	−1.1	4	−5.0	0	26	−0.2	6	−4.2	0	7.3	−1.3	4	−5.2	0
prometryn	H	21	−2.7	0	0.1	3	374	−3.0	0	−0.2	2	300	−3.9	0	−1.0	4
propyzamide	H						180	−2.6	0	−1.8	1					
Pyrimethanil	F	70	−1.6	1	−1.2	1	272	−2.2	0	−1.8	1	1390	−3.1	0	−2.7	0
simazine	H	15	−3.8	0	−1.1	1	260	−3.4	0	−0.7	3	6	−6.3	0	−3.6	0
trifloxystrobin	F	0.73	−1.4	1	−1.7	1	8	−2.0	1	−2.4	0	793	−0.8	6	−1.1	5

[a] I = insecticide; H = herbicide; F = fungicide. [b] 2,2,4-Trimethylpentane solvent recovered from the passive samplers.

Table 2. Environmental Variables Selected in Linear Model Building with Highest Explanatory Power for the Response Variables Using Explained Variance (r^2) and the Akaike Information Criterion (AIC) as Goodness of Fit Measures

response variable	log mTU_{DM}	T (°C)	conductivity (μS/cm)	turbidity (NTU)	r^2	AIC
$SPEAR_{pesticides}$	x				0.67	−34
SIGNAL	x				0.36	98
bacteria[a]						
flagellates[a]		x	x		0.49	434
ciliates[a]		x		x	0.59	209
amoebas[a]				x	0.78	200
nematodes[a]						
gastrotrichs[a]				x	0.59	182

[a] Per unit of leaf mass.

log TU as low as −3.[1] In our study, insecticides only exceeded −2 for log TU_{DM}, whereas herbicides only reached −2 for log TU_{SC}, indicating that insecticides and herbicides primarily represent a risk for invertebrates and for primary producers, respectively (Table 1). By contrast, the fungicides trifloxystrobin, chlorotholanil, iprodione, and pyrimethanil exceeded both the log TU_{DM} and log TU_{SC} of −2 (Table 1) and thus are ecotoxicologically relevant to both trophic levels. The mode of action of most fungicides is less selective compared to current-use herbicides and insecticides, and they may therefore exert negative impacts on a wide range of nontarget organisms.[22] The fungicide trifloxystrobin was most frequently identified as the most toxic compound when regarding both the maximum mTU_{DM} and mTU_{SC} per site (SI Table S1). The high relevance of fungicides in terms of toxicity may be a result of the type of agriculture of our study region (mainly grapevine, fruit, and vegetable production) as fungicides were less relevant in regions with soy bean, cereal, and oilseed production.[7,23] However, our study highlights that fungicides may play a larger role for toxicity on freshwater communities than is currently acknowledged within the risk assessment of pesticides given that for example (a) only insecticides and herbicides are among the 33 priority pollutants of the European Union,[24] and (b) few ecotoxicological studies on fungicides have been conducted compared to the number of studies dealing with insecticides and herbicides,[22] particularly in relation to aquatic fungi (the trophic group that fungicides are designed to affect).

Effects on Biota. Both biotic indicators for macroinvertebrates showed a decline in the abundance of sensitive taxa in the communities with an increase in estimated toxicity in terms of log mTU_{DM} aggregated from all sampling methods (Figure 2). This

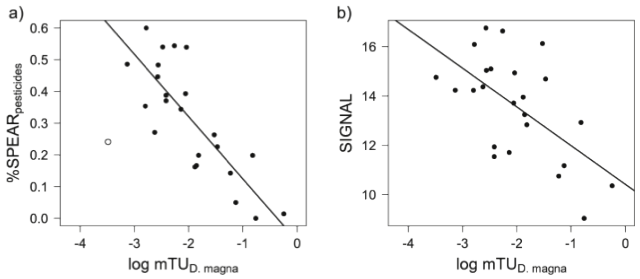

Figure 2. Relationship between the log of maximum toxic units (log mTUs) for *D. magna* and (a) $SPEAR_{pesticides}$ and (b) SIGNAL grades in the 24 sites. The log mTUs resulted from the log mTUs of all sampling methods for each site that were aggregated over the sampling methods and sampling dates using the maximum value. Linear model r^2 was 0.67 ($p < 0.001$) for $SPEAR_{pesticides}$ [0.51 when including one observation (○) that was removed because of unduly influencing the model according to Cook's distance] and 0.36 ($p = 0.002$) for SIGNAL.

result is in agreement with other studies that found strong linear relationships between pesticide toxicity and macroinvertebrate community composition in agricultural areas.[6,7] The $SPEAR_{pesticides}$ and the SIGNAL index both responded exclusively to estimated pesticide toxicity and not to other environmental gradients in the present study. Given that we recorded many relevant environmental variables that typically explain macroinvertebrate community composition (SI Table S1) and that the SIGNAL index is a general ecological index that responds to many environmental stressors,[18] our findings emphasize the importance of pesticides for the structure of freshwater communities in agricultural areas (Table 2). The relationship with toxicity was highest for the $SPEAR_{pesticides}$ indicator (Figure 2, Table 2) and confirms the suitability of this trait-based biomonitoring concept to selectively detect effects of pesticides.[5] The good performance of the $SPEAR_{pesticides}$ in our study is particularly remarkable given the potential inaccuracies arising from the use of (a) family level taxonomic and trait data and (b) physiological sensitivity data predominantly from European and North American taxa for the sensitivity assessment of Australian taxa (SI Table S6). Hence, the variation of the physiological sensitivity within macroinvertebrate families from different continents may not have major influences on the risk assessment of pesticides in a trait-based framework. Moreover, least polluted sites in our study (log $TU_{DM} < -2.5$) reached levels of $SPEAR_{pesticides}$ that were similar to those of least contaminated and reference sites (0.4−0.6) in five regions of Europe[8] suggesting that the trait composition may be relatively constant not only across biogeographic regions but even across continents, though further investigations are required.

For the density of selected microbial groups, we did not find a relationship with the log mTU_{SC} or log mTU_{DM}. Other studies detected change in the periphyton community[9] and the structure of the bacterial community[10] due to pesticide stress, whereas there is still a paucity of toxicity data for protozoa.[11] The absence of evidence for effects of pesticides in our study is not necessarily evidence for the absence of effects but may result from (a) using inaccurate toxicity estimates and (b) inaccurate effects end points. Regarding the former, we examined relationships of the microorganisms with estimated toxicity for invertebrates and primary producers, i.e., log TU_{DM} and log TU_{SC}, but a more specific assessment of the pesticide toxicity for microorganisms may be required, though no such data is currently available.[11] With respect to the inaccurate effect end points, we used the density for broad taxonomic microbial groups whereas specific biotic indicators, as for macroinvertebrates, and a more thorough taxonomic and functional characterization of the microbial community may be needed to detect effects of pesticides. However, in agreement with other studies[25,26] we found a relationship of temperature and conductivity with the density of several microbial groups (Table 2).

Performance of the Different Sampling Methods. In grab water samples, more compounds and approximately twice the number of total detections > LOQ were found compared to sediment samples and TRIMPS passive samplers (Figure 1, SI Table S3). This can be explained by the following: (a) several predominantly hydrophilic compounds were only (SI Table S3) or more frequently detected in grab water samples as indicated by a lower median K_{OC} for detected pesticides in grab water samples and (b) the LOQs for many compounds in sediment samples and TRIMPS were comparably higher than in grab water samples (SI Table S3) resulting in a lower number of detections. Due to the LOQ, the average toxicity of compounds in water samples was significantly lower than that for sediment samples and TRIMPS in terms of log TU_{DM} (Figure 1a) and significantly lower than that for sediment samples for log TU_{SC} (Figure 1b). In addition, the lower toxicity of grab water samples is also a consequence of point sampling since this does not detect episodic peak concentrations of pesticides[2] and in turn results in lower maximum pesticide concentrations and hence TUs. In fact, for only 3 and 4 of the 19 compounds detected concurrently in all three sampling methods the maximum TU_{DM} and TU_{SC} were found in water samples (SI Table S3). These findings are consistent with a study of 10 pesticides in a French agricultural area comparing event-driven water sampling, sediment sampling, and passive sampling.[4] In this study, the number of pesticides and the number of detections > LOQ were highest in water samples, whereas the highest toxicity in terms of TUs resulted from sediment samples. However, sediment samples in the French study had the lowest explanatory power for macroinvertebrate community change in terms of $SPEAR_{pesticides}$, and both event-driven water sampling and passive sampling performed reasonably well (r^2 = 0.38 and 0.51, respectively).[4] By contrast, the toxicity derived from sediment samples was most important to explain variation in $SPEAR_{pesticides}$ in the present study, whereas log mTUs derived from passive sampling exhibited only good explanatory power in

conjunction with the log mTUs from sediment samples and the log mTUs derived from grab water sampling were redundant to explain variation in biotic indices (SI Table S7). This can be explained either by sediment-mediated exposure that was the main cause of observed effects and that was not detected by water-based sampling methods or by methodological problems related to the water sampling methods. First, the low performance of passive sampling in the present study may be due to inaccuracies associated with the estimation of the passive sampler water concentrations (time-weighted average and equilibrium) since calibration data and K_{SW} for the TRIMPS were not available for many compounds (see Experimental Section). Furthermore, in the case of fluctuating pesticide concentrations involving multiple runoff events, the time-weighted average water concentrations from passive samplers are probably not strongly correlated with the peak pesticide concentration,[3] which can be most important for biota. The good performance of passive sampling in the study of Schäfer et al.[4] probably resulted from the restriction of the monitoring to a shorter time period where only one runoff event occurred. Finally, the low explanatory power of log mTUs from grab water sampling in contrast to the good performance of event-driven water sampling in the other study[4] demonstrates the relevance of employing sampling methods that are either continuous or triggered by exposure events. Overall, we suggest that a combination of sediment sampling and passive or event-driven water sampling is most suitable to assess the toxicity and to establish relationships with effects on biota, whereas grab water sampling is unreliable for determining the toxicity.

■ ASSOCIATED CONTENT

Supporting Information. Figures of the sampling sites, sampling schedule and of mTU over time, details on the derivation of the equation for time-weighted average concentrations, characteristics of the sampling sites including mTU and abundance of selected microorganisms, information of the extraction and cleanup procedures, a detailed list of all pesticides detected with physicochemical and ecotoxicological data, parameters of the instruments used for chemical analysis, the explanatory power of different sampling methods for ecological quality and an overview of the sampled macroinvertebrate taxa with classification for the biotic indicators. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Phone: ++49 (0) 6341 28031536; e-mail: senator@ecotoxicology.de.

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8. Publication VII

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Thresholds for the Effects of Pesticides on Invertebrate Communities and Leaf Breakdown in Stream Ecosystems

Ralf B. Schäfer,*[,†] Peter Carsten von der Ohe,[‡] Jes Rasmussen,[§] Ben J. Kefford,[∥] Mikhail A. Beketov,[⊥] Ralf Schulz,[†] and Matthias Liess[⊥]

[†]Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau, Fortstrasse 7, 76829 Landau, Germany

[‡]UFZ, Helmholtz Centre for Environmental Research, Department Effect-Directed Analysis, Permoserstrasse 15, 04318 Leipzig, Germany

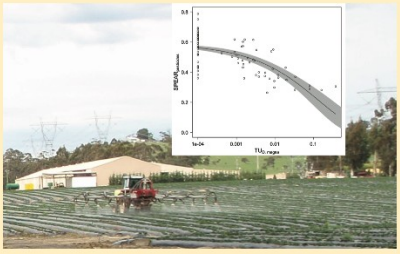
[§]Faculty of Science and Technology, University of Aarhus, Vejlsøvej 25, 8600 Aarhus, Denmark

[∥]Centre for Environmental Sustainability, School of the Environment, University of Technology Sydney, Broadway NSW 2007, Australia

[⊥]UFZ, Helmholtz Centre for Environmental Research, Department System-Ecotoxicology, Permoserstrasse 15, 04318 Leipzig, Germany

Supporting Information

ABSTRACT: We compiled data from eight field studies conducted between 1998 and 2010 in Europe, Siberia, and Australia to derive thresholds for the effects of pesticides on macroinvertebrate communities and the ecosystem function leaf breakdown. Dose−response models for the relationship of pesticide toxicity with the abundance of sensitive macroinvertebrate taxa showed significant differences to reference sites at 1/1000 to 1/10 000 of the median acute effect concentration (EC50) for *Daphnia magna*, depending on the model specification and whether forested upstream sections were present. Hence, the analysis revealed effects well below the threshold of 1/100 of the EC50 for *D. magna* incorporated in the European Union Uniform Principles (UP) for registration of single pesticides. Moreover, the abundances of sensitive macroinvertebrates in the communities were reduced by 27% to 61% at concentrations related to 1/100 of the EC50 for *D. magna*. The invertebrate leaf breakdown rate was positively linearly related to the abundance of pesticide-sensitive macroinvertebrate species in the communities, though only for two of the three countries examined. We argue that the low effect thresholds observed were not mainly because of an underestimation of field exposure or confounding factors. From the results gathered we derive that the UP threshold for single pesticides based on *D. magna* is not protective for field communities subject to multiple stressors, pesticide mixtures, and repeated exposures and that risk mitigation measures, such as forested landscape patches, can alleviate effects of pesticides.



■ INTRODUCTION

Freshwater ecosystems are among the most threatened ecosystems in terms of species extinctions and losses in ecosystem services. One of the major stressors for these ecosystems are pesticides, which are introduced via point and nonpoint sources.[1] An efficient protection of freshwater ecosystems requires the determination of a reliable threshold value for the effects of pesticides. For example, the Uniform Principles (UP) of the European Union (EU) state that for a single pesticide "no authorization shall be granted if the toxicity/exposure ratio for fish and *Daphnia* is less than 100 for acute exposure [...]".[2] A review of mesocosm studies on the effects of single insecticides (carbamates, organophosphates, and pyrethroids) by Wijngaarden et al.[3] suggested that this safety factor would be protective. They reported that insecticide concentrations below the above-mentioned toxicity/exposure ratio of 100 that relates to a concentration of 1/100 of the median effect concentration (EC50) for *Daphnia magna* are unlikely to cause notable effects.[3] However, the joint effects of multiple stressors, including mixtures of pesticides[4] are rarely considered in mesocosm studies,[5] though they may influence effect thresholds.[6] Indeed, a field study conducted in 20 agricultural streams showed a significant change in community structure already at an acute toxicity/exposure ratio for *D. magna* in the range of 100−1000 for the most toxic compound.[7]

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Table 1. Study Regions with Number (No.) of Sites, Biological End Points Reported, Number of Pesticides Measured and Range of Toxic Units (TU) Included in This Study

region	no. sites	biological end points reported	pesticide monitoring methods[d]	no. of pesticides measured	lowest log TU reported	highest log TU reported	ref
South Finland	13	$SPEAR_{abundance}$, $SPEARP_{PM\ abundance}$ and invertebrate leaf breakdown	PS and SPS	10	−5	−4.3	17
Brittany, France	16	$SPEAR_{abundance}$, $SPEARP_{PM\ abundance}$ and invertebrate leaf breakdown	EWS, PS, and SPS	10	−5	−0.4	17
Central Germany	20	$SPEAR_{abundance}$	2 EWS	21	−5	−0.7	7
Victoria, Australia	24	$SPEAR_{pesticides}$[b] and invertebrate leaf breakdown	GWS, PS, and sediment	97	−3.5	−0.2	8, 19
Island Funen, Denmark	14	$SPEAR_{pesticides}$[b] and invertebrate leaf breakdown	EWS, GWS, and sediment	31	−6.6	−1.7	22, 23
Flanders, Belgium	7	SPEAR[%][b]		0[e]			18
North Germany	11	SPEAR[%][b]		0[e]			18
Siberia, Russia	6[a]	$SPEAR_{organic}$[c]		0[e]			24

[a]Only reference sites included (sites 3, 5, 6, 7, 9, and 10 of original publication[24]). [b]Reported indicator values were calculated according to Liess and von der Ohe.[7] For this study, the $SPEAR_{pesticides}$ values were calculated from the original data as described below. [c]For this study, the $SPEAR_{pesticides}$ indicator was calculated from the original data as described below. [d]PS = passive sampling, SPS = suspended particle sampling, EWS = event-driven water sampling, GWS = grab water sampling [e]Sites were considered as having no pesticide contamination. See references for details.

Beside effects on the structure of freshwater communities, pesticides can impede important ecosystem functions such as leaf breakdown[8] that represents the main energy source in freshwater ecosystems beside gross primary production.[9] However, to which extent effects on biota propagate to effects on ecosystem functions has been ranked as one of the most important research questions for the conservation of biological diversity.[10] Theoretically, the biotic community and ecosystem functions can be linked in four ways.[11] First, in a near linear way implying effects on biota would lead to a similar decline in ecosystem functions. Second, there may be functional redundancy in the community and no effects on ecosystem functions would occur up to certain thresholds. Third, the loss of most species may be compensated whereas the loss of a few so-called keystone species or ecosystem engineers would result in changes in ecosystem functions. Thus the effects depend on the identity of the species lost. Fourth, a chemical may alter the functional capacity of species and hence affect ecosystem functioning without alteration of the community.[12] It is unknown which of these models applies for the relationship between effects of pesticides on biota and on ecosystem functions and whether this relationship would be universal.

Species traits have been suggested as a stressor-specific tool in ecological risk assessment[13,14] as they allow for a mechanistic link between stressors and communities, even under conditions of multiple stressors.[15,16] The SPEcies At Risk (SPEAR) indicator for pesticides[7] relies on species traits to calculate the fraction of pesticide-sensitive species in macroinvertebrate communities. The SPEAR index has been successfully linked to pesticide toxicity and the leaf breakdown rate in field studies, while being generally discriminative toward co-occurring stressors in agricultural regions,[7,14,17−19] as well as applicable over different biogeographical regions.[17−19] The latter is especially important because it enables the meta-analysis of studies from different regions.

In this study, we determined thresholds for the effects of pesticides on freshwater ecosystems from a meta-analysis of field studies. Therefore, we compiled data from various field studies in different regions on the effects of pesticides on freshwater macroinvertebrate communities as detected using the SPEAR approach as well as on the ecosystem function leaf breakdown. Macroinvertebrate communities were selected as structural end point since (a) they belong to the most sensitive group of organisms to pesticides in freshwater communities, (b) trait-based approaches in freshwater ecology are most advanced for macroinvertebrates, and (c) there is a paucity of field studies on the effects of pesticides on other groups of biota.[20] In addition, we examined how effects on the structure, in terms of the fraction of pesticide-sensitive species in the communities, are related to effects on the important ecosystem function of leaf breakdown.[20]

EXPERIMENTAL SECTION

Selection and Description of Field Studies. The following inclusion criteria were used for the selection of field studies on the effects of pesticides: (1) at least 5 different streams monitored, (2) selection of pesticides for chemical analysis that are most likely to represent a risk to macroinvertebrates in the respective region based on recommended pesticide use information for the respective year (and region) sampled, available toxicity data for *D. magna* or results from previous monitoring programs [see 7, 17, 19] and (3) the SPEAR values or leaf breakdown rates reported. In addition, we included reference sites from studies, where SPEAR values were reported (Table 1). We focused on studies reporting the trait-based SPEAR indicator, because in contrast to taxonomical data this indicator has been demonstrated to be applicable over different biogeographical regions.[17,18] However, we are not aware of other studies that met the first two criteria and presented macroinvertebrate community data to assess the effects of pesticides. For example, one study encompassing 29 different streams[21] was not included since only sediment concentrations for a limited set of pesticides were reported and the total sediment concentration of the monitored pesticides was used as a proxy for nonmonitored pesticide concentrations. Overall, 8 studies conducted between 1998 and 2010 (study duration between 2.5 and 36 months) with a total of 111 sites were included in the present study, of which 6 studies were conducted in different regions of Europe, and a study in each of Australia and Siberia (Table 1). Except for reference sites which were predominantly located in forested areas, the sites in the studies were located in agricultural areas. The sites were

selected to not receive discharge from wastewater treatment plants, industrial facilities or mines in order to exclude the input of toxicants other than pesticides. The pesticide monitoring was adjusted (1) to capture episodic runoff events and (2) to the properties of the pesticides selected for chemical analysis in the particular study. The selected pesticides varied among the study regions due to differences in crops, pests and authorized pesticides (Table 1).

Data preparation. Environmental concentrations of pesticides were scaled to acute effects of *D. magna* calculating Toxic Units (TU)[25] by dividing the compound concentration with the respective 48-h median effect concentration (EC50) for *D. magna*. Dose−response modeling was used to evaluate the relationship between TU and SPEAR, which represent pesticide toxicity and community change, respectively. The TUs used here were given as the maximum TUs of all pesticides across samples per site in the original studies and were reported to have similar explanatory power for biotic end points as the sum of TUs of all pesticides across each or all samples per site.[7,19,26] The maximum TU represents the simplest approach because the estimated pesticide toxicity relies solely on the most toxic pesticide concentration observed per site, whereas all pesticide concentrations per sample or site contribute to the calculation of the sum of TU. Carbamate and organophosphate insecticides and several fungicides were predominantly responsible for the maximum TU in the sites (Supporting Information Table S1).

We used the modified version of the original SPEAR indicator[7] as described in Schäfer et al.[17] (therein referred to as $SPEAR_{pM\ abundance}$) to compare the indicator values between different biogeographical regions. For terminological clarity, we refer to this indicator as $SPEAR_{pesticides}$ in the following as suggested by Beketov et al.[27] For sites for which this version of the SPEAR indicator was not reported (Table 1), we calculated the indicator according to:

$$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n} \log(x_i + 1)y}{\sum_{i=1}^{n} \log(x_i + 1)}$$

where n is the number of taxa observed in a sampling site, x_i is the abundance of taxon i and y is 1 if taxon i is classified as Species At Risk (SPEAR) regarding the traits "physiological sensitivity" and "dispersal capacity", otherwise 0. The trait data used were derived from the database associated with the SPEAR online calculator (http://www.systemecology.eu/SPEAR/index.php).

To characterize the propagation of effects from pesticide-driven structural changes to ecosystem functions, the response of the invertebrate-driven leaf breakdown rate ($k_{invertebrate}$)[28] to changes in $SPEAR_{pesticides}$ was investigated. The leaf breakdown rate is not stressor-specific and hence responds to different environmental conditions.[29] In contrast to species traits,[30] $k_{invertebrate}$ varies over biogeographical regions and would not be expected to be similar across sites without pesticide contamination because of the influence of other environmental gradients.[31] Indeed, no relevant pesticide toxicity and only minor variation of $SPEAR_{pesticides}$ was detected in the sites from South Finland, whereas the invertebrate leaf breakdown rate varied strongly between sites in response to temperature.[17] Since the aim was to examine the link between pesticide-driven community change and invertebrate leaf breakdown rate, these sites were not considered for further analysis. The invertebrate leaf breakdown rates from the French, Danish and Australian streams (Table 1) were not comparable as leafs of different tree species were employed. Therefore, the % change in the leaf breakdown rate $k_{invertebrate}$ was calculated for each data set by dividing all values for $k_{invertebrate}$ by the maximum value obtained from individually fitted models for $k_{invertebrate}$ as explained by $SPEAR_{pesticides}$ (see below). We used the maximum value from the models fitted with all data points instead of the maximum value for $k_{invertebrate}$ from the respective raw data in order to avoid undue influence of a single data point.

Data Analysis. Before analysis the data were divided into sites with and without forested upstream sections, as defined in the original publications, and analyzed separately (Table 1). This was done because the presence of forested upstream sections was demonstrated to alleviate the effects of pesticides on the macroinvertebrate community as indicated by $SPEAR_{pesticides}$.[7,17,26] S-shaped dose−response curves with TU as concentration and $SPEAR_{pesticides}$ as response variable were computed using two-parameter log−logistic, Weibull I and Weibull II models with the upper limit fixed to the arithmetic mean of the $SPEAR_{pesticides}$ values for reference sites and the lower limit fixed to 0,[32] representing the lowest possible indicator value. In addition, linear, quadratic, and cubic regression models were computed to check for the fit of more parsimonious models. The best-fit model among the s-shaped dose response models and the polynomial regression models was selected using the Bayesian Information Criterion (BIC). If a s-shaped dose−response curve represented the best-fit model, we calculated the effect concentration (EC) for the percentage (p) of reduction in $SPEAR_{pesticides}$ for p = 10, 50 and 90%. In addition, the p value was computed for the EC related to the log TU of −2 that is equal to the safety factor of 100, employed in the UP of the EU. Moreover, we derived the lowest concentration at which significant differences (α = 0.05) to reference sites occur in the best-fit dose−response model using 95% confidence intervals. Technically, we determined the lowest concentration for which the 95% confidence interval of the fitted model did not overlap with the 95% confidence interval for the reference sites. Several of the original studies assigned log TUs of either −5 or −4 to sites where no pesticides were found assuming that this would represent the minimum log TU for which no pesticide effects would occur (Table 2). Since the minimum TU influences the dose−response modeling, all analyses were conducted for both minimum reported TUs, that is, we assigned either a log TU of −5 or −4 to all sites with no pesticide detections. To confirm the results of the dose−response modeling, analysis of variance (ANOVA) with a priori treatment contrasts was used to identify significant differences in $SPEAR_{pesticides}$ values between reference sites and groups of contaminated sites in terms of TU. The class boundaries of log TU ≤ −3.5 (reference sites),

Table 2. Estimated Effect Concentrations (EC) in Terms of log TU for p = 10%, 50%, and 90% Reduction in $SPEAR_{pesticides}$ for the Models with and without Forested Upstream Sections (FUS and WFUS, Respectively) and with a Minimum log TU of −5 (low min.) or −4 (high min.)

	estimated EC (in log TU)			
p	FUS low min.	FUS high min.	WFUS low min.	WFUS high min.
10	−3.6	−2.9	−4.2	−3.5
50	−1.7	−1.4	−2.5	−2.1
90	−0.4	−0.4	−0.6	−0.6

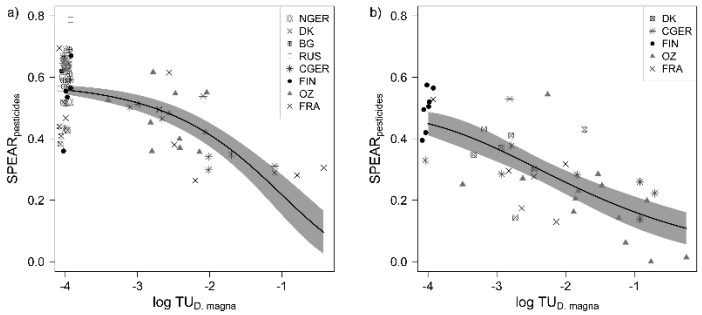

Figure 1. Dose−response curves with 95% confidence bands (gray) for the relationship between log TU and SPEAR$_{pesticides}$ for sites with forested upstream sections (a) and for sites without forested upstream sections (b). Reference sites were assigned a minimum log TU of −4. Random noise (0.00002) was added (jittering) to the TU values in order to show all data points in the plot. This affected primarily the sites with minimum TUs.

−3.5 < log TU ≤ −2.5 (lightly contaminated sites), −2.5 < log TU ≤ −1.5 (moderately contaminated sites) and log TU > −1.5 (highly contaminated sites) were selected in order to have similar class widths and at least 5 observations in each class. A similar classification was used in the study of Schäfer et al.[17]

For the examination of the propagation of effects of pesticides on ecosystem functions, the relationship between the invertebrate-driven leaf breakdown rate (% $k_{invertebrate}$) and SPEAR$_{pesticides}$ was modeled using s-shaped dose−response models and polynomial regression models as described above, except that three-parameter s-shaped dose response models were fitted since no upper limit could be fixed. First, the modeling was done separately for each country, because in the original studies this relationship had only been examined for the sites from Brittany, France. Subsequently, the data were modeled jointly. For best-fit linear regression models, significance of the slope was tested with the t test and in the case of data from different countries, analysis of covariance (ANCOVA) was used to detect significant differences between slopes and intercepts from the countries. ANOVA, ANCOVA and linear regression models were checked for normal distribution of residuals, homoscedasticity and unusual observations.[33] All computations and graphics were created with the free and open source software R (version 2.13.1 for Mac OS X, 10.6.8)[34] including supplemental packages such as "drc" for dose−response modeling.[35]

■ RESULTS

Weibull I and II as well as log−logistic models were identified as best-fit dose−response models for TU and SPEAR$_{pesticides}$ (Supporting Information Table S2). The estimated EC$_{10}$ for the different models ranged from a log TU of −2.9 to a log TU of −4.2 depending on a) the availability of forested upstream sections and b) which minimum TU was assigned (Table 2). The estimated EC$_{50}$ were identical for the two minimum TUs (Table 2). For an EC related to a log TU of −2, the fraction of species at risk in the communities in terms of abundance was reduced by 27% and 41% in sites with forested upstream sections for models with a minimum log TU of −4 and −5, respectively (Figure 1, Figure S1). In sites without forested upstream sections, this EC corresponded to 54% and 61% reduction in SPEAR$_{pesticides}$, respectively (Figure 1, Figure S1). Significant differences (nonoverlapping 95% confidence intervals) to reference sites were observed for log TUs ≥ −3 and −3.5 in sites with forested upstream sections and for log TUs ≥ −3 and −3.6 in sites without forested upstream sections when assigning a minimum log TU of −4 and −5, respectively. Similar results were obtained for the ANOVAs, in which all sites in classes with a log TU > −3.5 exhibited a significant difference (all $p < 0.01$) to reference sites (Figure 2).

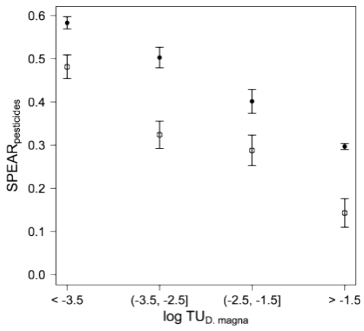

Figure 2. Arithmetic mean of SPEAR$_{pesticides}$ values with standard errors for different classes of toxic units (TU). The sampling sites were divided into sites with forested upstream sections (filled points) and sites without forested upstream sections (open points). Sample sizes of the different classes were 44 and 9 sites with a log TU ≤ −3.5, 10, and 12 sites with −3.5 < log TU ≤ −2.5, 12, and 11 sites with −2.5 < log TU ≤ −1.5 and 4 and 9 sites with log TU > −1.5 for sites with forested upstream sections and sites without forested upstream sections, respectively. All classes with TUs > −3.5 were significantly different (all $p < 0.001$) to reference sites in ANOVA with treatment contrasts.

The linear models exhibited the best-fit for the relationships between % $k_{invertebrate}$ and SPEAR$_{pesticides}$, except for sites from Brittany, France, for which a Weibull II model yielded a slightly better fit (Supporting Information Table S2). However, the slope for linear regression models was only significant for the sites from Victoria, Australia ($r^2 = 0.46$, $p < 0.001$, $n = 23$) and Brittany, France ($r^2 = 0.87$, $p < 0.001$, $n = 11$), but not for the

sites from Denmark (r^2 = 0.09, p = 0.31, n = 13), for which no plausible relationship between $SPEAR_{pesticides}$ and $\%k_{invertebrate}$ could be established (data not shown). The sites from Denmark were not included in the joint dose−response modeling, because the aim was to derive a joint relationship between $SPEAR_{pesticides}$ and $\%k_{invertebrate}$. The best-fit model for the joint data from France and Australia was linear (Supporting Information Table S2) and exhibited a good fit between $\%k_{invertebrate}$ and $SPEAR_{pesticides}$ (r^2 = 0.51, p < 0.001, n = 34) (Figure 3). In ANCOVA, the intercepts for data from Brittany and Victoria were significantly different (p = 0.02), whereas the slopes exhibited no significant differences (p = 0.25). However, if the data of each region were autoscaled before ANCOVA, neither the intercepts (p = 0.89) nor slopes (p = 0.15) were significantly different.

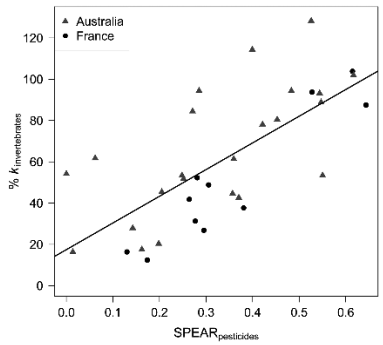

Figure 3. Linear regression model for the relationship between $SPEAR_{pesticides}$ and $\%k_{invertebrate}$ and for sampling sites from Brittany, France and Victoria, Australia. The linear model explained 51% of the variation (p < 0.001, n = 34). Note that the intercepts for data from Brittany and Victoria were significantly different (p = 0.02) in ANCOVA (see Results for details).

■ DISCUSSION

Effect Thresholds for Macroinvertebrate Communities. In our analysis, pesticide effects on the abundance of sensitive invertebrates were found at TUs for *D. magna* below 0.01. Concentrations related to the safety factor incorporated in the UP resulted in a 27% to 61% decline in the abundance of sensitive taxa, depending on the presence of forested upstream sections and which minimum TU was selected in modeling (Figure 1, Figure S1). Similarly, both of the latter factors (presence of forested upstream sections and minimum TU) influenced the estimated effect concentrations (Table 2) and the concentration at which significant differences to reference sites occurred. The models with a lower minimum TU exhibited a better fit in terms of the BIC compared to models with a higher minimum TU (Table S2). Nevertheless, more field data in the log TU range of −3 to −5 would be needed to substantiate a selection between both minimum TUs. The effect threshold was determined to be approximately 1 to 1.5 orders of magnitude lower (log TU of −3 to −3.6) than the safety factor of the UP. However, field studies and results from the joint analysis of studies with differing methodologies are subject to random and systematic uncertainties that can lead to wider confidence bands or bias in the dose−response models and would consequently affect the derived effect threshold. Several uncertainties were identified as random (Supporting Information Table S4) and presumably resulted in wider confidence bands of the fitted dose−response curves (Figure 1, Figure S1) as for example, the joint analysis of data obtained from different countries, years and pesticide sampling methods (Table1). Nevertheless, there were no significant differences between the studies regarding the relationship of $SPEAR_{pesticides}$ and TU (linear model for studies with pesticide gradients of at least 1 log unit in terms of TU, p = 0.23, n = 58). Moreover, some of the variation in the relationship between pesticide toxicity and $SPEAR_{pesticides}$ may result from differences in the dose−response relationship of individual compounds i.e. concentrations of different compounds relating to for example 1/100 of their EC50 for *D. magna* may exert different effects on the abundance of sensitive taxa. Two studies found that the toxicity of a range of different organic toxicants also explained between 68% and 87% of the variance in terms of r^2 in SPEAR,[24,26,36] suggesting that the use of toxic units for *D. magna* as benchmark for the toxicity of different organic toxicants is adequate and that the associated uncertainty is of minor importance.

Two sources of uncertainty could result in a systematic bias in the derived effect threshold. First, the underestimation of pesticide toxicity due to underestimated pesticide concentrations or the nonmeasurement of ecotoxicologically relevant compounds would lead to a left shift of the dose−response curve and consequently a decrease in the effect threshold. However, underestimation of the real concentrations by a factor of 10 to 100 would be required in order to yield similar effect thresholds than incorporated in the UP. We consider an underestimation of this order of magnitude as highly unlikely given that the field studies employed sampling techniques especially targeted at capturing episodic pesticide exposures (Table 1). Although the sampling techniques varied due to differences in the pesticides monitored and regional conditions, the relationship between pesticide toxicity in terms of TU and $SPEAR_{pesticides}$ was not significantly different between the studies (see above). Furthermore, the concentrations responsible for the estimated TUs are in agreement with studies monitoring pesticide runoff with 15-min or 1-h resolution in single agricultural streams. Two studies in Central Europe found peak herbicide concentrations of 2.5[37] and 3.5 μg/L,[38,39] and a study in South Africa found insecticide concentrations ranging from 0.2 to 2.9 μg/L in runoff events.[40] In the studies with relevant pesticide contamination (TU > −4) included in our meta-analysis, peak insecticide concentrations were in the range between 0.3 and 1.2 μg/L (i.e., Brittany, France = 0.7 μg/L; Victoria, Australia = 1.2 μg/L; Denmark = 0.3 μg/L; Germany = 0.5 μg/L). Moreover, it would not be expected that all compounds are underestimated in equal measure and underestimation should therefore increase the variability in pesticide toxicity that relied on different compounds in all included studies. In fact, all individual studies exhibited a very good fit (all r^2 from linear models between 0.62 and 0.68) of pesticide toxicity with the respective biotic end point. Finally, laboratory toxicity experiments with single pyrethoid, organochlorine and organophosphate insecticides demonstrated that chronic population effects can occur 3 to 4 orders of magnitude below acute toxicity concentrations.[41−44] This means that effects may be expected above a TU for *D. magna* of −4 or −3,

which is in line with the effect thresholds derived here. Overall, although we agree that methodical advancements in the characterization of episodic pesticide exposure are desirable and that underestimation of true peak concentrations to a certain degree may occur in the field with currently available sampling techniques, we consider it highly unlikely that true pesticide concentrations were 1 or 2 orders of magnitude higher.

Second, the response of the SPEAR index to a confounding factor that is highly correlated with pesticide toxicity would lead to a decrease in effect thresholds (Supporting Information Table S4). The original studies included in our analysis (Table 1) and two recent studies[45,46] identified the toxicity of the observed stressor as the most important explanatory variable for the respective version of the SPEAR index, whereas 8 and 9 respectively measured confounding factors exhibited no explanatory power for SPEAR. Furthermore, three studies demonstrated that $SPEAR_{pesticides}$ decreased only in contaminated sites and after beginning of the pesticide application period,[7,17,47] reinforcing that pesticides are the culprit because other agricultural stressors such as eutrophication or sedimentation are present throughout the year. Moreover, $SPEAR_{pesticides}$ showed no response to physicochemical, habitat or landscape variables in reference sites.[18,48] Finally, albeit one study found a difference in $SPEAR_{pesticides}$ between samplings of heterogeneous and homogeneous habitats in 13 streams in agricultural areas, there was nevertheless a strong relationship between $SPEAR_{pesticides}$ and TU for each habitat type (r^2 = 0.68 and 0.6 in heterogeneous and homogeneous habitats, respectively).[49] Overall, there is only low uncertainty that the indicated effects were not due to pesticide toxicity. Higher uncertainty remains regarding the mechanisms causing the observed effects of pesticides in the field. A review of mesocosm studies regarding effects of carbamate, pyrethroid and organophosphate insecticides suggested that a log TU for *D. magna* of −2 would be protective for individual insecticides in the field.[3] Beside methodical[46] (and see debate[50,51]) reasons, the difference between the field and mesocosm studies could result from different community composition,[52] repeated exposures, pesticide mixtures[5,53] and the joint effects of different stressors,[4,46,54,55] all of which can enhance the effects of pesticides and are rarely considered in mesocosms. Moreover, chronic long-term effects on merolimnic insects occurring at concentrations related to a TU of −3 to −4 as outlined above[41−44] may not be detected in mesocosm studies, which rarely exceed a study period of several months. However, studies with a high temporal and spatial resolution would be needed to clarify the mechanisms in the field [see ref 56]. Overall, we suggest that there is low uncertainty that our derived effect threshold for effects of pesticides on macroinvertebrate communities is too low and we therefore conclude that the safety factor related to *D. magna* incorporated in the EU Uniform Principles for single pesticides is not protective for freshwater ecosystems, though the mechanisms should be elucidated in future studies.

Effects Thresholds for the Ecosystem Function of Leaf Breakdown. The relationship between the community structure in terms of $SPEAR_{pesticides}$ and the percentage of invertebrate leaf breakdown was linear in Brittany, France and Victoria, Australia (Figure 3). This means that of several suggested links between the community structure and ecosystem functions (see Introduction), pesticide effects on the abundance of sensitive macroinvertebrates seem to translate to a similar effect on the breakdown rate of leafs by invertebrates. Hence, in these regions is no greater tolerance of this important ecosystem function to pesticide contamination and the effect thresholds for the abundance of SPEAR taxa may also apply. Given that regional case studies on the relationship between pesticides and ecosystem functions are scarce, this result may not hold for different ecosystem functions,[8] and other regions. In fact, no plausible relationship between $SPEAR_{pesticides}$ and the invertebrate leaf breakdown rate was found for the Danish sites. Similarly, the original study on the Danish streams only reported a statistically significant relationship between pesticide toxicity and microbial leaf breakdown but not with invertebrate leaf breakdown.[23] Hence, although pesticide toxicity lead to community change in terms of $SPEAR_{pesticides}$ in the Danish sites (Figure 1), this did not translate to effects on the ecosystem function of leaf breakdown. This can be explained by the domination of the shredder community by *Gammarus pulex*, which is a rather tolerant species due to its ecological traits and is consequently not classified as SPEAR.[23] In fact, the density of *Gammarus pulex* was significantly correlated only to the leaf breakdown rate (p = 0.02, test for Pearson correlation, n = 13). Thus, the effect threshold for the invertebrate leaf breakdown presumably depends on the composition of the shredder community, and if non-SPEAR taxa such as *Gammarus pulex* dominate, there may be functional redundancy up to a certain threshold before pesticides affect invertebrate leaf breakdown. Finally, the question is to which extent a temporal difference between pesticide application and leaf input from deciduous trees affects the relationship between pesticide-driven structural changes and invertebrate leaf breakdown (see ref 57). The studies in France and Denmark were conducted in the period of peak insecticide application in these regions, which precedes the period of main input of leafs (late autumn) by several months. Although it is known that community alterations can persist over months,[7,20] it remains to be shown that the invertebrate leaf breakdown is affected outside of the main season of pesticide application. However, for streams receiving a relatively constant leaf input from evergreen forests (e.g., Australian streams),[58] the influence of seasonality on the effects of pesticides on invertebrate leaf breakdown should be of minor importance.

Relevance for Ecological Risk Assessment of Aquatic Ecosystems. The thresholds obtained in our study may be relevant for pesticides and other organic compounds where macroinvertebrates represent the most sensitive group of taxa. In a study on the concentrations of 331 organic toxicants in large rivers of North Germany, invertebrates were considered as most sensitive for 110 compounds, among them many insecticides and fungicides, whereas algae and fish represented the most sensitive group for 142 and 79 compounds, respectively.[59] Another study reported that invertebrates were most sensitive for 225 organic toxicants, whereas algae and fish exhibited highest sensitivity for 158 and 104 organic toxicants, respectively.[36] In this study, an effect threshold of 1/1000 of the acute EC50 for *D. magna* was suggested for the derivation of environmental quality standards (EQS) for river basin specific pollutants, based on an analysis of macroinvertebrate biomonitoring and chemical monitoring data. Hence, effect thresholds for macroinvertebrates would also be protective of other aquatic organisms for a wide range of compounds. The relatively good relationship between pesticide toxicity in terms of TU and SPEAR in our study is remarkable, considering that

the macroinvertebrate data originated from different regions in Europe, Siberia and Australia and the low explanatory power for biotic end points often seen in ecological meta-analyses.[60] Our study therefore supports the use of trait-based approaches in risk assessment to identify the impact of anthropogenic stressors on a continental or even global scale.[11,16]

Without knowing the temporal and spatial dimension of the reduction in the abundance of sensitive macroinvertebrate populations in this study, it is not possible to decide whether the observed effects on the communities were transient or long-term, defined as no complete recovery until the spraying period in the consecutive year. In the latter case, the effects would be unacceptable for the requirements of the EU directive for the placement of plant protection products on the market.[2,61] However, we suggest that current exposure of freshwater ecosystems to pesticides may be unacceptable for the requirements of this and other EU directives. First, one field study showed long-term effects, that is, that no recovery of the communities occurred until the prespraying period of the following year.[7] Second, given that pesticides are widely applied in agriculture, which represents the dominant land use in the EU and elsewhere, and that pesticides frequently occur in streams and rivers in concentrations above effect thresholds,[20] the associated reduction in the abundance of sensitive taxa may lead to losses in biodiversity on a regional scale (γ-diversity) as also indicated by other studies.[62,63] Since a recent EU Directive requires that the risks for biodiversity from pesticides be minimized,[64] more pesticide mitigation measures may be needed to comply with this Directive. Our study highlighted on the basis of a comprehensive data set that forested upstream sections can reduce adverse effects of pesticides on the macroinvertebrate community, especially under low pesticide contamination as indicated by higher effect thresholds (Table 2). Hence, together with other risk mitigation measures such as pesticide use reduction, buffer strips and vegetated treatment systems,[22,65,66] the conservation and increase of landscape patches without agricultural disturbance may somewhat alleviate the effects of pesticides in aquatic ecosystems.

■ ASSOCIATED CONTENT

Supporting Information

A figure with the relationship between pesticide toxicity and $SPEAR_{pesticides}$ for a minimum log TU of −5, a table with the goodness of fit results for all models, a table with the parameters of the fitted dose−response models and a table giving sources of potential uncertainties. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: ++49 (0) 6341 28031536. E-mail: senator@ecotoxicology.de.

Author Contributions

Study design: R.B.S., P.C.O., B.J.K., M.B., M.L. Provision of data: R.B.S., P.C.O., J.R., M.B., M.L. Data analysis: R.B.S. Discussion and interpretation of results: all. Drafting of manuscript: R.B.S. Revising manuscript: R.B.S., M.B., B.J.K., P.C.O., M.L.

Notes

The authors declare no competing financial interest.

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9. Publication VIII

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ARTICLE

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Occurrence and Toxicity of 331 Organic Pollutants in Large Rivers of North Germany over a Decade (1994 to 2004)

Ralf B. Schäfer,*[,†] Peter Carsten von der Ohe,[‡] Ralph Kühne,[§] Gerrit Schüürmann,[§,∥] and Matthias Liess[⊥]

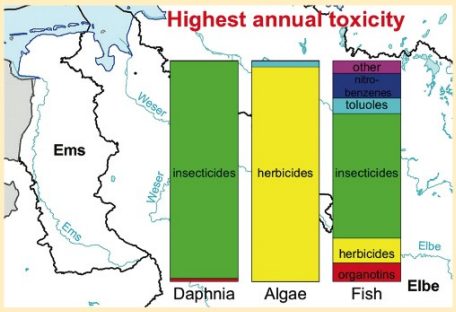
[†]Institute for Environmental Sciences, University Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany

[‡]Department of Effect-Directed Analysis, [§]Department of Ecological Chemistry, and [⊥]Department of System Ecotoxicology, UFZ, Helmholtz Centre for Environmental Research, Permoserstrasse 15, 04318 Leipzig, Germany

[∥]Institute for Organic Chemistry, Technical University Bergakademie Freiberg, Leipziger Strasse 29, 09596 Freiberg, Germany

Supporting Information

ABSTRACT: We analyzed the detection frequencies and concentrations for 331 organic compounds measured between 1994 and 2004 in the four largest rivers of north Germany, the Elbe, Weser, Aller, and Ems Rivers, and we assessed the potential risk for aquatic fauna using experimental and predicted acute toxicity data for the green alga *Pseudokirchneriella subcapitata*, the crustacean *Daphnia magna*, and the fish *Pimephales promelas*. The detection frequency for most compounds decreased significantly from 1994 to 2004. Polycyclic aromatic hydrocarbons (PAHs) were most frequently detected, while pesticides were the most important chemical group concerning toxicity for the standard test organisms. The predicted toxicity for *D. magna* was significantly higher than for the other organisms and reached levels envisaging acute toxic effects on the invertebrate fauna, still in 2004. Most of the compounds responsible for potential acute effects on aquatic organisms are currently not considered as priority substances in the European Union, while only 2 of 25 priority substances that have been measured occurred at levels that may be relevant in terms of toxicity for the selected test organisms. We conclude that attenuation of pesticides and other organic toxicants should play an increased role in river basin management.



INTRODUCTION

A wide range of activities in agriculture, industry, and households is associated with the use of organic chemicals. Depending on the physicochemical properties and usage, these compounds may be released to the environment and can result in the pollution of freshwater ecosystems.[1] In the European Union (EU), the Water Framework Directive (WFD) was established in 2000 and aims at achieving good ecological and good chemical status of surface waters by 2015.[2] Besides water chemistry characteristics such as nutrient concentrations, the evaluation of chemical status relies on compliance with Environmental Quality Standards (EQS) for 33 so-called priority substances (PS), among them metals, pesticides, polycyclic aromatic hydrocarbons (PAH), and organotin compounds.[3] Moreover, the EU member states are requested to identify further compounds of concern for the respective river basins, which could compromise good ecological status.

One approach for the identification of ecologically relevant compounds is long-term screening of the environment for a large set of chemicals in concert with an assessment of the potential toxicity of the observed concentrations, which can be done by use of measured or predicted effect concentrations for standard test species.[4] Given the high costs involved, long-term river basin monitoring studies on hundreds of organic contaminants in freshwater ecosystems, with a few exceptions,[5] have been conducted only through governmental monitoring programs. However, there is a paucity of meta-analyses of such data concerning the toxicity of contaminants for freshwater ecosystems. This may be because the toxicity assessment of monitoring data is hampered by the scarcity of acute toxicity data for aquatic species for many compounds. For example, toxicity data for algae, invertebrates, and fish were available for only 16% of 500 compounds.[4] However, quantitative structure−activity relationships (QSAR) and read-across methods can be used to estimate the toxicity of chemicals if experimental toxicity data for compounds of similar structure are available.

To our knowledge, this paper presents the most comprehensive long-term study on the concentrations and associated

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Table 1. Location of Sampling Sites with Discharge and Statistics on Measurement of Compounds

sampling site	river	basin	median discharge[a] (m^3/s)	measurement frequency	total no. of samples measured	total no. of compds measured	compds ≥ LOQ[b]	total no. of measurements
Cuxhaven	Elbe	Elbe	[c]	monthly[d]	138	238	167	21 185
Grauerort	Elbe	Elbe	[c]	monthly[d]	143	319	225	25 013
Schnackenburg	Elbe	Elbe	493	monthly[d]	149	319	229	31 508
Herbrum	Ems	Ems	57	monthly[d,e]	123	284	183	22 631
Farge	Weser	Weser	223	monthly[d,e]	122	284	179	21 241
Hemeln	Weser	Weser	74	monthly[f]	189	272	152	23 552
Verden	Aller	Weser	72	monthly[f]	189	272	154	23 400

[a] For monthly average data of the years 1994−2001. Data were kindly provided by the Federal Institute for Hydrology (BFG), Koblenz, Germany. [b] Compounds detected above the level of quantification (LOQ). [c] Tidal zone. [d] Only 9 or 10 measurements in 1995 and 1996. [e] No measurements in 1994. [f] No measurements in 1994 and 2004, only 9 or 10 measurements in 1995 and 2003.

ecological risks of organic toxicants in large rivers in central Europe. In detail, we describe the exposure of four large rivers of north Germany to 331 organic compounds over a period of 10 years, using the results of monthly monitoring programs from governmental agencies. The compounds mainly belonged to the following chemical groups: (A) pesticides and transformation products; (B) polychlorinated biphenyls (PCB); (C) polycyclic aromatic hydrocarbons (PAH) and derivatives; (D) halogenated benzenes and nitrobenzenes; (E) halogenated alkanes; (F) phenols and chlorophenols; (G) anilines, anisoles, and alkylated benzenes; (H) toluoles, toluenes, and halogenated derivatives; and (I) organotins. A risk assessment of the measured compound concentrations for fish, invertebrates, and algae was conducted by use of experimental acute toxicity data for standard test organisms: fathead minnow *Pimephales promelas*, waterflea *Daphnia magna*, and green alga *Pseudokirchneriella subcapitata*. Where no experimental data were available we used estimates of a novel QSAR approach as an approximation. Finally, the identified compounds of concern were compared to the assessment of chemical status based on priority substances as outlined in the WFD.

EXPERIMENTAL SECTION

Description of Sampling Sites. Seven sampling sites in four of the five largest rivers of north Germany were sampled monthly as part of environmental monitoring programs from 1994 to 2004 (Table 1; Figure S1, Supporting Information). The median of the mean monthly discharge in the sites ranged from 57 to 493 m^3/s (Table 1). In general, all rivers are heavily modified, were dredged for shipping, and receive inputs of inorganic and organic pollutants from industry, agriculture, households, and sewage treatment plants, although the magnitude of impact may differ between the rivers.

Data Acquisition and Quality. The results of the chemical water monitoring in the seven sampling sites were kindly provided by the Lower Saxony Water Management, Coastal Defense and Nature Conservation Agency (NLWKN) and comprised monthly observations of a total of 331 organic pollutants (see Table S1 in Supporting Information for complete compound list) measured from 1994 to 2004.[6,7] Between 78 and 300 compounds were analyzed in each of the 1053 samples from all sampling sites (Table 1), except for five samples where only 15−61 compounds were analyzed. The monitoring program in the seven sites exhibited differences in the total numbers of measured compounds and samples per site and to a minor extent in the measurement frequency (Table 1).

All steps of the monitoring program, for example, sampling, sample storage, sample treatment, and chemical analysis, were carried out according to certified methods and in compliance with a quality assurance program to ensure reliability and compatibility of the results (see Table S1, Supporting Information, for details on the chemical analysis). Briefly, the sampling consisted of taking a nonfiltered whole water grab sample. In the laboratory the samples were handled according to the respective method and measured by gas chromatographic or high-performance liquid chromatographic methods. The chemical analysis was conducted solely in accredited laboratories. Due to the long observation time and different laboratories involved, the levels of quantification (LOQ) exhibited variation (Table 2; see Table S2, Supporting Information for full table).

Consideration of Compound Partitioning between Water and Suspended Particles. The concentrations of compounds in the monitoring data referred to the nonfiltered whole water sample. Therefore these concentrations could overestimate the concentrations in the water phase since a significant proportion of a hydrophobic substance may be adsorbed or bound to suspended organic particles, which can reduce the toxicity of that compound and should be accounted for in the risk assessment for aquatic organisms.[8] We used a reformulation of the equilibrium partitioning approach to approximate the freely available concentration C_d (in micrograms per liter) of organic compounds:[9]

$$C_d = \frac{C_{tot}}{(f_{OC}K_{OC} + 1)}$$

where C_{tot} is the total concentration in the whole water sample in micrograms per liter, K_{OC} is the dimensionless soil organic carbon−water partitioning coefficient, and f_{OC} is the fraction of organic carbon that was approximated with the total organic carbon content (TOC).

Compilation and Estimation of Toxicity Data for Invertebrates, Algae, and Fish. If available, we used laboratory-derived acute toxicity data for standard test organisms [48-h median effect concentration (EC_{50}) for *D. magna*, 48-h to 96-h EC_{50} for *P. subcapitata*, and 96-h median lethal effect concentration (LC_{50}) for *P. promelas*] to assess the risk of a compound. In the following, we do not distinguish between EC_{50} and LC_{50} and only use the term EC_{50} to enhance readability. The toxicity data were compiled from peer-reviewed literature as well as available databases,[4] and wherever possible peer-reviewed literature was consulted to confirm toxicity data from databases (see Table S2, Supporting Information, for further details).

Table 2. Compounds with Higher Than 40% Detection Frequencies with Their Minimum and Maximum Levels of Quantification, Maximum Concentration, and Number of Measurements in All Sites

CAS no.	English name	chemical group[a]	min LOQ[b] (μg/L)	max LOQ[b] (μg/L)	max concn (μg/L)	n[c]	detection frequency[d] (%)	priority substance no.[e]
206-44-0	fluoranthene	C	0.002	0.002	0.053	270	99	15
129-00-0	pyrene	C	0.002	0.002	0.046	224	99	
58-89-9	γ-hexachlorocyclohexane	A	0.000 08	0.000 08	0.03	795	98	18
60-00-4	EDTA	J	0.1	0.3	35	387	93	
85-01-8	phenanthrene	C	0.002	0.002	0.038	224	91	
205-99-2	benzo[*b*]fluoranthene	C	0.002	0.005	0.025	270	89	28
50-32-8	benzo[*a*]pyrene	C	0.002	0.005	0.024	270	88	28
193-39-5	indeno[1,2,3-*c*,*d*]pyrene	C	0.002	0.002	0.033	268	88	28
127-18-4	tetrachloroethylene	E	0.0002	0.002	1.2	697	88	29a
191-24-2	benzo[*ghi*]perylene	C	0.002	0.018	0.2	270	87	28
218-01-9	chrysene	C	0.002	0.002	0.024	224	85	
56-55-3	benz[*a*]anthracene	C	0.002	0.002	0.023	224	78	
139-13-9	nitrilotriacetic acid	J	0.1	0.5	10	421	77	
319-84-6	α-hexachlorocyclohexane	A	0.000 07	0.000 07	0.2	719	67	
207-08-9	benzo[*k*]fluoranthene	C	0.002	0.014	0.012	269	65	28
1912-24-9	atrazine	A	0.001	0.006	0.6	1017	62	3
118-74-1	hexachlorobenzene	A	0.000 04	0.000 08	0.03	673	58	16
75-25-2	bromoform	E	0.002	0.008	0.3	696	56	
56-23-5	carbon tetrachloride	E	0.0002	0.004	0.05	697	52	6a
118-96-7	2,4,6-trinitrotoluene	H	0.000 09	0.02	3.5	104	52	
67-66-3	trichloromethane	E	0.004	0.02	1.5	696	48	32
79-01-6	trichloroethylene	E	0.001	0.006	0.5	697	46	29b
1007-28-9	desisopropylatrazine	A	0.003	0.09	0.6	1037	44	
5915-41-3	terbutylazine	A	0.003	0.006	0.5	1037	43	

[a] A, pesticides and transformation products; B, polychlorinated biphenyls; C, polycyclic aromatic hydrocarbons and derivatives; D, halogenated benzenes and nitrobenzenes; E, halogenated alkanes; F, phenols and chlorophenols; G, anilines, anisoles, and alkylated benzenes; H, toluoles, toluenes, and halogenated derivatives; I, organotin compounds; J, miscellaneous. [b] LOQ = limit of quantification. [c] *n* = total number of measurements. [d] Ratio for observations above LOQ. [e] As outlined in ref 3.

However, for only 207, 107, and 100 of the 331 compounds were experimental toxicity data found for *D. magna*, *P. subcapitata*, and *P. promelas*, respectively. Missing toxicity data were estimated from experimental values for similar compounds as described in Schüürmann et al.[10] Initially, a data set containing available experimental toxicity values for each of the above-mentioned standard test species, containing about 1000, 550, and 700 experimental toxicity values, respectively, together with the respective chemical structures was recorded. To predict the toxicity of a compound not part of this set, the arithmetic average of the experimental values for the three most similar compounds from the data set was calculated. The similarity of compounds was evaluated by employing a atom-centered fragments (ACF) based approach.[11] Actually, the results of two different levels of ACF determination were combined and filtered by thresholds regarding similarity and number of similar compounds. The particular weights for the combination of these levels as well as the respective thresholds were fitted individually for each species by means of cross-validation with the training set. The averaged prediction error of the model ranged from 0.5 to 1.0 logarithmic unit for data where experimental values were available for reasonably similar compounds, that is, a similarity ≥ 0.75 on a scale from 0 to 1[10] (see Table S2, Supporting Information, for the categories of similarity of the read-across compounds). In case sufficiently similar compounds were not available, baseline toxicity estimated from the octanol−water partitioning coefficient (K_{ow}) was used, employing established QSAR models for the three standard test organisms.[12,13] The structural alerts of compounds for which the baseline toxicity was estimated did not indicate enhanced toxicity.[13] Compounds with a predicted toxicity 10 times higher than the estimated water solubility[12] and a melting point of more than 100 °C were excluded from the assessment.[14]

Toxicity Assessment. The toxicity of the dissolved water concentrations C_d for each of the three trophic groups was predicted by the toxic unit approach,[15] where the toxic unit (TU) for a compound is the compound concentration divided by the respective 48-h or 96-h EC_{50} for the standard test species. We used the maximum TU (mTU), which is the highest TU of all observed individual compound concentrations in each sample, as an indicator for the minimal expected toxicity of the respective sample. We did not use the sum of all TUs (sumTU) in a sample because the sumTU exhibits a stronger dependency on the number of compounds measured and could overestimate toxicity of compounds with a dissimilar mode of action. The mTU accounted for >50% of the sumTU of a sample for 80%, 61%, and 40% of samples ($n = 1052$, one sample with no detection of compounds excluded from analysis) for *D. magna*, *P. subcapitata*, and *P. promelas*, respectively. This means that, for the majority of samples in the risk assessment for invertebrates and primary producers and a considerable fraction of the samples for the risk assessment of

fish, the most toxic individual compound as represented by the mTU would also dominate the toxicity of all compounds in terms of sumTU.

Chemical Status Assessment with Regard to Priority Substances. According to the WFD, a good chemical status requires compliance with either the maximum allowable concentration (MAC) EQS and the so-called annual average (AA) EQS values for the set of 33 priority substances. Of the 33 priority substances, a total of 25 organic compounds were measured in the basins of the study (see Table S2, Supporting Information). The number of measured priority substances varied between years and sites, with 6−18 and 9−22 measured compounds per site in the years 1994−1997 and 1998−2004, respectively (see Table S3, Supporting Information, for details). The latest available EQS values were used for compliance checking.[3]

Data Analysis. For the analysis of differences in the detection of chemicals between sites, years, and months, the compounds were split into 10 chemical groups (Table 2). Since the number of total measured compounds varied between sites, years, and months (Table 1), we used the relative detection frequency per sample for comparisons among these variables. Generalized linear models (GLM) with logit link were used to identify which of the variables (i.e., year, site, month, basin) and their interactions are relevant to explain variation in the response variable detection frequency, which was assumed to be binomial distributed.[16] Stepwise model selection with the Akaike information criterion (AIC) as goodness-of-fit measure and starting with the intercept-only null model was used in order to identify the best-fit model. Since the best-fit model for the full data set [AIC 14 610, deviance 4807, degrees of freedom (df) 3828] contained several two-way interaction terms with the variable chemical groups, the analysis was conducted separately for each of the chemical groups in order to ease interpretation. In case of over- or underdispersion of a GLM, a quasi-binomial model was employed to verify the results of the binomial model.[16] Significant differences between factor levels of sites and months were identified by a multiple comparison procedure with Tukey's all-pairwise comparison contrasts (TALC) as described in ref 17.

Given that the chemical status with regard to priority substances is based on annual values, we calculated maximum annual mTUs for each site for the risk assessment based on the mTU. Analysis of variance was employed to identify significant differences between basins, test species, and years for the response variable annual mTU, by the same model selection and multiple comparison procedures as described for the GLMs. In order to detect differences in the sensitivity of the three standard test organisms to the 331 organic compounds, the logarithmic ratio of their acute toxicity data (ECR) was calculated:

$$\mathrm{ECR}_{x,y}(c) = log \frac{\mathrm{EC}_{50_x}(c)}{\mathrm{EC}_{50_y}(c)}$$

for each compound c and for each of two species x and y. A similar sensitivity of two organisms to the compounds in the data set would translate to a median of 0 and an even spread toward positive and negative values for the ECR values.

All statistical computations and graphics were created with the open-source software package R (www.r-project.org) using version 2.8.0 (for Mac OS X, 10.5.5).

RESULTS AND DISCUSSION

Spatiotemporal Patterns of Occurrence of Organic Pollutants. Of the 331 compounds, 257 compounds were detected at equal or higher levels than the LOQ in the water samples of all rivers, whereas 74 compounds were detected only below the level of quantification (see Table S2, Supporting Information). Twenty-four compounds were detected in more than 40% of the water samples (Table 2). With the exception of one sample (March 2003 in Cuxhaven), at least one substance was detected in each water sample. On average, 14% of the measured compounds were found in a sample, while this ratio varied strongly between samples [53% relative standard deviation (RSD)]. The detection frequency was not correlated with the number of measurements or the limit of quantification (all Pearson r between −0.04 and −0.09; all $p > 0.17$; $n = 249$; compounds without concentrations above the LOQ were excluded; see Table S2, Supporting Information, for values). The GLMs for detection frequency as response variable indicated significant differences between the sampling sites for all chemical groups, between years for all chemical groups except for polychlorinated biphenyls, and between months for 6 of the 10 chemical groups (Table S4, Supporting Information). By contrast, any chemical group exhibited significant differences in the detection frequencies between basins (Table S4, Supporting Information).

The chemical group with the highest total detection frequency (43%) was the polycyclic aromatic hydrocarbons (PAH); 10 of the 15 most frequently detected compounds belonged to this group (Table 2). Our results are in accordance with other studies that highlighted the high frequency of detection of PAHs in aquatic environments due to their diffusive input pathway.[5,18] Significantly higher detection frequencies of PAHs were observed in the months January−March compared to the rest of the year, especially to the months July−September (all $p < 0.01$, TALC). Similar patterns were observed in a study on PAHs in the Seine River in France and may result from higher combustion activities, remobilization due to flooding, or decreased photodecomposition in winter.[19]

Except for Cuxhaven, where the tidal influence presumably led to dilution by seawater, the sites in the Elbe exhibited significantly higher detection frequencies compared to the sites in the Ems and Weser basin for some groups of chemicals such as pesticides and halogenated alkanes ($p < 0.05$, TALC, see Table S4, Supporting Information, for details). This is in line with the fact that, in the 20th century, the Elbe was among the most polluted rivers in Germany.[20]

Except for pesticides and PAHs (see above), no patterns in the detection frequency for months were observed. Pesticides showed significantly higher detection frequencies from June to July compared to the months October−April (all $p < 0.02$, TALC) with the exception of January (all $p > 0.05$, TALC). This period of higher detection frequencies matches the application period of pesticides in central Europe. The elevated detection frequency at the beginning of the year was also observed for several pesticides in a 1-year study in the Humble River in northeast England[18] and may result from field runoff associated with flooding as suggested for PAHs (see above).

From 1994 to 2004, several chemical groups showed a reduction in detection frequencies over all sampling sites (Table S4, Supporting Information). Pesticides and halogenated alkanes exhibited a continuous decline, while the groups of (B) polychlorinated biphenyls, (D) halogenated benzenes and nitrobenzenes, (H)

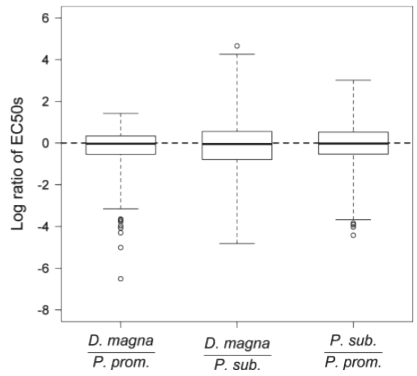

Figure 1. Box-and-whisker plots for relative toxicity of monitored compounds for the test organisms *D. magna*, *P. subcapitata*, and *P. promelas*. The whiskers extend out from the box to a maximum of 3 times the interquartile range.

toluoles, toluenes, and halogenated derivatives showed significantly lower detection frequencies in the period 1998−2004 compared to years 1994−1997 (Table S4, Supporting Information). These observations match with long-term sediment quality studies in the river Elbe from 1991 to 2001 that reported a general decline for several inorganic and organic pollutants.[20]

Toxicity Assessment for Standard Test Species and Comparison with Biological Monitoring Results. The three standard test organisms *D. magna*, *P. promelas*, and *P. subcapitata* were relatively equal in their sensitivity to most of the 331 organic compounds in terms of the logarithmic ratio of EC_{50} values (ECR). This was indicated by medians between −0.05 and −0.03 and a relatively even spread of the ECR values between the upper and lower quartile around the median (Figure 1). The compounds related to these ECR values were presumably narcotics, that is, they exhibited baseline toxicity.[13] The spread of ECR values was less even toward the maximum and minimum ECR values (Figure 1). *P. promelas* was only a maximum of 26-fold and 1024-fold more sensitive than *D. magna* and *P. subcapitata*, respectively, while both *D. magna* and *P. subcapitata* showed a 10 000-fold higher sensitivity than *P. promelas* for several compounds (Figure 1). A total of 72 different compounds exhibited at least 100-fold lower or higher EC_{50} values (i.e., ECR ≥ 2 or ECR ≤ −2) for a test species in relation to another test species. Interestingly, 58 of the 63 compounds for *D. magna*, 55 of the 59 compounds for *P. subcapitata*, and 5 of the 7 compounds for *P. promelas* with ECR values ≥ 2 or ≤ −2 were pesticides. This can be explained by the fact that the majority of current-use pesticides, especially herbicides and insecticides, are specifically designed to eliminate certain groups of target organisms and therefore exhibit excess toxicity to these groups while being relatively nontoxic to other groups of organisms.[21]

The annual mTU values for *D. magna* were significantly higher than for *P. subcapitata* and *P. promelas* (both $p < 0.001$, TALC for best-fit model for annual mTU, AIC = −746, $n = 213$), whereas they were not significantly different between the latter two species ($p = 0.44$) (Figure 2). By contrast, a study on the toxicity of 83 pesticides in 17 streams reported 5−10-fold higher median mTU values for primary producers (*P. subcapitata* and *Lemna gibba*) compared to *D. magna*,[22] though the fish species (*Lepomis macrochirus*) was also at lowest risk to be acutely affected by toxicants.[22] This suggests that the group of organisms at highest risk from organic toxicants varies between basins and the risk assessment should therefore always include organisms from all different trophic levels.

Predominantly, herbicides and organophosphate insecticides were accountable for the highest annual risk of toxicity for *P. subcapitata* and *D. magna*, respectively (Table 3; Table S5, Supporting Information). For *P. promelas*, four nonpesticides were responsible for the highest annual mTUs, but pesticides were still accountable for 48 of the 71 annual mTUs (Table S5, Supporting Information). Although several studies have highlighted the ecotoxicological relevance of pesticide input for small streams in agricultural areas[24,25] and that pesticide concentrations decrease with the size of surface water bodies,[26] this shows that pesticides can be the most potent toxicants even in large rivers.

The concentrations of several compounds reached levels that were within 1 order of magnitude of the EC_{50} values for *D. magna* and *P. subcapitata* and were even higher than the acute EC_{50} for *D. magna* for the insecticide dichlorvos for one site in 1994 and four sites in 1996 (Figure 2; Table S6, Supporting Information). Meta-analyses of results from freshwater mesocosm studies suggest that effects of insecticide and herbicide contamination on the invertebrate community can, depending on the mode of action of the respective substance, occur above a TU of 0.01 for *D. magna*, while this threshold is higher for phytoplankton (algae) and fish with a TU of 0.1.[27,28] Though these thresholds should be interpreted with caution, they clearly indicate a risk of acute toxic effects when exceeded. Based on these thresholds, the risk of acute effects on fish and algae was minor as 0% and 8% of the annual mTU exceeded 0.1, respectively (Figure 2; Table S6, Supporting Information). By contrast, the measured concentrations of organic compounds were related to a high risk of acute toxic effects on the invertebrate community for the majority of sites and years (89% of observations with annual mTU for *D. magna* > 0.01; Figure 2; Table S6, Supporting Information). Annual mTU values for *D. magna* below 0.01 were only observed for single sites in the Elbe and Weser basin between 2000 and 2003, except for Cuxhaven in 1998. Nevertheless, in 2004 the concentrations exceeded the threshold again at all sites and thus it remains unclear if there is an ongoing decrease. The best-fit linear models for the annual mTU values of the three species indicated significant differences between years for *D. magna*, while there were significant differences between sites for *P. subcapitata* and *P. promelas* (AIC = −205, −371, and −757, respectively; all $p < 0.002$; $n = 71$ for each test species). Overall, our results are in accordance with ecotoxicity tests for several sites along the Elbe between 1992 and 2001 that also found serious mortality for daphnids with no clear temporal trend.[20]

Although our results suggest a high risk for acute toxic effects on the aquatic fauna and especially invertebrates, the risk assessment approach used here, relying on the mTU for the bioavailable water concentrations and mesocosm thresholds, may still underestimate real acute toxic effects. First of all, the concentrations used were derived from monthly point water samples that are not suitable to assess the peak water concentrations, especially for compounds with varying exposure patterns such as pesticides.[29] Hence, the actual TUs will be higher than the ones based on the measured concentrations. In addition, we do not consider (1) additive or synergistic

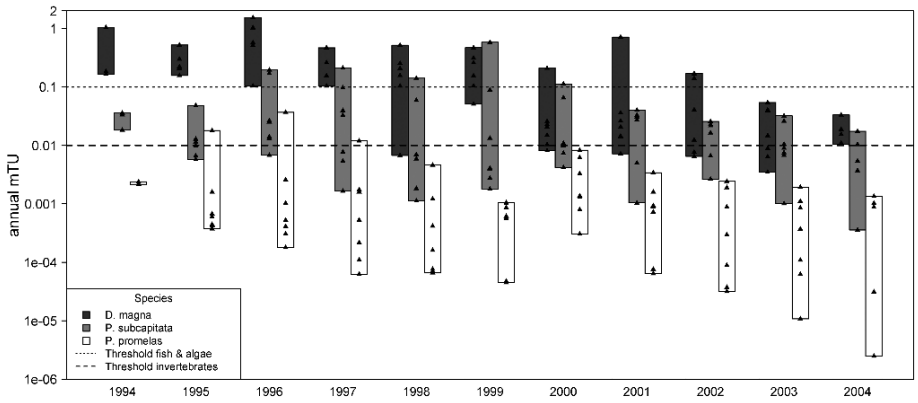

Figure 2. Min−max bar plot of annual maximum toxicity (mTU) for *D. magna*, *P. subcapitata*, and *P. promelas* for all sites from 1994 to 2004 and the respective thresholds for aquatic organisms (see text for details). Triangles display the variation in mTU values for the seven sampling sites.

Table 3. Compounds Accountable for Threshold Annual mTU with EC_{50}, Number of Times Accountable for Annual mTU, Pesticide Group, and Highest Annual mTU[a]

compd[b]	EC_{50} (μg/L)	source EC_{50}[c]	no. annual mTU[d]	pesticide group[e]	highest annual mTU
		Annual mTU > 0.01 for *D. magna*			
dichlorvos	0.19	E	35	insecticide (OP)	1.5
diazinon	1.00	E	6	insecticide (OP)	0.71
pirimiphos-ethyl	0.03	P/3	13	insecticide (OP)	0.57
azinphos-ethyl	0.20	E	2	insecticide (OP)	0.21
pirimiphos-methyl	0.21	E	5	insecticide (OP)	0.18
chlorpyrifos-methyl	0.60	E	1	insecticide (OP)	0.033
fonofos	2.30	E	1	insecticide (OP)	0.021
fenclorfos	0.73	P/2	1	insecticide (OP)	0.019
ethion	0.06	E	1	insecticide (OP)	0.015
malathion	0.7	E	2	insecticide (OP)	0.011
		Annual mTU > 0.1 for *P. subcapitata*			
diuron	2	E	17	herbicide	0.58
alachlor	5	E	17	herbicide	0.19

[a] See Table S5, Supporting Information, for all compounds accountable for annual mTU. [b] For all compounds listed, chemical group is A = pesticide. [c] E = experimental data from literature (see Table S2, Supporting Information, for details); P = predicted data from read across, together with the level of similarity (los) to compounds with experimental data (1, los $\leq$ 0.5; 2, 0.5 < los $\leq$ 0.7; 3, 0.7 < los $\leq$ 0.85; and 4, los > 0.85). [d] Number of times accountable for annual mTU for all seven sampling sites and for all years (n = 71). [e] Pesticide group as given in the FOOTPRINT pesticide properties database;[23] OP = organophosphate.

effects between compounds, (2) chronic effects, or (3) recurring pulses of toxicants, all of which may increase the toxicity. Furthermore, not all existing organic toxicants were measured, and relevant compounds in terms of toxicity may have been missed.[5] Moreover, the annual mTU values were predominantly associated with compounds for which experimental EC_{50} values were available, whereas compounds with predicted EC_{50} values accounted only to a minor extent for annual mTU values (Table 3; Table S5, Supporting Information). With respect to prediction errors, an underestimation of the real EC_{50} (i.e., higher real EC_{50} values) would only lead to minor changes in the toxicity assessment using annual mTUs. By contrast, overestimation of the real EC_{50} values (i.e., lower real EC_{50} values) would result in even higher annual mTU values. Finally, uncertainties remain whether the effect thresholds derived from existing mesocosm studies are protective for aquatic communities since field studies demonstrated effects at lower concentrations[24,30] and these effects may not have been detected in previous mesocosm studies due to the low proportion of sensitive long-living taxa in mesocosm communities.[31] However, a similar study in a Spanish river basin also indicated significant effects of organic pollutants on the macroinvertebrate community for mTU > 0.01.[31]

Significance of Results for River Basin Management. In the European Union, current management of the chemical pollution of freshwater ecosystems focuses on the assessment of 33 priority

substances that are assumed to present a specific risk for the environment when they exceed the environmental quality standards (EQS).[32] Indeed, 13 of the 24 compounds most frequently detected in the rivers of north Germany were priority substances (Table 2). Moreover, the chemical status indicated potential effects of the organic priority substances for several years and sites in terms of exceedance of EQS values (Table S3, Supporting Information). Nevertheless, only five of the 25 organic priority substances that have been measured exceeded the EQS values in the years 1994−2004 for the seven sampling sites: alachlor, trifluralin, and tributyltin as well as the PAHs benzo[*ghi*]perylene and indeno[1,2,3-*c*,*d*]pyrene. However, not all organic priority substances were measured in the monitoring programs, so there may be other priority substances that exceed their respective EQS values. This possibility is supported by the fact that we found a significant correlation between the number of measured priority substances and the number of exceedances of EQS values for our data (Pearson $r = 0.75$, $p < 0.001$, $n = 71$).

Since EQS values integrate protection goals that are not related to ecotoxicological effects (e.g., fish consumption, drinking water production), an exceedance of EQS values does not necessarily indicate ecotoxicological risk. Indeed, of the priority substances that exceeded the EQS values, only two (alachlor and trifluralin) occurred in concentrations that exceeded a TU of 0.1 for *P. subcapitata* and none reached a TU of 0.01 for *D. magna* or of 0.1 for *P. promelas* (Table S2, Supporting Information). Conversely, although diuron accounted 17 times for the highest annual toxicity to *P. subcapitata*, with concentrations up to 58% of the EC_{50} value (Table 3), this did not lead to an exceedance of the respective EQS value (1.8 μg/L). Given that mesocosm studies demonstrated effects for concentrations of this order of magnitude, the current EQS value may not be protective for phytoplankton communities.[27,28] We suggest that the EQS values should be revised to consider the ecotoxicological risk of priority substances to all trophic groups.

Only two of the substances most relevant for the risk of acute toxic effect to the standard test organisms were priority substances (alachlor and diuron) (Table 3). Hence, priority substances were of only minor importance for the risk assessment for primary producers, invertebrates, and fish. Our study is in accordance with a review by Brack et al.,[33] which highlighted that in several investigations compounds other than priority substances were relevant for toxicity to the aquatic biocenosis. This result is especially important for river conservation and restoration measures. In the case that the current practice, relying on the assessment of priority substances, assumes a good chemical status, this may lead to measures to improve the ecological quality that may not be successful since nonpriority organic substances can still have acute toxic effects. For example, some studies reported that conservation and restoration measures showed only minor improvement of the ecological quality of the stream, though this is not necessarily due to organic toxicants.[34,35] Overall, our study highlights that organic toxicants and especially pesticides may play a more important role for the ecological conditions in river systems than is currently acknowledged. Therefore, we suggest (1) to include these compounds in the list of river basin specific pollutants and (2) in general to use approaches such as the one outlined here to identify, based on chemical monitoring data, ecotoxicologically relevant compounds in other river basins.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. One figure of the sampling sites and six tables giving a full list of measured compounds with methods used in chemical analysis and toxicity data, overview of priority substance measurements and the respective exceedances of EQS values, a description of the GLM models, and a list of compounds accountable for highest annual toxicity per site and per organism. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Telephone: ++49 (0) 6341 28031536; e-mail: senator@ecotoxicology.de.

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10. Publication IX

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A SIMILARITY-INDEX–BASED METHOD TO ESTIMATE CHEMICAL CONCENTRATION LIMITS PROTECTIVE FOR ECOLOGICAL COMMUNITIES

BEN J. KEFFORD,*†‡ RALF B. SCHÄFER,† MATTHIAS LIESS,§ PETER GOONAN,|| LEON METZELING,# and DAYANTHI NUGEGODA†
†Biotechnology and Environmental Biology, School of Applied Sciences, RMIT University, Bundoora, 3083 Victoria, Australia
‡Centre for Environmental Sustainability, Department of Environmental Science, University of Technology, Sydney, 2007 New South Wales, Australia
§UFZ, Helmholtz Centre for Environmental Research, Department of System Ecotoxicology, 04318 Leipzig, Germany
||Environment Protection Authority, Adelaide, 5001 South Australia, Australia
#Environmental Protection Authority Victoria, Macleod, 3085 Victoria, Australia

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Abstract—A new method is presented to determine retrospectively proportional changes of species composition in a community at risk from particular concentrations of chemical stressors. The method makes estimates with some similarities to those claimed by species sensitivity distributions (SSDs) but is based on species presence/absence field data and requires assumptions that are more likely to be met. The method uses Jaccard's index (JI), the proportion of species in common to two samples. At a similar level of contamination, the occurrence of species is usually highly variable, and thus JI values between individual pairs of samples can be low. However, by pooling samples with a similar contamination level, an increasingly complete set of species present at this level of contamination is gained. Our method involves calculating JI between randomly selected groups of samples (pooled sample sets) with similar and different levels of contamination. It then relates changes in JI to the difference in contamination and produces estimates of the proportional change in species between preselected categories of contamination. The application of the method is illustrated by using data on riverine freshwater macroinvertebrates exposed to salinity in southeastern Australia; pesticide runoff potential in the Aller River Catchment, Germany; and metal pollution (principle Cu) in the Clark Fork River Catchment, Montana, USA. Environ. Toxicol. Chem. 2010;29:2123–2131. © 2010 SETAC

Keywords—Risk assessment Community change Stream invertebrates Similarity indexes

INTRODUCTION

Anthropogenic chemical contamination is widely recognized as an important stressor on biota globally. Environmental quality guidelines (EQGs) and risk assessments are widely used to manage contamination in water, sediments, soils, and biota. Guidelines should be set accurately, i.e., at levels that will protect the environment but also avoid unnecessary constraints of economic activity. One method of calculating EQGs is the use of species sensitivity distributions (SSDs), which are cumulative distribution functions of data on the sensitivities of species to some toxicant [1]. This sensitivity data stems usually from single-species laboratory experiments (e.g., lethal concentration to x% of the population, LC_x, or no-observed-effect concentration NOEC values), but Leung et al. [2] suggested using declines in the abundance of taxa in nature. One attractive aspect of SSDs is that they operate within a risk assessment framework, allowing estimates of the concentration that will protect a given proportion of species. This proportion is expressed as a protective concentration (PCp) or conversely a hazardous concentration ($HC100 - p$), where $100 - p$ is the maximum acceptable loss of species, often 5%. Alternatively for a given chemical concentration, the potentially affected fraction (PAF) of species can be estimated.

What is considered to be an acceptable environmental risk is context dependent. In a pristine environment, for example, any risk may be judged unacceptable, whereas in an already heavily modified system some risks may be acceptable. A useful attribute of SSDs is that the maximum acceptable loss of species ($100 - p$) can be varied depending on societal preferences. For pristine environments, p might be set at 99% and thus PC_{99} values estimated, whereas in highly modified systems p would be set at a lower percentage.

In estimating PCp values, SSDs rely on a number of assumptions, and their logic has been criticized [3–5]. Critical assumptions are mentioned here. The sample of species in the SSD is an unbiased sample of the communities for which conclusions will be drawn [4]. This assumption would appear to be rarely met in the conventional use of SSDs [6]. The number of species with sensitivity data included in the SSD is adequate [4]. For most toxicants, this assumption is not met [6]. The mathematical model used for estimating the SSD is appropriate. This assumption is difficult to test when the above-mentioned assumptions are not met. Ecological interactions between species do not influence species' sensitivity [4], despite conflicting evidence [7]. The endpoint is ecologically relevant [4]. In many cases, individual-level laboratory-measured endpoints are not ecologically relevant [8].

Recently, some modifications to SSDs [2,6,9–13] have been suggested that should result in SSDs that better meet some of these assumptions. However, none of these modifications has attempted to address all assumptions of SSDs, and thus it will be controversial whether PCp values really protect p% of species. Here we suggest a new method of estimating the chemical

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* To whom correspondence may be addressed (ben.kefford@rmit.edu.au).
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concentration that results in *p*% of species present in a community not changing, which includes all but the rarest species. Although this is not the same target of concern as that used by SSDs, it is similar in that both aim to protect community structure from having no more than a predetermined proportional change. We use an index of similarity, Jaccard's index (JI), which determines the proportion of species present in two samples to estimate retrospectively the change in community composition across gradients of contamination in nature. Estimating EQG based on indexes of similarity between individual sampling units is problematic, because the biotic communities present vary widely as a result of many biotic, abiotic, and stochastic factors unconnected with chemical contamination. We overcame this problem by pooling samples of a particular level contamination and comparing these pooled samples. The end result is estimates of community change associated with changes in contamination.

MATERIALS AND METHODS

The concept

Our approach relies on the Jaccard's index (JI), which is given by

$$\mathrm{JI} = j/(a+b-j),$$

where j is the number of joint species recorded in both samples A and B, a is the total number of species in sample A, and b is the total number of species in sample B. Thus JI is the proportion of species in common between two samples. Jaccard's index ranges from zero when both samples have no species in common to one where each sample has the identical species list.

Jaccard's index can be calculated between all pairs of samples across a pollution gradient. If the contaminant concentration affects species composition, then samples with similar concentrations would tend to have higher values of JI than samples with dissimilar concentrations. However, the occurrences of species are affected by many factors unrelated to chemical contamination, so the species present at two uncontaminated sites (or two sites with similar levels of contamination) are unlikely to have a JI near 1.

Moreover, we would also not expect 5% fewer species at a contaminated site with an exposure related to the PC_{95} value [14]. This rather would hold regarding exposure related to the PC_{95} value across a larger region and the complete list of species present (across this region). Usually, it is impractical to compile a complete list of species across a region. However, by pooling or amalgamating samples with similar contaminant concentrations, an approximation of the complete list of species across a contaminant concentration range can be obtained. This follows from species accumulation curves that increase at a decreasing rate as sampling effort increases. Jaccard's index can then be calculated between lists of species from pooled samples with discrete levels of contamination. These lists of species are composed from multiple sites with differences in physical habitat and other factors unconnected with contamination. Therefore JI calculated between (multiple) pooled samples with similar levels of contamination would tend to have higher and less variable JI values than between single samples. When the pooled samples span different sites and sampling episodes, the set of pooled samples will combine samples across a range of habitat and other attributes. When these sets of pooled samples represent different levels of contamination, their JI values indicate the similarity of the community between the indicated contamination levels and not between particular sites that differ in many respects unconnected the contamination.

In practice, the first step of the proposed method involves selecting categories or classes of contamination (concentration). In the present study, a sample is considered to be a single sampling unit at a site and at a point in time, regardless of any subsampling. Sampling episodes at the same site on different occasions are treated as separate samples. Samples within each contamination category are then randomly allocated into what we call pooled sample sets (collections of pooled samples without replacement), and JI is calculated between all pairs of pooled sample sets. Next, the mean JI between each category is calculated. Finally, the relative change in the mean JI between categories, which we call relative species retention (RSR), is calculated. If we have the ordinal contamination categories i ranging from 1 to n, referring to least (1) and most (n) contaminated, then $j_{x,y}$ with $x \neq y$ is the mean JI between categories x and y, and $j_{x,x}$ and $j_{y,y}$ are the mean JIs within categories x and y, respectively. The RSR between contamination categories x and y is $j_{x,y}/j_{x,x}$. We note that RSR cannot determine whether species are lost or gained as contamination increases.

Jaccard's index was calculated, and significant differences in JI between contamination categories were assessed by analysis of similarity (ANOSIM) in Primer 6.16 (www.primer-e.com) with a critical p value (the probability of a type 1 error) of 0.05. However, regardless of the statistical significance in JI between categories, we suggest that the practical significance of RSR should be considered. This is because, with very large sample sizes, tiny changes in the community may be statistical significant. All species that occurred in only one sample were excluded, because the occurrence of such species gives no information on their sensitivity. Other, rare species were included, because any other rule would be subjective. The effects of different pooled sample set sizes were examined for the salinity data set (see below) using the R statistical computing language (r-project.org; see Supplemental Data for script).

Data sets

Salinity in southeastern Australia. Two macroinvertebrate data sets from streams in the adjoining Australian states of Victoria and South Australia were merged. The data were collected by each state's Environmental Protection Authority (EPA) [14,15]. Salinity was measured from electrical conductivity (EC) as mS/cm adjusted to 25°C (hereafter mS/cm), because most saline waters in southeast Australia have ionic proportions similar to sea water [16]. The analysis conducted was restricted to samples identified to species (when practicable) from the edge/pool habitat (sample size [n] = 2,966) collected by sweeping a net (mesh size 0.25 mm) through all subhabitats over approximately 10 m. These samples were split into predefined [14] EC categories (Table 1). The least sampled EC category (>30 mS/cm) had 21 samples, so analysis of this data set was conducted using a pooled sample set size of 21. By 21 samples, the number of new species in an EC category detected with each additional sample was decreasing.

Agriculture pesticide intensity. We used a macroinvertebrate data set [17] from sites sampled in tributaries of the Aller River, northern Germany, during the main pesticide application period of May to June from 1985 to 2002. Sampling was conducted according to the German standard for biological stream investigations: DIN 38410 *Bestimmung des Sprobienindex in Fliessgewässern*; detailed at http://www.fliessgewaesserbewertung.de/en/. At each sampling episode,

Table 1. The top right triangle shows the mean Jaccard's index (JI) of similarity between pooled sample sets (or 21 samples) and in parentheses analysis of similarity (ANOSIM) pairwise R between the different electrical conductivity EC (mS/cm) categories. Relative species retention (RSR) across 21 samples between all pairs of categories is in the bottom left triangle. Data refer to freshwater macroinvertebrates from Victoria and South Australia from edge/pool habitats, using all species present at >1 sample.[a]

EC category	<0.05	0.050–0.099	0.10–0.19	0.20–0.29	0.30–0.49	0.50–0.99	1.0–1.49	1.5–1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–6.9	7.0–9.9	10–14.9	15–30	>30
n (samples)	390	429	296	166	187	317	196	194	211	122	94	140	104	71	28	21
<0.05	0.44	0.43 (0.29[d])	0.37 (0.85[d])	0.32 (1.00[d])	0.26 (1.00[d])	0.21 (1.00[d])	0.19 (1.00[d])	0.18 (1.00[d])	0.16 (1.00[d])	0.17 (1.00[d])	0.15 (1.00[d])	0.15 (1.00[d])	0.14 (1.00[d])	0.11 (1.00[d])	0.07 (1.00)	0.04 (1.00)
0.050–0.099	0.97	0.44	0.40 (0.58[d])	0.35 (0.99[d])	0.29 (1.00[d])	0.24 (1.00[d])	0.21 (1.00[d])	0.20 (1.00[d])	0.18 (1.00[d])	0.19 (1.00[d])	0.17 (1.00[d])	0.17 (1.00[d])	0.15 (1.00[d])	0.12 (1.00[d])	0.09 (1.00[b])	0.04 (1.00[b])
0.10–0.19	0.84	0.90	0.40	0.39 (0.42[d])	0.34 (0.94[d])	0.29 (1.00[d])	0.27 (1.00[d])	0.25 (1.00[d])	0.23 (1.00[d])	0.24 (1.00[d])	0.22 (1.00[d])	0.22 (1.00[d])	0.20 (1.00[d])	0.17 (1.00[d])	0.11 (1.00)	0.06 (1.00)
0.20–0.29	0.72	0.80	0.96	0.42	0.39 (0.77[d])	0.34 (0.98[d])	0.33 (0.99[d])	0.30 (1.00[d])	0.29 (1.00[d])	0.30 (1.00[d])	0.27 (1.00[d])	0.27 (1.00[d])	0.26 (1.00[d])	0.21 (1.00[d])	0.15 (1.00)	0.08 (1.00)
0.30–0.49	0.60	0.67	0.85	0.92	0.43	0.40 (0.49[d])	0.39 (0.76[d])	0.38 (0.88[d])	0.36 (0.98[d])	0.37 (0.97[d])	0.35 (0.99[d])	0.33 (1.00[d])	0.31 (1.00[d])	0.27 (1.00[d])	0.21 (1.00)	0.10 (1.00)
0.50–0.99	0.48	0.54	0.71	0.81	0.93	0.42	0.42 (0.05)	0.42 (0.23[c])	0.40 (0.57[d])	0.42 (0.22[d])	0.40 (0.59[d])	0.38 (0.83[d])	0.36 (0.96[d])	0.31 (1.00[d])	0.25 (1.00)	0.13 (1.00)
1.0–1.49	0.43	0.48	0.67	0.77	0.91	1.00	0.44	0.44 (0.06)	0.42 (0.30[d])	0.44 (0.08)	0.42 (0.35[b])	0.40 (0.75[d])	0.39 (0.82[c])	0.34 (0.98[c])	0.27 (1.00)	0.13 (1.00)
1.5–1.9	0.40	0.46	0.63	0.72	0.87	0.98	1.00	0.44	0.44 (0.08)	0.46 (−0.08)	0.43 (0.16)	0.42 (0.54[d])	0.40 (0.67[c])	0.35 (0.90[c])	0.29 (1.00)	0.14 (1.00)
2.0–2.9	0.36	0.41	0.57	0.69	0.84	0.94	0.97	0.99	0.44	0.46 (−0.23)	0.44 (0.08)	0.44 (0.17)	0.42 (0.59[c])	0.37 (0.85[c])	0.32 (0.99)	0.15 (1.00)
3.0–3.9	0.38	0.43	0.59	0.71	0.86	0.98	1.01	1.03	1.04	0.48	0.46 (0.21)	0.46 (0.20)	0.44 (0.84[c])	0.44 (1.00[c])	0.34 (1.00)	0.16 (1.00)
4.0–4.9	0.34	0.39	0.55	0.65	0.81	0.93	0.97	0.98	1.00	0.96	0.45	0.45 (0.22)	0.44 (0.51[b])	0.40 (0.94[b])	0.34 (1.00)	0.17 (1.00)
5.0–6.9	0.34	0.38	0.54	0.64	0.77	0.89	0.92	0.95	1.00	0.96	1.00	0.46	0.45 (0.41[c])	0.43 (0.80[b])	0.37 (1.00)	0.18 (1.00)
7.0–9.9	0.31	0.35	0.50	0.62	0.73	0.85	0.89	0.91	0.95	0.92	0.98	0.98	0.49	0.45 (0.69[b])	0.40 (1.00)	0.20 (1.00)
10–14.9	0.25	0.28	0.41	0.50	0.61	0.74	0.77	0.80	0.85	0.83	0.88	0.92	0.93	0.50	0.48 (0.33)	0.26 (1.00)
15–30	0.17	0.20	0.28	0.36	0.49	0.60	0.62	0.67	0.72	0.71	0.74	0.80	0.82	0.97	UD	0.30 (UD)
>30	0.08	0.10	0.16	0.19	0.23	0.31	0.30	0.32	0.34	0.34	0.37	0.39	0.42	0.53	UD	UD

[a] UD = undefined (a result of only one pooled sample set in both categories).
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.

20 subsamples (0.25 × 0.25 m) were taken to reflect habitat composition (one subsample unit per 5% substrate coverage) across a 20- to 100-m river reach. Samples were sorted in the field.

We related change in species occurrence to an index of runoff potential (RP) [17]. The RP of a site was based on the $\log_{10}$-transformed load (g) of a generic compound that could enter the water body along a 1,500-m stretch upstream. Runoff potential was correlated with other indicators of agriculture intensity, but the major effect of RP on macroinvertebrates in the Aller Catchment is from pesticide runoff and not from other causes [17]. We split the data set into five RP categories: very low, defined as RP > -10 but ≤ 4 ($n = 40$ samples); low, > -4 but ≤ 3 (60); moderate, > -3 but ≤ 2 (100); high, > -2 but ≤ 1 (120); and very high, > -1 but $0 \leq$ (40). A pooled sample set size of 10 was used.

Metal pollution in the Clark Fork River. The Clark Fork River, in Montana, USA, has been contaminated by metals, principally Cu but also As, Ag, Cd, Pb, and Zn from mining and smelting for >125 years [18]. We analyzed concurrent data on freshwater benthic macroinvertebrates and Cu in fine (<0.064 mm) stream bed sediment collected (by the State of Montana and the U.S. Geological Survey, respectively) annually (1993–2003) during base flow (typically August) from riffles at 10 sites including three uncontaminated and a gradient of seven contaminated sites. We restricted our analysis to Cu as an indication of the effect of total metal pollution. Each macroinvertebrate sample consisted of a composite of four collections using a modified 0.1-m^2 Hess sampler (1-mm mesh) [19]. At each site and sampling episode, three Cu subsamples were taken, each subsample being a composite of three to five discrete collections of the surface (top 1 cm) sediment collected from different depositional areas [20]. The values are presented in micrograms Cu/gram dry weight of sediment.

We treated all 27 samples from uncontaminated sites as a single category reference (mean Cu: 24.8 μg/g, range 10–85 μg/g, $n = 19$, for eight samples from reference sites no Cu data were available). We pooled nine randomly selected samples to create three reference pooled sample sets. For Cu, we first sorted by sediment Cu and selected seven pooled sample sets each containing nine samples in increasing sediment Cu concentration. Thus the contaminated pooled sample sets had no replication. After analysis across these categories, three new categories were selected, and the analysis was repeated.

RESULTS

Salinity in southeastern Australia

Effect of pooled sample set size. Increasing the number of samples in pooled sample sets resulted in greater similarity in species lists between pooled sample sets within EC categories. This was demonstrated by a negative relationship between the size of pooled sample sets and a decrease in the standard deviation in JI between pooled sample sets within EC categories (Fig. 1a).

The number of samples pooled had a slight effect on the proportion of species estimated to be at risk from salinity. With ≤100 samples pooled into sampling sets, the mean squared deviation (from that obtained with 21 pooled sample sets) was always <0.0014, or RSR was ±3.7% ($\sqrt{0.0014}$) of that obtained with 21 samples (Fig. 1b). Furthermore, across pooled sample sets from seven to 50, mean squared deviation in RSR was always <0.00038 i.e., ±1.9%. In a second step, we recalculated the mean square deviation in RSR only for data points where RSR is ≥0.9, assuming that for the establishment

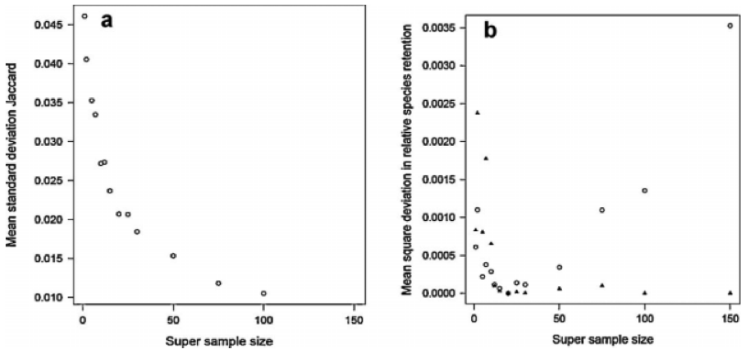

Fig. 1. Effect of pooled sample set size in the southeast Australian salinity data set. (**a**) Mean standard deviation of Jarcard's Index within electrical conductivity (EC) categories with increasing level of pooling. (**b**) Mean squared deviation in relative species retention (RSR) from that obtained from a pooled sample set size of 21, that is, mean of $(RSR_n - RSR_{21})^2$, where RSR = relative species retention and the subscript n refers to the plotted pooled sample set size plotted on the x-axis and 21 to a pooled sample set size of 21. Circles indicate all data used, triangles only data with $RSR_n = 0.9$.

of environmental quality guidelines most interest will be in the range of RSR that protects ≥90% of species. Similarly, pooled sample set size had only a small effect on RSR. For 10 to 150 sampled pooled into sampling sets, mean square deviation in RSR was always <0.00065, i.e., ±2.5%.

Change in relative species retention. The community structures in pooled sample sets (of 21 samples) between the EC categories were significantly different (ANOSIM global $R = 0.771$, $p < 0.001$). Not all comparisons between salinity categories resulted in significant changes in JI between the categories. For example, a rise in salinity from 1.0 to 1.49 mS/cm to 1.5 to 1.9 mS/cm would not result in changes of species composition across 21 samples indicated by an ANOSIM pairwise *R* of 0.06 and an RSR of 1.00 between these categories (Table 1). By contrast, a rise from 1.0 to 1.49 mS/cm to 5.0 to 6.9 mS/cm resulted in an 8% change in species across 21 samples.

Agriculture pesticide intensity in the Aller River Catchment

Significant differences were noted in JI between the RP categories (ANOSIM global $R = 0.361$, $p < 0.001$). The RSR between very low and low RP categories is >1 (Table 2), so all species were retained between these RP categories. With further increases in runoff potential, however, changes were noted in species composition (Table 2): between the very low and moderate RP categories, for example, with an RSR value of 0.85, indicating a 15% change in species across 10 samples.

Metal pollution in the Clark Fork River

Significant differences were observed in JI obtained between the categories of increasing sediment Cu (ANOSIM global $R = 0.984$, $p = 0.008$). Given that only reference sample sets were replicated and no replication of the contaminated sample sets occurred, no significant pairwise comparisons were noted. The RSR was similar (0.80–0.83) between reference sites and contaminated categories with ≤978 μg/g (Table 3). The two higher Cu categories (991–1095 and 1,110–1,537 μg/g) showed similar RSRs (0.69–0.73) in comparison with the reference sites. Thus the concentration response appears to be a step-function relationship, with a threshold occurring between the upper reference bound (85 μg/g) and the lower contaminated bound (169 μg/g) and a second threshold at approximately 978 to 991 μg/g.

We recalculated JI using three sediment Cu categories that reflected these differences: reference, 169 to 978 μg/g, and 991 to 1,537 μg/g. For each category, pooled sample sets consisting of nine randomly selected samples were chosen. Significant differences occurred in JI using these categories (ANOSIM global $R = 0.82$, $P_{\text{experiment wise}} = 0.016$). A significant difference ($P_{\text{experiment wise}} = 0.036$) occurred in JI between reference sites and the sediment Cu category 169 to 978 μg/g,

Table 2. The top right triangle gives the mean Jaccard's index (JI) within and between runoff potential (RP) categories, and in parentheses are analysis of similarity (ANOSIM) pairwise *R* statistics. In the bottom left triangle relative species retention (RSR) across 10 samples between runoff potential (RP) categories is given. Data refer to freshwater macroinvertebrates from the Aller River Catchment, Germany, using all species present at >1 site.

	Very low (RP −10 to −4)	Low (RP −4 to −3)	Moderate (RP −3 to −2)	High (RP −2 to −1)	Very high (RP −1 to 0)
Very low	0.37	0.39 (0.27[a])	0.31 (0.34[a])	0.29 (0.80[c])	0.28 (0.84[c])
Low	1.04	0.41	0.35 (0.12)	0.32 (0.59[c])	0.30 (0.83[c])
Moderate	0.85	0.85	0.34	0.34 (0.23[c])	0.33 (0.22[a])
High	0.79	0.78	1.01	0.38	0.36 (0.15)
Very high	0.77	0.73	0.96	0.96	0.38

[a] $p < 0.05$.
[c] $p < 0.01$.
[c] $p < 0.001$.

Table 3. The top right triangle gives the mean Jaccard's index (JI) within and between fine sediment Cu (μg/g dry weight) categories, and in parentheses are analysis of similarity (ANOSIM) pairwise *R* statistics (where this is defined).[a] In the bottom left triangle relative species retention (RSR) between the categories across nine samples is given where it is defined. Data refer to freshwater macroinvertebrates from the Clark Fork Catchment, Montana, USA, with Cu sediment categories defined as reference (three pooled sample sets) and pooled sample sets of increasing sediment Cu concentration.

	Reference (10–85)	169–277	286–391	393–609	614–779	780–978	991–1,095	1,110–1,537
Reference (10–85)	0.70	0.56 (1)	0.55 (1)	0.57 (1)	0.57 (1)	0.58 (1)	0.51 (1)	0.48 (1)
169–277	0.80	UD	0.68	0.61	0.56	0.60	0.63	0.60
286–391	0.79		UD	0.66	0.65	0.61	0.64	0.65
393–609	0.81			UD	0.65	0.62	0.56	0.52
614–779	0.81				UD	0.70	0.65	0.66
780–978	0.83					UD	0.63	0.63
991–1,095	0.73						UD	0.67
1,110–1,537	0.69							UD

[a] UN = undefined.

with an RSR of 0.82 between these categories (Table 4). Note that the *p* values given are experiment-wise to account for the product of multiple comparisons given the reclassification of categories.

DISCUSSION

We have shown that JI calculated between pooled sample sets of two different contamination categories can be used to estimate retrospectively the proportions of species present that do not change as concentrations of chemicals move between categories. The proportion of species present that do not change with contamination is subtly different from that estimated from SSDs, the proportion of species protected (PCp values). The PCp values consider only the proportion of species that will be excluded from a community from contamination levels that are assumed to prevent physiological tolerance. Ecological change, however, consists of both the loss of sensitive species and the gain of tolerant species as contamination increases. No ecological reason exists for why anthropogenic stress causing the gain of tolerant species would be a beneficial change [21]. We suggest that an appropriate goal of managing contamination is to prevent changes to ecological communities rather than protect species from exceeding their physiological sensitivity.

Nevertheless, especially for nonessential substances, the gaining of new species with increasing contamination may be relatively rare, and thus SSDs may be able to make a good approximation of community-level change. The hormesis effects are typically modest [22], and whether this effect commonly results in community-level change has largely been uninvestigated [21]. Although competitive exclusion resulting in apparent tolerance from indirect effects occurs [7], the question of their importance in structuring communities remains open. Thus, though not identical, for nonessential substances, it is possible that the JI approach may be broadly similar to that claimed by SSDs.

Table 4. The upper shaded triangle gives the mean Jaccard's Index (JI) within and between fine sediment Cu (μg/g dry weight) categories, and in parentheses are analysis of similarity (ANOSIM) pairwise *R* statistics. In the bottom left triangle relative species retention (RSR) between the categories across nine samples is given. Data refer to freshwater macroinvertebrates from the Clark Fork Catchment, Montana, USA, with Cu sediment at contaminated sites chosen after the analysis given in Table 3.

	Reference (10–85)	169–978	991–1,537
Reference (10–85)	0.68	0.56 (0.98[a])	0.49 (1)
169–978	0.82	0.67	0.64 (0.4)
991–1,537	0.71	0.95	0.70

[a] $p < 0.05$ (experiment-wise p values).

We thus compare the new JI method with some recently suggested modifications to conventional SSDs that are designed to meet better the assumptions of SSDs [4]. We use the term conventional SSD [1] to imply that species sensitivity values are obtained from standardized laboratory tests of a usually small number of standard test species that have not been selected to sample the sensitivities of species in specific natural communities. Laboratory tests designed to assess approximately the sensitivity of many nonstandard and rare test species sampled from specific communities, known as rapid tests, have been suggested [6,11,23]. Sensitivity of many nonstandard test species can also be estimated from expert opinion calibrated with Bayesian statistics against experimental conventional [10] or rapid [11] tests. The sensitivity of standard test species to a particular chemical can be derived from their relative sensitivity to other chemicals (statistical deviations) [9,13]. The sensitivity of sometimes abundant and widespread species can be estimated from abundance declines associated with increasing contamination and expressed in field-based SSDs [2,12,24–26]. Abundance declines cannot be reliably estimated on species that are found in low abundances or are infrequently collected. This is problematic for obtaining unbiased samples of the sensitivity of species in a community, insofar as most species are found both in low abundances and at low frequencies of occurrence, and rarity is not randomly distributed between taxa [27]. Furthermore, Hewitt et al. [28] showed that a field-based SSD could not in fact predict chemical concentrations that protected community structure. These modifications are based on three premises (Table 5) to meet some of Forbes and Calow's [4] requirements for a realistic risk assessment. However, only the method presented in this paper employs all of the premises (Table 5). In our opinion, all of the aforementioned new methods result in more realistic assessment of risk to ecological communities relative to conventional SSDs. While our approach fulfills all of these premises, it does have some of its own assumptions (see below). The chief limitation of the new method is that it can only be used retrospectively on chemicals already in the environment and cannot be used in prospective risk assessment.

The method presented here differs from existing analyses of biota communities along pollution gradients (e.g., [5]), which aim to determine concentrations of chemical stressors that lead to a departure from reference conditions. Although existing multivariate analyses consider samples taken across spatial and/or temporal gradients, the proposed method randomly pools samples (into pooled sample sets) with similar contamination

Table 5. Comparison of recently suggested approaches to improve estimates of the protective concentration for *p*% of species (PCp) values[a]

Premise (more accurate PCp values if)	Rapid tests	Expert opinion and Bayesian statistics	Statistical deviations	Abundance declines	This paper
Considering the sensitivity of more species	×	×	×	×	×
Sensitivity sampled from real communities include nonstandard test and rare species	×	×			×
Species sensitivity assessed in the field				×	×

[a] Crosses indicate that an approach uses the premise that rapid tests are approximate toxicity tests conducted on many field-collected species sampled to represent specific communities [6,23]. Expert opinion and Bayesian statistics refer to the use of expert opinion on the sensitivity of taxa calibrated against toxicity data using Bayesian statistics [10,11]. Statistical deviations involve estimation of the sensitivity of species based on their sensitivity to other substances relative to other species [9,13]. Abundance declines refer to estimation of the sensitivity of species based on the declines in their abundance in nature along a pollution gradient [2,12,24–26].

level, regardless of when and where samples were taken. Thus the presented method indicates the effect of chemical stressors on what we call the contamination category community. We define this construct as the biotic community that occupies a particular range of contamination regardless of spatial and temporal patterns (unconnected with the contaminant of interest).

How does RSR compare with existing guidelines/analyses?

The method presented showed altered macroinvertebrate community structure in the Clark Fork Catchment at sediment Cu concentrations higher than the U.S. Environmental Protection Agency (U.S. EPA) [29] screening concentration. A Cu concentration of 157 μg/g gives a response in 50% of species ([29]; http://epa.gov/waterscience/cs/report/2004/nsqs2ed-complete.pdf); our study suggests only approximately 20% species changing between reference and contaminated sites with sediment Cu in a broad range of 169 to 978 Cu μg/g. Especially considering that the Clark Fork is contaminated with metals other than Cu [18] that might have contributed to the community change in our study, the U.S. EPA screening concentrations may be overprotective, at least for the Clark Fork Catchment. Naturally high levels of metals at reference sites in the Clark Fork Catchment (reference sites had up to 85 μg Cu/g) might have already excluded some metal-sensitive species. Ingersoll and others [30], however, list a range of guidelines suggesting threshold concentrations of 86 to 390 μg Cu/g for North American freshwater sediments. This range overlaps with the frange that we estimated to protect 20% change in community.

For the Aller Catchment, we observed that all species were retained when pesticide runoff potential (RP) increased from very low to low, but, if RP reached moderate or higher levels, RSR declined. Similarly, no change in an index of pesticide impacts on macroinvertebrates, $SPEAR_{pesticide}$, occurred at very low and low RP from the period before the main pesticide application season (April) to the main pesticide application season (May–June) [17]. However, at moderate and higher RP, $SPEAR_{pesticide}$ decreased over this period. Thus the proposed method is in accordance with previous results.

$SPEAR_{pesticide}$ measures the proportion of individuals in a community belonging to species at risk (SPEAR) because of their physiological and ecological sensitivity [8,31]. $SPEAR_{pesticide}$ could logically be constant despite large changes in community structure and/or loss of species. The proposed method and $SPEAR_{pesticide}$ have completely different targets of concern for retrospective risk assessment; the former considers community structure change, whereas SPEAR considers changes in the fraction of sensitive individuals in the community.

Our findings, however, show substantial changes in community structure in southeastern Australian streams well below salinity levels predicted from an SSD. The 5th percentile of macroinvertebrates' 72-h mentioned median lethal concentration (LC50) values is approximately 11.6 mS/cm [14]. Even after division by a safety factor within the range of 3.33 to 20 (based on acute to chronic ratios), the PC_{95} values range from 0.58 to 3.5 mS/cm [14]. We observed a 3% and 16% change in species occurrence as salinity rises from <0.05 mS/cm to within the ranges of 0.050 to 0.099 mS/cm and 0.10 to 0.19 mS/cm, respectively. Indeed, when salinity changed from <0.05 mS/cm to 0.50 to 0.99 mS/cm, only 48% of species are retained. From 0.05 mS/cm to 3.0 to 3.9 mS/cm, only 38% of species are retained. This discrepancy probably is due to differences in how changes in community structure are implicitly defined between SSDs and the proposed method. The RSR between two contamination categories is calculated from JI, which combines both sensitive species lost and tolerant species gained as contamination increases. All methods of estimating PCp values consider only the number of sensitive species lost as contamination increases. These methods assume that all species have a threshold response to contaminants and that, below their threshold, species are unaffected. Hence, they do not include other responses such as hormesis, essentiality [21], or competitive exclusion resulting in apparent tolerance from indirect effects [7]. This assumption may be reasonable for synthetic chemicals and naturally occurring nonessential substances, even when hormesis exists, because its effects are typically modest [22], and as an approximation it may be reasonable to assume that competitive exclusion causing apparent tolerance to chemicals does not have an important effect on structuring communities. Nevertheless, essentiality responses are important for essential elements (e.g., selenium [32]) and mixtures of essential elements (e.g., salinity [33]) and result in a species having an optimal concentration range and suffering outside this range.

Low, but naturally occurring, salinity levels are stressful to many freshwater invertebrates [33]. Up to a salinity of about 0.5 to 1 mS/cm, the macroinvertebrate species richness in the EC categories used here and salinity are positively related (B.J. Kefford, personal observations). Some of the differences in RSR for EC categories <0.5 mS/cm observed thus are due to the addition of new species with increasing salinity. Predictions of ecological change from SSDs assume that sensitivity values (e.g., LC_x, EC_x, NOEC, or abundance declines) of species represent the ecological threshold of each species [4]. When the concentration is less than or equal to the sensitivity value of each species, the assumption is that their populations will be unaffected by the particular chemical. The apparent difference between the method presented here and SSDs for salinity are caused by different value judgments for how to define changes

in community structure. We believe that anthropogenic effects of chemicals on biotic communities should be defined in terms of all changes (i.e., both losses and gains of species) [21]. Although it is not always easy to determine the natural level of a chemical, RSR should be calculated with respect to the assumed natural level of the contaminant of interest. Hence an advantage of the method proposed is that, unlike SSDs, it does not assume that species exhibit a threshold response to a contaminant.

The assumptions of SSDs include the value judgments that the loss of any species is of equal importance and community structure is the target of concern not community function [4]. The method that we present here is not without its value judgments. Community structure is the target of concern, which is defined in terms of the occurrence of species in which gain and loss of species are equally important. Changes in the abundance of species are not considered, so the method cannot detect declines in species abundances, which may precede the loss of species. Nevertheless, the method presented here could easily be modified by using indices other than JI that incorporate abundance of species (e.g., Bray-Curtis) or that consider only losses of species. Species could be weighted so that the loss or gain of some species is more important than the loss or gain others. Organisms could be classified not taxonomically but according to functional groups or ecological traits and similarity calculated between pooled sample sets, so concentrations that result in an unacceptable change in functional groups or traits could be estimated. Species sensitivity distributions may be compiled with different endpoints and taxa, and the SSD concept could in principle be modified to weight species so that the loss of some species is more important than the loss of others. The SSDs are, however, able to make predictions only for loss of community structure [4]. Hence, methods based on similarity indexes are open for a greater range of targets of concerns and their implicit value judgments than SSDs.

Dependence of the method on sampling effort and bias

The analysis conducted suggests that dependence on pooled sample set size is relatively minor. In general, the greater the proportion of species with low, but nonzero, probability of occurrence, the greater the discrepancy between the RSR and the real species retention (that calculated if all species could be reported). The larger the number of samples per pooled sample sets, the smaller this discrepancy. We showed that RSR (in the southeast Australian macroinvertebrate data set) is not independent of pooled sample set size. However, when very small or large pooled sample set sizes were excluded, only relatively minor changes occurred in RSR with pooled sample set size ($\pm$1.9 to $\pm$3.7%). A solution to the (slight) dependence of RSR on pooled sample set size is to interpret RSR relative to the used pooled sample set size.

The RSR may also be dependent on the spatial/temporal extent of the sampling program according to the distribution of sensitive and tolerant species. Suppose that many contaminant-sensitive species are confined to restricted habitats or geographic ranges, whereas tolerant species tend to be widespread. In this case, increasing sampling extent would result in detecting the presence of relatively more sensitive species than tolerant species. Thus sampling extent and RSR would be inversely correlated. If the reverse were the case, species with restricted distribution tended to be relatively tolerant, then sampling extent and RSR would be positively correlated.

Guidelines estimated from SSDs, especially those composed exclusively of results from conventional laboratory tests, have uncertainties with regard to whether these guidelines really protect the proportion of species that they claim to protect. Indeed, although conventional SSDs may be precise (repeatable), they are unlikely to be accurate (really to protect the proportion of species claimed) [4]. Given that some dependency is observed in number of samples pooled, and possibly sampling extent, the method presented may be less precise. However, insofar as it assesses effects in nature, it is likely to be more accurate. We suggest that accuracy is more important than precision for community-level risk assessment. The dependence of RSR on pooled sample set size and sampling extent is, however, quantifiable. This can be done by comparing data sets split into different pooled sample set sizes, geographic areas, habitats, etc., to determine the influence on RSR. Such analyses would reveal the precision of the proposed method. It is, however, more difficult to determine the accuracy of conventional SSDs without comparing predictions from SSDs with effects in nature [14,28].

Categorical levels of contamination

The concentrations of chemical contaminants are continuous, yet we categorize continuous data into ordinal categories. Environmental quality guidelines and risk assessment may be somewhat affected by the categories chosen. We therefore suggest repeating the analysis with different categorizations. This allows for the dependence of EQGs on particular categories to be evaluated. Expert decisions are also made by alternative methods of setting EQG for protecting communities. For example, EQGs set using SSDs are dependent on decisions for what species to test, the exposure duration, endpoints, etc.

Categorizing also results in greater uncertainty regarding the precise value of the threshold in chemical concentrations that results in community-levels effects. This is especially relevant when categories span a wide concentration range as in Table 4. We suggest that the threshold be set at the maximum concentration in the highest category that has an acceptable change in RSR. We do acknowledge that some uncertainty regarding will remain as to whether this value corresponds to a real ecological threshold. This reduction in uncertainty has to be weighed against the fact that the method that we present identifies community-level ecological thresholds directly, thus making fewer assumptions. All other proposed methods indirectly identify community level. Laboratory- and field-based [2] SSDs use data on the response of individual organisms and population responses, respectively. By making the ordinal categories of contamination that are a requirement for this method, JI can be calculated across pooled sample sets. We thus suggest that any disadvantage of categorization is outweighed by the method's other advantages.

Causality associated with community change in field methods

As with any field study, the method presented requires the assumption that the change in biota associated with the contaminant of interest is causal. Although we acknowledge this assumption, it should not be viewed as an impediment [2,25]. Pooling samples implies that pooled sample sets encompass a range of water qualities and biotic and abiotic habitats. For there to be confounding variables with pooled samples sets, very strong associations between the contaminant of interest and the confounding variables would have to occur. Furthermore, rarely are field studies conducted without knowledge of factors affecting the biota. It is thus possible to analyze data sets for potential effects of other stressors and hence account for factors that are known to affect the biota. We suggest (for large data sets) calculating RSR for different subsets in which

samples with modalities of factors such as poor water or habitat quality are excluded [34]. If the chemical stressor of interest really causes the change in the community, then RSR values should be similar regardless of whether they are calculated from the full data set or from only those samples with good water and habitat quality.

Furthermore, many physical and chemical measurements covary. Salinity, for example, is causally connected to dissolved oxygen saturation and the concentration of major ions, and in many cases it is of limited use to differentiate for their individual effects. Other confounding factors might not be causally connected with a contaminant but nevertheless covary because both are mechanistically related. The Clark Fork is polluted by metals other than Cu [18], but we calculated RSR against Cu only. Because metal contamination in the Clark Fork has a common cause, it seems valid to set EQGs for this catchment from the RSR values calculated here based on Cu even if the RSR values are partially caused by other metals. Under these circumstances, however, the applicability of the EQGs for different regions may be limited.

CONCLUSIONS

Several methods exist for estimating the proportion of species potential change in communities by a stressor: conventional SSDs or other recently proposed laboratory-, statistical-, and field-based improvements to SSDs and the method presented here (Table 5). All approaches have their strengths, weaknesses, assumptions, and value judgments. It is important that these are acknowledged and considered in the risk assessment process. We suggest that the method we present using JI has some advantages over other methods for chemicals already in the environment. One useful aspect of similarity-based index methods is to validate other methods against actual changes in communities in nature. When results from different methods are similar, confidence that the guidelines protect what they claim to protect is increased. When the results are divergent, the causes of the discrepancies should be investigated and may lead to a greater understanding of the ecological effects of contaminants and ultimately more appropriate management of chemicals.

SUPPLEMENTAL DATA

R routine for considering the effect of pooled sample set size (77 KB PDF).

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Is there an interaction of the effects of salinity and pesticides on the community structure of macroinvertebrates?

Eduard Szöcs [a,*], Ben J. Kefford [b], Ralf B. Schäfer [a]

[a] Institute for Environmental Sciences, University of Koblenz-Landau, Forststraße 7, 76829 Landau, Germany
[b] Centre for Environmental Sustainability, School of the Environment, University of Technology Sydney, Broadway NSW 2007, Australia

HIGHLIGHTS

- We investigated effects of pesticides and salinity on macroinvertebrate communities.
- Both salinity and pesticides influenced community structure.
- Salinity discriminated on a higher taxonomic level than pesticides
- We found no interactions between salinity and pesticides.

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ABSTRACT

Salinization of freshwater ecosystems is a global problem affecting many regions worldwide and can co-occur with pesticides in agricultural regions. Given that both stressors are potent to affect macroinvertebrate communities, their effects could interact.

We investigated the effects of salinity and pesticides at 24 sites in an agricultural region of southern Victoria, South-East Australia. We used distance-based redundancy analysis to determine the influence of pesticides, salinity and other environmental variables on the composition of macroinvertebrate communities.

Salinity and pesticide toxicity had a statistically significant effect on communities as had the substrate composition and the percentage of pool and riffle sections in the sampled stream reaches. We did not find evidence for interactive effects between salinity and pesticides, i.e. the effect of one of these variables did not depend on the level of the other.

Nevertheless, our results show that salinization and exposure to pesticides can be major factors for the structure of macroinvertebrate communities in agricultural regions. Pesticide toxicity acted on a lower taxonomic level compared to salinity, potentially indicating evolutionary adaptation to salinity stress.

1. Introduction

Macroinvertebrates play an important role in the functioning of freshwater ecosystems, for example they regulate rates of leaf litter decomposition (Graça, 2001) and nutrient cycling (Vanni, 2002). In addition, they are an important food source for fish (Wallace and Webster, 1996) and other animals living in or around the streams (Baxter et al., 2005). Hence they represent an important link in food webs.

In Australia salinization due to rising saline watertables is considered as one of the most serious environmental problems (Lovett et al., 2007) and increasing stream salinity may have adverse effects on macroinvertebrate communities (Metzeling, 1993). The problem of salinization is not restricted to Australia but occurs globally, including the Iberian Peninsula (Gallardo-Mayenco, 1994), the USA (Griffith et al., 2001) and Central Mexico (Sarma et al., 2002).

In agriculture, pesticides are used to increase agricultural productivity (Wilson and Tisdell, 2001), but can have adverse effects on non-agricultural systems, including macroinvertebrates in streams (Liess and von der Ohe, 2005; Schulz, 2004). Runoff during rainfall events and spray-drift are the main entry routes of pesticides from fields into surface waters (Schäfer et al., 2011c).

In agricultural landscapes, e.g. around Melbourne, Australia, salinization (Williams, 2001) and pesticide-exposure (Wightwick and Allinson, 2007) may occur concurrently and lead to interactive non-additive (i.e. antagonistic or synergistic) effects (Davies et al., 2004). A synergistic effect arises between two stressors when their joint effect is greater than the sum of the individual effects, whereas an antagonistic effect means that the joint effect is smaller than the sum of the individual effects.

Analyzing ecological communities is clearly a multivariate problem and complicated by simultaneously acting and correlated explanatory variables (Graham, 2003). Schäfer et al. (2011b) analyzed the effects of pesticides on macroinvertebrate communities using a trait-based

* Corresponding author at: Tel.: +49 6341 280 31536; fax: +49 6341 280 31326.
E-mail address: szoe8822@uni-landau.de (E. Szöcs).

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indicator (SPEAR indicator for pesticides (Liess and von der Ohe, 2005)). They demonstrated that pesticides can lead to shifts in the proportion of sensitive species in communities. However, no study has examined how pesticides and salinity in combination affect stream macroinvertebrate communities. This is despite the likely common occurrence of these stressors and laboratory studies showing non-additive effects of organophosphate pesticides and salinity (Hall and Anderson, 1995).

Hence in this study we used multivariate techniques, to address the following questions:

- Both pesticide toxicity and salinity are expected to affect invertebrate communities, but is there an interaction between both stresses?
- How important are salinity and pesticide toxicity in comparison to other environmental variables in shaping the macroinvertebrate community composition?

2. Material and methods

2.1. General description of the dataset

The data from 24 sites investigated by Schäfer et al. (2011b, 2012) situated in a 120 km radius around Melbourne, Victoria, Australia were used here (see Supplement for coordinates). The sites covered gradients of both pesticide exposure and salinization. Pesticide toxicity was expressed in terms of Toxic Units (TU) with respect to *Daphnia magna* (concentration/EC_{50}, (Sprague, 1970)) and salinity in terms of electrical conductivity (μS/cm at 25 °C, hereafter μS/cm).

Importantly, this dataset contained sites with both low salinity (<0.2 mS/cm) and pesticide toxicity (<0.001 $TU_{D.magna}$), low salinity but high pesticide toxicity (>0.01 $TU_{D.magna}$), high salinity (>1 mS/cm) and high pesticide toxicity and high salinity but low pesticide toxicity. Since no industrial facilities or waste-water treatment plants were located upstream of the sampling sites, any organic toxicants in surface waters most likely originated from agricultural pesticide use.

Macroinvertebrates and environmental variables other than pesticides (see below) were collected three times in: September (2008), November (2008) and February (2009), but in February three sites were not sampled (i.e. n = 21) due to inaccessibility and stream drying. Macroinvertebrates were sampled according to the rapid bioassessment method (EPA Victoria, 2003) which gives a semi-quantitative measure of the community: samples were taken from edge/pool (sweep sampling, 250 μm mesh size) and where present (=6 sites) in riffle habitats (kick sampling, 500 μm mesh) over a reach of at least 10 m (per habitat), live picked on site (minimum 30 person-minutes per habitat) and identified in the laboratory. The abundance of each taxon was estimated as suggested for the German sampling protocol which is compliant with the Water Framework Directive (WFD) (Meier et al., 2006). If applicable, the samples from both pool and riffle habitats from each site were pooled for data analysis.

Twenty-four water quality and hydrological characteristics were measured in the field (see Table 1). The data consisted of 4 datasets: toxicity, water chemistry, habitat and macroinvertebrate abundance data (all raw data available in Supplement).

Exposure to 97 pesticides was assessed using three methods: grab water samples, sediment samples, and 2,2,4-trimethylpentane passive samplers (TRIMPS). All three methods were used on 4 to 6 occasions between September (2008) and February (2009). The maximum toxicity (in terms of TU) derived from all three sampling methods and sampling periods was used as measure for pesticide stress. Standard physico-chemical parameters were measured: nitrate, nitrite, ammonium, phosphate and dissolved oxygen concentrations, as well as temperature, pH, electric conductivity and turbidity. Measured and estimated stream characteristics included maximum and minimum widths, sampling reach wide current velocity, depth, proportion of pools and riffle and substrate composition as outlined in detail in the rapid bioassessment protocol (EPA Victoria, 2003). Discharge was estimated by multiplying depth with mean width and the spatially-weighted average current velocity (see Supplement).

Table 1
Summary of environmental variables.

	Variable	Unit	Range
Water variables	Temperature	°C	8.2–22.8
	pH	–	6.21–8.94
	Salinity[a]	μS/cm (25 °C)	39.7–5530.0
	Dissolved oxygen	% sat.	4.0–141.2
	Ammonia[a]	mg/L	0–8.0
	Nitrite[a]	mg/L	0–2.7
	Nitrate[a]	mg/L	0–9.4
	Phosphate[a]	mg/L	0–40.0
	Turbidity[a]	NTU	1.2–33.1
Habitat variables	Depth[b]	m	0.05–1.00
	$Width_{max}$[b]	m	2–30
	$Width_{min}$[b]	m	1–17
	Pool	%	20–100
	Riffle[b]	%	0–80
	Bedrock	%	0–30
	Boulder (>25.6 cm)	%	0–30
	Cobble (6.4–26.5 cm)[b]	%	0–40
	Pebble (1.6–6.4 cm)[b]	%	0–30
	Gravel (0.2–1.6 cm)[b]	%	0–60
	Sand (0.06–0.2 cm)	%	0–70
	Clay (<0.06 cm)[b]	%	5–100
	Velocity[a]	cm/s	0.02–50.00
	Discharge[a]	L/s	0.2–2000.0
Toxicity variables	TU max	log $TU_{D.manga}$	−5.14 to −0.95

[a] Variables log10 transformed prior to analysis.
[b] Variables excluded from analysis due to high correlation with other variables.

2.2. Data analysis

Where concentrations of water chemistry variables were less than the limit of detection (LOD) the values were set to LOD/2 (Clarke, 1998). Skewed and wide spread environmental variables were $\log_{10}(x)$ transformed before analysis (Table 1).

For statistical analyses invertebrate data was aggregated (from mostly genus) to family level in order to have a consistent taxonomic resolution. Previous studies showed that similar results are found for family and lower level ordinations (Jones, 2008; Metzeling et al., 2006).

Variables measured at the same time and site may be collinear. Hierarchical variable clustering was used to identify and eliminate redundant variables from the dataset (Khattree and Naik, 2000). Variables with a strong correlation to other variables (Spearman's Rho > 0.7) were removed from the dataset, based on expert judgment.

We used distance-based redundancy analysis (db-RDA) (Legendre and Anderson, 1999; McArdle and Anderson, 2001) to examine the effects of environmental variables on macroinvertebrate communities after confirming a monotone or linear univariate response to salinity and pesticides for the majority of the 20 most abundant taxa. Db-RDA is a constrained ordination method (showing only the variation that can be explained by constraining variables), which allows the usage of every distance measure. Since 82% of the abundance data were zero entries, we used the Bray–Curtis dissimilarity. Abundance data was 4th root transformed prior to calculating dissimilarity to focus on composition rather than on abundance (Anderson et al., 2011). Forward selection of the explanatory variables was performed to find a parsimonious model and determine the most influential environmental variables. Two stopping criteria were used in forward selection: (1) permutation p-values (1000 permutations per step) and (2) adjusted R-squared of the global model as proposed by Blanchet et al. (2008).

For investigating the effects of salinity (as electrical conductivity), pesticide toxicity (as $TU_{D.\ magna}$) and their interaction we used manual model building with salinity, pesticide toxicity and their interaction as predictors. Permutation p-values were calculated for every sampling

event separately, because missing data in February (n = 21) did not allow for a permutation design taking temporal autocorrelation into account. Ordinations were made pooling all three sampling events and removing a temporal effect in order to show the effects.

R-Code and data, which were used for computations and graphics (R, version 2.14.2 on Linux, 64bit (R Development Core Team, 2012) and "vegan, version 2.0–3" (Oksanen et al., 2012)) are supplied in the Supplemental material, in order to reproduce our analysis (Barnes, 2010).

3. Results

3.1. Influential environmental variables

Forward selection revealed that salinity, pesticide toxicity, substratum and flow conditions were important factors shaping the invertebrate assemblages. Of the selected variables, velocity, discharge and %pool in the reach explained most of the variance. Water chemistry parameters other than salinity and pesticide toxicity showed no correlation to macroinvertebrate communities (Table 2).

Corixidae spp. were found only at sites with a high amount of pools, whereas Leptophlebiidae spp. were associated with fast flowing habitats. Hydropsychidae spp. were found at sites with riffles but medium velocity (Fig. 1).

3.2. Effects of salinity, toxicity and their interaction

Both salinity and pesticide toxicity had a statistically significant relationship with macroinvertebrate community structure. However, the interaction between both stressors was not statistically significant (Table 2).

Ceinidea spp. and Lymnaeidae spp. were most abundant at saline sites, whereas mayflies of the family Leptophlebiidae were sensitive to increasing salinity. Baetidae spp. and Simuliidae spp. reacted sensitive to pesticide pollution, whereas the snail species *Physa acuta* (Family Physidae) was not affected by increasing pesticide toxicity. Taxonomic groups like molluscs and crustaceans were salt tolerant, whereas mayflies, caddisflies and stoneflies were sensitive. Such discrimination between taxonomic groups was not apparent for pesticide toxicity because within a taxonomic group there were different tolerances towards pesticides: For example within the mayflies, Caenidae spp. were relatively tolerant to pesticides in contrast to Baetidae spp. but both were relatively sensitive to salinity (Fig. 2).

Table 2
Results of marginal permutation tests of db-RDA (1000 permutations). Bold values indicate statistically significant effects ($p<0.05$).

Forward selection	F	p	cum[a]
Pool %	4.49	**0.001**	0.039
Discharge	3.58	**0.001**	0.095
Sand	3.24	**0.001**	0.135
Conductivity	3.17	**0.001**	0.179
Velocity	3.05	**0.001**	0.216
Boulder	1.86	**0.015**	0.239
TU max	1.59	**0.038**	0.259

Interaction	September		November		February	
	F	p	F	p	F	p
Conductivity	3.02	**0.002**	2.36	**0.005**	2.43	**0.008**
TU max	1.83	**0.043**	2.70	**0.005**	0.63	0.868
Conductivity x TU max	0.54	0.917	1.21	0.249	0.99	0.436

[a] Cumulative proportion of explained variance.

4. Discussion

4.1. Salinity

Salinity is a major factor shaping macroinvertebrate communities and increasing salinity due to agriculture may adversely affect these communities. Db-RDA showed that salinity explained a high amount of variation in the community data (Table 2). Stream invertebrate communities have been shown in a number of other studies to respond to salinity (Kefford et al., 2010, 2011; Kefford, 1998; Metzeling et al., 2006).

Tolerance differences between major taxonomic groups were observed (Fig. 2): crustaceans and molluscs were tolerant and ephemeropterans were sensitive to increasing salinity. These results are partly supported by other studies showing that crustaceans are the most salt tolerant order (Berezina, 2003; Kefford et al., 2003; Piscart et al., 2005) and ephemeropterans the most sensitive order (Dunlop et al., 2008; Kefford et al., 2003, 2005, 2006, 2012; Short et al., 1991).

Hart et al. (1990) expected molluscs, especially pulmonate gastropods (Hart et al., 1991), like Lymnaeidae spp., to be sensitive to increasing salinities. In contrast to laboratory studies supporting this conjecture (Kefford et al., 2003), our results suggest that in the field this family reacts less sensitively than the other families. A possible explanation is the problem of extrapolation from laboratory tests to community effects in the field due to indirect effects (Seitz and Ratte, 1991).

As Kefford et al. (2004) pointed out laboratory tests of salinity tolerance reflect the maximum salinity a species can inhabit, which is in agreement with our findings. In the current study Baetidae spp. were one of the most sensitive families with none observed above 1000 µS/cm. This reflects the relatively low maximum field distribution (Kefford et al., 2004) and results of laboratory tests of Baetidae spp. reported elsewhere (Dunlop et al., 2008; Hassell et al., 2006; Kefford et al., 2003, 2005, 2006).

4.2. Pesticides

Pesticides affected macroinvertebrate communities, which has been shown in several other studies (Liess and von der Ohe, 2005; Schäfer et al., 2007; Schäfer et al., 2011d). But compared to salinity pesticide toxicity explained less of the observed variation (Table 2) and may therefore have less importance for shaping the communities, at least in the region studied. However it must be noted that the study took place during a drought in 2008/2009, with no precipitation in January and February 2009 (BOM, 2009). This could also influence our findings, since lower precipitation would likely reduce pesticide input due to reduced run-off from fields and in turn increase salinity due to evaporation.

In a mesocosm study Beketov et al. (2008) found that the baetid mayfly *Cloeon dipterum* and *Simulium latigonium* were the most affected species by the insecticide thiacloprid. Baetidae spp. and Simuliidae spp. were also among the most pesticide sensitive families in our study (Fig. 2). In a field study in Germany Berenzen et al. (2005) found that the abundance of *Radix ovata* (Family: Lymnaeidae) was positively correlated with increasing pesticide toxicity. We made similar observations in Australian streams.

Laboratory data (as compiled by von der Ohe and Liess (2004)) also suggest that molluscs are among the most tolerant taxa towards pesticides. Plecoptera spp. and Trichoptera spp. are considered being the most pesticide sensitive insects, which was also the case in the current study. Fig. 2 suggests that Calamoceratidae spp. and Austroperlidae spp. being exceptions, but these two families were found only occasionally. In laboratory acute toxicity tests Corixidae spp. and Baetidae spp. had a similar sensitivity to pesticides (von der Ohe and Liess, 2004). Daam et al. (2009) found Corixidae spp. being the most sensitive family using outdoor microcosms, but as they remark this may be a result of emigration rather than toxicity. Our field data suggests a higher tolerance of Corixidae spp. then predicted from this experimental data.

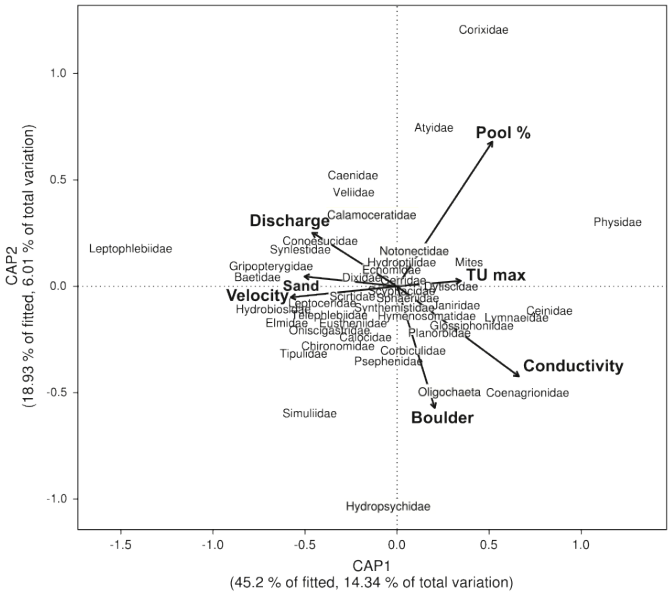

Fig. 1. Forward selection of environmental variables using distance-based redundancy analysis. Only the first two axes of the correlation biplot are shown, site scores not displayed for clarity, species scores displayed as weighted sums. Only families with a minimum abundance greater than 10 individuals are displayed. Ordination based on 4th root transformed abundance data and Bray–Curtis dissimilarity.

Corixidae spp. as air-breathing organisms (a) have a reduced toxicant uptake (Buchwalter et al., 2003) and (b) could avoid pesticide peaks on land, which may explain the observed differences between experimental and field data.

In contrast to salinity, pesticide toxicity acted on a lower taxonomic level. For example Ephemeroptera contained a broad range of pesticide sensitive and tolerant families but all were salinity sensitive. Likewise toxicity tests of stream macroinvertebrates with pesticides (von der Ohe and Liess, 2004) and salinity, showed that the sensitivity to salinity is less variable within orders than to pesticides (Kefford et al., 2012). Some authors argued that taxa with a more recent divergence from marine ancestors may be more tolerant to salinity (Hart et al., 1991; James et al., 2003). The fact that salinity discriminates on a high taxonomic level might be a legacy of these marine ancestors, with crustaceans and molluscs having near marine ancestors (Vermeij and Dudley, 2000). Salinity has been also naturally occurring in Australia and been acting on organism for a long time relative to organic pesticides which have only been used intensively after World War II (Schäfer et al., 2011d). The much shorter exposure period of communities can be an explanation why no adaption could evolve.

4.3. Other factors

Besides pesticides and salinity, factors describing the physical habitat (substratum, presence of pools, discharge and velocity) were identified as influential for macroinvertebrate communities. These local factors are partially interrelated and well known to shape the distribution of species (Costa and Melo, 2008; Minshall, 1984; Rempel et al., 2000). For example filter feeders like Hydropsychidae spp. and Simuliidae spp. were found at sites with riffles, rough substrate and medium velocity (Fig. 1), since they need some flow to gather their food. However the distinction of the taxa between riffles and pools was not prominent. This may be due to riffles only being present in six sites and therefore data pooling of pool and riffle habitats. Hence, differences between pools and riffles were probably masked in this analysis. There was no correlation to other water chemistry variables. This can be explained as the study was designed to cover primarily a broad range of salinities and pesticide exposure with other gradients being as small as possible.

In other parts of the world such as Northern Europe lotic communities vary seasonally because of life histories of various species (Šporka et al., 2006). In Victoria, Australia, we did not find major differences between September, November and February — all common families were present in all samplings. This lack of seasonality may be attributed to the taxonomic resolution at family level and a higher taxonomic level could reveal a seasonal pattern (Marchant, 1990).

4.4. Salinity and pesticides combined

Our results show that salinization and exposure to pesticides can be important factors for the structure of macroinvertebrate communities in agricultural regions. We did not, however, find evidence for non-additive effects between salinity and pesticides on macroinvertebrate communities. That is the effect of salinity and pesticide combined is equal to the sum of both stressors.

There have been many laboratory studies investigating the combined effect of pesticides and salinity (Hall and Anderson, 1995). However there was no clear trend identifiable from these single species and single substance tests. Schäfer et al. (2011a) correlated biomonitoring data from the Australian River Assessment System (AUSRIVAS) program with pesticide-exposure estimated by a runoff-model (Burgert et al., 2011; Schriever and Liess, 2007). They likewise observed no interaction effect of salinity and estimated pesticide risk. Moreover, the sampling method could be another possible explanation for not detecting

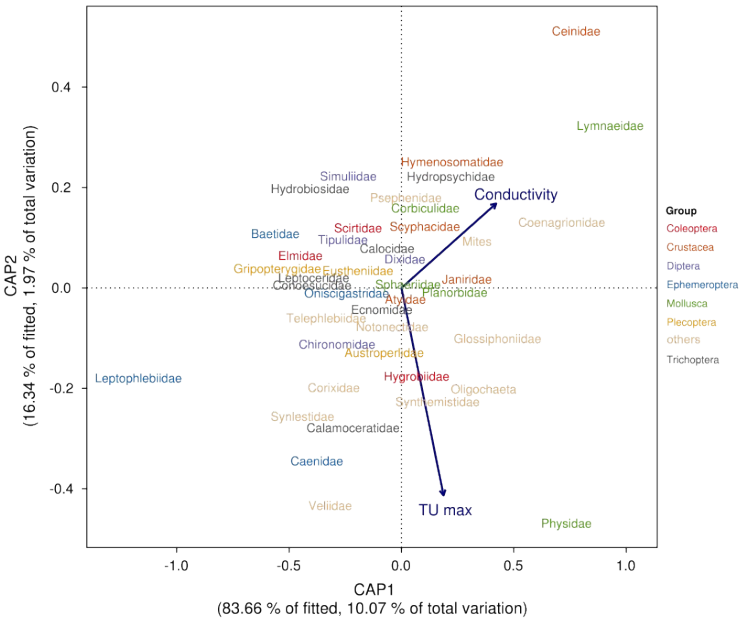

Fig. 2. Distance based redundancy analysis of salinity and pesticide toxicity, see Fig. 1 for details.

interactions between pesticides and salinity. The sampling was conducted only semi-quantitatively and the taxonomic resolution was homogenized at the family level. A quantitative sampling (i.e. surber sampling) with species level taxonomic resolution could reveal interaction effects.

Overall, controlled experiments like stream mesocosms isolating the two factors salinity and pesticides would be required for a deeper understanding of the underlying mechanisms.

5. Conclusions

Salinization and exposure to pesticides can be important factors for the structure of macroinvertebrate communities in agricultural regions. In the region and year studied, salinity was more important than pesticide toxicity for community composition. No interaction between salinity and pesticide toxicity was apparent: therefore we suggest no stronger effects of pesticides when used in salinization-prone regions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2012.07.066.

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12. Publication XI

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Biodiversity, ecosystem functions and services in environmental risk assessment: Introduction to the special issue

Ralf B. Schäfer *

Institute for Environmental Sciences, University Koblenz-Landau, Fortstraße 7, 76829 Landau, Germany

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ABSTRACT

This Special Issue focuses on the questions if and how biodiversity, ecosystem functions and resulting services could be incorporated into the Ecological Risk Assessment (ERA). Therefore, three articles provide a framework for the integration of ecosystem services into ERA of soils, sediments and pesticides. Further articles demonstrate ways how stakeholders can be integrated into an ecosystem service-based ERA for soils and describe how the current monitoring could be adapted to new assessment endpoints that are directly linked to ecosystem services. Case studies show that the current ERA may not be protective for biodiversity, ecosystem functions and resulting services and that both pesticides and salinity currently adversely affect ecosystem functions in the field. Moreover, ecological models can be used for prediction of new protection goals and could finally support their implementation into the ERA. Overall, the Special Issue stresses the urgent need to enhance current procedures of ERA if biodiversity, ecosystem functions and resulting services are to be protected.

Ecosystems deliver various goods and services to human societies such as clean water, food, recreation and spiritual values (Costanza et al., 1997; MEA, 2005). These ecosystem services are the product of ecosystems functions that are considered beneficial to human societies. They usually result from complex interactions between and within abiotic (environment) and biotic (species) components of ecosystems; the frameworks for evaluating ecosystem services explicitly include the role of humans in abiotic/biotic interactions. In general, a reduction in the diversity of species has been linked to a decrease in ecosystem functions and consequently services (Cardinale et al., 2006; Hooper et al., 2005; MEA, 2005), which emphasises the relevance of maintaining biodiversity. However, human land and resource use, especially in the last century (Steffen et al., 2005), have caused dramatic changes in ecosystems that were often associated with a decline in the abundance of many species (Secretariat of the Convention on Biological Diversity, 2010). This compromises the provisioning of ecosystem services with many services facing degradation due to unsustainable use (MEA, 2005). Hence, there is an urgent need to incorporate the effects of human activities on biodiversity, ecosystem functions and services into the ecological risk assessment (ERA) framework, which is discussed in this Special Issue. It consists of several articles on the topic "Biodiversity, ecosystem functions and services in Ecological Risk Assessment" that are associated with a corresponding Session on the 20th Annual Meeting of the Society for Environmental Toxicology and Chemistry (SETAC) Europe in Seville 2010. Although most of the contributions to this Special Issue share a focus on agricultural ecosystems, the topic of this Special Issue is not limited to these types of ecosystems.

The Special Issue commences with three articles presenting a framework for the integration of ecosystem services into the ERA for soils (Faber and Van Wensem, 2012–this issue) and for two specific stressors in aquatic ecosystems, i.e. sediments (Apitz, 2012–this issue) and pesticides (Nienstedt et al., 2012–this issue). Faber and Van Wensem (2012–this issue) describe the scientific and political developments that promote the integration of ecosystem services in ERA, which should be realised by selecting reliable assessment endpoints providing a clear link to ecosystem services. This is outlined exemplarily for the ERA of soil ecosystem services. Apitz (2012–this issue) stresses the importance of beneficial and adverse effects of sediments on species and hence the ecosystem services they provide. Moreover, she suggests frameworks for the identification of exposures associated with human activities (on land and within aquatic ecosystems) and effect endpoints for the Sediment-Ecological Regional Assessment (SEcoRA). The quantity, quality, transport and location of sediments should be considered in the assessment, as well as the multi-scale aspects of landscape/sediment/ecosystem interactions. In the contribution of Nienstedt et al. (2012–this issue) the derivation of specific protection goals for the ERA of pesticides is described. The authors suggest that the seven groups of taxa that are known as the key drivers of ecosystem services are included in the ERA of pesticides. Moreover, they suggest that protection goals are defined in terms of the ecological entities, spatial and temporal scales as well as in terms of the magnitudes of effects.

* Tel.: +49 6341 280 31536; fax: +49 6341 280 31326.
E-mail address: schaefer-ralf@uni-landau.de.

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The two articles by Rutgers et al. (2012–this issue) and van Wijnen et al. (2012–this issue) outline a different approach on how to define ecosystem services in that they include stakeholders in the process. The authors interviewed stakeholders from four farms and defined site-specific ecosystem services that were later compared to reference situations. Generally, soil ecosystem services in the four farm sites were relatively degraded compared to the reference sites. The authors describe land management measures that were implemented to improve the soil ecosystem services. Maps played an important role in the whole process and the methodology to use maps to communicate degradation of ecosystem services is detailed in van Wijnen et al. (2012–this issue).

All five papers have in common that they suggest new indicators and assessment endpoints for ecosystem services within an ERA context or for the assessment of ecological quality. This would also require changes in monitoring approaches and the article of Chapman (2012–this issue) outlines a monitoring framework based on ecosystem services. Such monitoring should adapt to new assessment endpoints but should not compromise the continuity of long-term data records. Chapman (2012–this issue) describes the points to consider when setting up a monitoring programme and provides suggestions on how to integrate ecosystem service endpoints into such a monitoring programme.

Most of the articles in this Special issue highlight the importance of defining the spatial scale at which biotic or abiotic endpoint should be protected. The article by Kefford et al. (2012–this issue) asks whether ecosystem functions or services are likely to be protected using current ERA frameworks which focus on the protection of species at a regional spatial scale (e.g. species sensitivity distributions (Posthuma et al., 2002)). Given that the traits of organisms have been suggested as links between species and ecosystem functions and services (Bello et al., 2010; McGill et al., 2006), this study investigates whether the protection of taxonomic structures on the regional scale also protects trait structures on the local scale. The study suggests that ERAs for salinity and turbidity based on SSDs (Posthuma et al., 2002) or relative species retention (Kefford et al., 2010) do not necessarily protect the trait structures and hence ecosystem functions and services and that other assessment approaches are needed to reach these protection goals.

The study of Schäfer et al. (2012–this issue) describes the responses of the ecosystem functions allochthonous organic matter breakdown and stream metabolism to the stressors pesticide exposure and elevated levels of salinity in 24 stream sites in southeast Australia. Both stressors lead to decreases in the ecosystem function allochthonous organic matter breakdown whereas no effects were detected on the function of stream metabolism. The authors suggest that the observed effects on ecosystem functions can impair important ecosystem services of freshwater ecosystems. Similarly, the article of Boutin et al. (Boutin et al., 2012–this issue) raises concerns over whether the current ERA guidelines for herbicides protect biodiversity and the ecosystem services associated with terrestrial primary producers. They review existing data and present new data on the effects of herbicides on wild plants and conclude that the current ERA approach does not guarantee the protection of biodiversity of wild species and their ecosystem functions and services. The inclusion of test species selected based on traits rather than only crop species and the consideration of other ecologically relevant test endpoints may be a first step in order to achieve a better protection of these goals.

However, not all combinations of stressors and endpoints are amenable to testing in the laboratory or field and therefore the article of Galic et al. (2012–this issue) examines to what extent ecological modelling can help to predict effects of stressors on new protection goals such as ecosystem services. The authors use different case studies to demonstrate how ecological models may enhance the ERA of agro-ecosystem services as well as to tackle the issue of trade-offs between services. They highlight the possibility of ecological models to obtain information on endpoints that are not measurable or are difficult to measure.

Overall, the articles in this Special issue (1) propose frameworks and methods to integrate ecosystem services and biodiversity within ERA, ecological monitoring and ecological modelling and (2) highlight that current risk assessment practice may not protect biodiversity, ecosystem functions and services. It is hoped that this collection of papers will stimulate further discussion; it is critical that ERA continues to evolve if we are to use it to protect biodiversity, ecosystems and the services upon which we depend.

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13. Publication XII

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Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services

Ralf B. Schäfer [a,*], Mirco Bundschuh [a], Duncan A. Rouch [b], Eduard Szöcs [a], Peter C. von der Ohe [c], Vincent Pettigrove [d], Ralf Schulz [a], Dayanthi Nugegoda [b], Ben J. Kefford [e]

[a] Institute for Environmental Sciences, University Koblenz-Landau, Forststraße 7, 76829 Landau, Germany
[b] Biotechnology and Environmental Biology, School of Applied Sciences, RMIT University, PO Box 71, Bundoora, Victoria 3083, Australia
[c] UFZ-Helmholtz Centre for Environmental Research, Department Effect-Directed Analysis, Permoser Straße 15, 04318 Leipzig, Germany
[d] Centre for Aquatic Pollution Identification & Management, The University of Melbourne, 30 Flemington Road, Parkville, Victoria 3052 Australia
[e] Centre for Environmental Sustainability, Department of Environmental Science, University of Technology Sydney, P.O. Box 123, Broadway NSW 2007, Australia

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ABSTRACT

Effects of anthropogenic and environmental stressors on freshwater communities can propagate to ecosystem functions and may in turn impede ecosystem services. We investigated potential shifts in ecosystem functions that provide energy for freshwater ecosystems due to pesticides and salinity in 24 sites in streams of southeast Australia. First, effects on allochthonous organic matter (AOM) breakdown using three different substrates (leaves, cotton strips, wood sticks) in coarse and fine bags were investigated. Second, we examined effects on stream metabolism that delivers information on the ecosystem functions of gross primary production and ecosystem respiration. We found up to a fourfold reduction in AOM breakdown due to exposure to pesticides and salinity, where both stressors contributed approximately equally to the reduction. The effect was additive as, no interaction or correlation between the two stressors was found. Leaf breakdown responded strongly and exclusively to exposure to pesticides and salinity, whereas cotton strip breakdown was less sensitive and responded also to other stressors such as nutrients. No functional redundancy for the effects of pesticides and salinity on leaf breakdown was observed. For wood stick breakdown, no relationship to environmental gradients was found, however, the sample size was lower. We did not detect effects of pesticides or salinity on gross primary production or ecosystem respiration. A reduction in AOM breakdown by pesticides and salinity may impair the ecosystem services of food provision and possibly water purification. Hence, future studies should examine the spatial extent of these effects.

1. Introduction

Freshwater ecosystems deliver various goods and services for human societies such as clean water, food (e.g. fish), purification of wastes, recreation and spiritual values. However, freshwater biota are severely threatened as outlined in the Millennium Ecosystem Assessment (MEA) which identified among others organic pollution, heavy metals and pesticides as anthropogenic stressors of major importance (MEA, 2005). For most anthropogenic stressors it is unclear to which extent effects on freshwater biota (structural changes) propagate to effects on ecosystem functions and potentially ecosystem services (Covich et al., 2004; MEA, 2005).

Organic matter represents the basic energy for ecosystems and is mainly provided by the ecosystem functions of organic matter breakdown and primary production via photosynthesis. In freshwater ecosystems, these functions deliver organic matter resulting from (1) the breakdown of allochthonous organic matter (AOM) and (2) the photosynthesis or breakdown of aquatic biota (autochthonous organic matter) (Tank et al., 2010; Webster, 2007). Since a proportion of the organic matter in lotic ecosystems is exported downstream, both ecosystem functions deliver energy for local as well as downstream food webs (Allan and Castillo, 2007; Webster, 2007). Hence, any alteration in one or both of these functions may also propagate downstream (Delong and Brusven, 1994; Wallace et al., 1997).

Macroinvertebrates (especially shredders) and microorganisms (bacteria and fungi) are the main decomposers of AOM (Graca et al., 2001; Hieber and Gessner, 2002). To determine the ecosystem function of AOM breakdown, breakdown of leaves, cotton strips or wood sticks were suggested as measures (Young and Collier, 2009). For these three measures, with a few exceptions, only leaf breakdown has been investigated with respect to anthropogenic stressors (Young et al., 2008). The leaf breakdown is especially inhibited by toxicants, whereas other stressors such as excess nutrients may increase breakdown (Gessner and Chauvet, 2002; Young et al., 2008).

* Corresponding author. Tel.: +49 6341 280 31536; fax: +49 6341 280 31326.
E-mail address: schaefer-ralf@uni-landau.de (R.B. Schäfer).

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While the AOM breakdown is mainly relevant for first- to fifth-order streams, the relevance of photosynthesis as energy source increases along the stream network (Vannote et al., 1980). For example, a study of Webster (2007) assigned approximately 81% of available organic carbon of the whole stream network of a medium-sized US river to gross primary production (GPP), primarily in the lower reaches. The contribution of GPP to the local energy budget can be estimated by dividing GPP by the ecosystem respiration (ER) (Tank et al., 2010). GPP and ER can be calculated by measuring the stream metabolism, for which several methods are available (Tank et al., 2010). Despite the major importance of GPP for freshwater ecosystems, only few studies have examined potential adverse effects of anthropogenic stressors on this ecosystem function (Gücker et al., 2009; Tank et al., 2010), except for several studies on the effects of agricultural land-use on GPP (Bernot et al., 2010; Gücker et al., 2009; McTammany et al., 2007; Young and Huryn, 1999).

Pesticides represent an important stressor for freshwater ecosystems and can impact all groups of organisms (Liess et al., 2008; Schäfer et al., 2011c). Nevertheless, to date only one field study examined the relationship between leaf breakdown and estimated site specific pesticide toxicity as derived from measured pesticide concentrations (Schäfer et al., 2007). This study found a reduction in leaf breakdown by invertebrates with increased pesticide toxicity (Schäfer et al., 2007). We are not aware of a field study on the effects of pesticides on the breakdown of cotton or wood on stream metabolism.

Beside the input of pesticides, agriculture in arid and semi-arid regions such as the Middle East, central Asia and southeast Australia is also a leading cause for anthropogenic salinisation that can result in a rise of electrical conductivity (EC) from below 500 µS/cm to several thousand µS/cm in freshwater ecosystems (Williams, 1987). Additionally salinity can be elevated by saline effluent from industry or mining (Piscart et al., 2005). Although changes in conductivity to several thousand µS/cm affect all major groups of freshwater biota (Hart et al., 1990), the consequences for ecosystem functions such as AOM breakdown or GPP are largely unknown (Gutierrez-Canovas et al., 2009).

In the present study, we investigated whether these two stressor pesticides and salinisation affect the ecosystem functions of AOM breakdown, GPP and ER. Furthermore, we compared different methods for the determination of the breakdown of AOM with respect to their sensitivity for both stressors. Finally, we assessed the relevance of observed effects on ecosystem functions for associated ecosystem services.

2. Methods

2.1. Study design and sampling schedule

The study was conducted in 24 sites in streams in an agriculturally dominated region of southern Victoria in southeast Australia (Fig. 1). The streams were selected to exhibit a gradient in the exposure to pesticides and salinity. The sampling was scheduled for the expected main time of pesticide application in spring and summer of 2008/2009 and encompassed six pesticide samplings, two times monitoring of leaf and cotton strip breakdown and one monitoring of wood breakdown and stream metabolism (Fig. 2). Due to weather extremes during the summer 2008/2009 in Victoria, Australia (0 mm precipitation and highest temperatures on record in 120 years across several regions between 1/1/2009 and 28/2/2009) and due to catastrophic forest fires (Schäfer et al., 2010), some stream sites fell dry or were not accessible so that the samplings in February and March comprised only 16 of the 24 sites (BOM, 2009a,b,c). This lead to a reduced sample size for the monitoring methods employed in this period (Fig. 2, Appendix Table A.1). The study presented here was complemented by a study on the effects of pesticides on macroinvertebrates and densities of microorganisms, and these results as well as further details on the sampling sites and region are given in Schäfer et al. (2011b).

2.2. Pesticide monitoring and recording of environmental variables

A total of 97 insecticides, herbicides and fungicides were monitored in the sampling period from September 2008 to March 2009 (Fig. 2) using grab water sampling, sediment sampling and passive sampling with trimethylpentane passive samples (TRIMPS) (Leonard et al., 2002). A detailed overview of the substances, sampling methods, chemical analysis and pesticide detections is described elsewhere (Schäfer et al., 2011b). The pesticide concentrations were

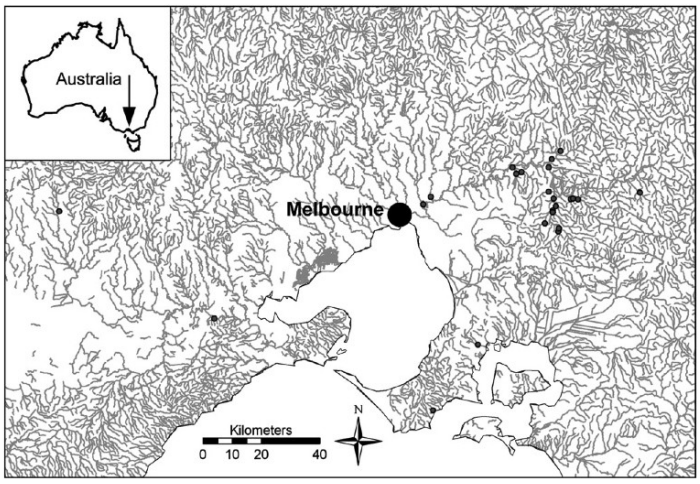

Fig. 1. Location of the sampling sites (small dots) in the stream network in the region around Melbourne (large dot) in Victoria, Australia.

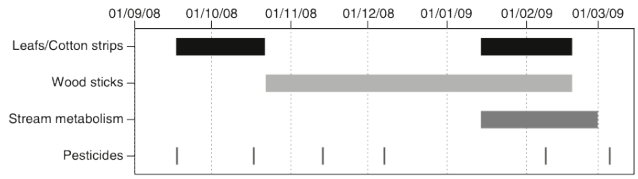

Fig. 2. Schedule for the monitoring of leaf and cotton strip breakdown, wood stick breakdown and stream metabolism as well as pesticide sampling. Bars indicate the period of substrate or logger deployment in the 24 sites. For pesticide sampling, the bars refer to the time points of sediment and water sampling or retrieval of continuous TRIMPS passive samplers (see Schäfer et al., 2011b for details on pesticide sampling).

used to estimate the toxicity in each site in terms of the maximum toxic unit (mTU):

$$\mathrm{mTU} = max_{i=1}^{n}\left(\frac{c_i}{\mathrm{EC50}_i}\right) \quad (1)$$

where c_i is the concentration of pesticide i, $\mathrm{EC50}_i$ is the corresponding 48-h to 96-h median effect concentration for a given standard test species and n is the number of pesticide detections in the site. The standard test species were selected with respect to the organism groups that are involved in the ecosystem functions investigated in this study, which were macroinvertebrates (AOM breakdown), microorganisms (AOM breakdown) and primary producers (GPP). Therefore, *Daphnia magna* and *Selenastrum capricornutum* were selected as standard test species for macroinvertebrates and primary producers, respectively. In addition, the predictions of both species were used as measure for microorganisms, as for the study compounds no sufficient toxicity data for microbial (e.g. *Vibrio fischeri*)

Table 1
Maximum (Max.), minimum (Min.), median, mean, % standard deviation (SD), potential transformations (Trans.) and category for the biotic and environmental variables.

Variable[a]	Min.	Max.	Median	Mean	%SD	Trans./category[b]
$k_{leaves\ invertebrates}$ ($dday^{-1}$)	0.0002	0.0016	0.0008	0.0008	48	n/Ef (AOM)
$k_{leaves\ microorganisms}$ ($dday^{-1}$)	0.001	0.002	0.0016	0.0015	19	n/Ef (AOM)
$k_{cotton\ invertebrates}$ ($dday^{-1}$)	0.00001	0.00026	0.00009	0.0001	82	n/Ef (AOM)
$k_{cotton\ microorganisms}$ ($dday^{-1}$)	0.00007	0.0003	0.00016	0.00017	36	n/Ef (AOM)
$k_{wood\ sticks}$ ($dday^{-1}$)	0.00004	0.00033	0.00017	0.00019	48	n/Ef (AOM)
Ergosterol (μg g^{-1})	0.01	123	3	29	133	log/Ef (Ass.)
Functional microbial richness (after 72 h)	13	26	20	21	18	n/Ef (Ass.)
GPP (mg O_2 L^{-1} d^{-1})	1	44	5	7	147	n/Ef (GPP)
ER (mg O_2 L^{-1} d^{-1})	3	101	19	28	93	n/Ef (ER)
T (°C)	11.9	19.3	15.2	15.4	10	n/PC
pH	6.7	8	7.2	7.3	5	n/PC
EC at 25 °C (μS cm^{-1})	47	3563	181	798	139	log/PC
Dissolved oxygen (% sat.)	22	87	73	72	21	n/PC
NH4[c] (mg L^{-1})	0.2	4.4	0.4	0.7	132	log/PC
NO2[c] (mg L^{-1})	0.001	0.9	0.005	0.098	232	log/PC
NO3[c] (mg L^{-1})	0.1	4	0.48	0.62	131	log/PC
PO4[c] (mg L^{-1})	0.15	21	9	8.6	66	log/PC
Alkalinity (mmol)	0.9	13.4	2	3.7	91	n/PC
Turbidity (NTU)	3.7	18.9	8	9.1	49	n/PC
Depth (m)	0.08	0.87	0.33	0.38	58	n/PC
Current velocity (m s^{-1})	0.01	0.35	0.15	0.16	73	n/PC
PAR (mol m^{-2} d^{-1})	2.71	24.8	8.09	9.12	61	n/PC
Width left bank (m)	3	50	10	15	81	log/Geo
Width right bank (m)	3	35	15	16	65	log/Geo
Pool sections (%)	20	100	96	88	24	n/Habitat
Bedrock (%)	0	23	0	1	345	n/Habitat
Boulder (%)	0	20	0	4	164	n/Habitat
Cobble (%)	0	20	4	7	107	n/Habitat
Pebble (%)	0	25	1	7	133	n/Habitat
Gravel (%)	0	30	6	9	104	n/Habitat
Sand (%)	5	50	15	20	65	n/Habitat
Clay (%)	10	95	44	52	63	n/Habitat
$mTU_{D.\ magna}$	0.0003	0.57	0.008	0.049	242	log/PC
$mTU_{S.\ capricornutum}$	0.00002	1.76	0.0078	0.18	244	log/PC
Recovery section[d]	0	1	0	n	n	n/Geo
Left bank cover[d]	1	5	4	n	n	n/Geo
Right bank cover[d]	1	5	4	n	n	n/Geo
Shading[d]	1	5	3	n	n	n/Habitat
Filamentous algae[d]	0	3	1	n	n	n/Habitat
Total macrophytes[d]	1	4	2	n	n	n/Habitat
Coarse particular organic matter[d]	1	3	2	n	n	n/Habitat

[a] See EPA (2003) for details on the measurement of habitat, geographical and physicochemical variables.
[b] Transformation: n = no; log = log-transformed. Category: Ef = ecosystem function; AOM = allochthonous organic matter; GPP = gross primary production; ER = ecosystem respiration; Ass. = associated with ecosystem function AOM breakdown; PC = physicochemical variable; habitat = habitat variable; Geo = geographical variables.
[c] NH4 = ammonium; NO2 = nitrite; NO3 = nitrate; PO4 = phosphate.
[d] Ordinal variables classifying the prevalence/coverage from 0 (absent) to 5 (very high), except for Recovery section where 1 refers to the presence of undisturbed upstream sections, else 0. See Schäfer et al. (2011b) for details.

standard test species were available. For further details on the compilation of toxicity data and site-specific results for toxic units see Schäfer et al. (2011b).

Environmental parameters were recorded in concert with the pesticide sampling in September, November and February (Fig. 2) and included physicochemical, landscape and habitat variables (Table 1). Temperature, pH, salinity as EC and dissolved oxygen were measured in the field with a TPS FL90 (Brisbane, Australia) water quality metre. Ammonium, nitrite, nitrate and phosphate concentrations were determined on site using a HI 83200 photometer (Hanna Instruments, Melbourne, Australia) with the respective reagents. We used a Hach 2100P turbidimeter (Loveland, USA) to measure turbidity in the field and an Aquamerck (Merck, Melbourne, Australia) test kit to measure alkalinity. Further habitat and landscape variables (Table 1) were recorded with a ruler, visual inspection or maps according to protocols of the Environment Protection Authority (EPA) Victoria (EPA, 2003). Summary statistics for all environmental variables are given in Table 1, site-specific information are reported in Schäfer et al. (2011b).

2.3. Determination of AOM breakdown rates

We determined the breakdown of leaves, cotton strips and wood sticks in order to compare different methods that have been suggested for the assessment of AOM breakdown (Tiegs et al., 2007; Young and Collier, 2009). For leaf breakdown, *Eucalyptus camaldulensis* leaves from a locally common riparian tree that were prior to abscission were collected in spring and oven-dried (48 h at 60 °C). Approximately 2.5 g of dried leaves was placed into coarse polyethylene mesh bags (mesh size: app. 6 mm; bag size: 20×20 cm) and into fine cylindrical nylon bags (mesh size: 50 µm; cylinder length: 15 cm). Leaves in the coarse bags were accessible to invertebrates and microorganisms, whereas leaves in the fine bags were accessible for microorganisms only and served as control for microbial degradation and leaching (Gessner and Chauvet, 2002). The fine bags consisted of two separate sections, where one section contained the 2.5 g of leaves and the second section contained leaves that were used to estimate the fungal biomass and functional groups of microorganisms according to the carbon sources metabolised by them (see below). Triplicate coarse and fine bags were deployed in each site approximately 10 cm above the stream bed so that they touched the bottom. The bags were retrieved after approximately 5 weeks (Fig. 2). The remaining litter was carefully taken out, washed to remove deposits, oven-dried at 60 °C (48 h), reweighed and averaged for each type of bag for every site. To correct for handling losses three coarse and fine bags were treated the same way as the others but returned immediately to the laboratory after a brief immersion in the stream. Physical abrasion may also contribute to leaf breakdown but was not measured. However, a study on streams in the same region reported only minor influence (3–7%) of physical abrasion on the leaf weight mass loss (Imberger et al., 2008).

For cotton strip breakdown, unbleached standardised cotton was obtained from EMPA (St. Gallen, Switzerland), cut into 5×10 cm strips and autoclaved for 1 h at 120 °C. Subsequently, one cotton strip was placed in the coarse bags and in each section of the fine bags. After retrieval the cotton strips were cleaned, soaked in 70% ethanol for a few minutes to inhibit microbial decay during storage, air dried and stored at −18 °C. Three strips were treated the same way as the others but returned immediately to the laboratory after a brief immersion in the stream to serve as control for handling losses. Tensile strength was measured using an Instron Series IX Tensiometer (Instron, Melbourne, Australia) after cutting 1×5 cm strips from the centre of the cotton strips. The instrument parameters were: 3 mm s^{-1} crosshead speed, 23 °C temperature and 50% relative humidity.

Birch wood ice cream sticks (length: 12 cm, width: 1 cm, depth: 0.2 cm) were used to determine wood breakdown. They were weighed after drilling a hole in the stick that allowed for securing with wire. In each sampling site four sticks were tied to the stream bottom using a metal peg in the same spot where the coarse and fine bags were deployed. The sticks were retrieved after approximately 3.5 months, cleaned, oven-dried at 60 °C (24 h) and re-weighed. Triplicate wood sticks were treated as outlined but returned immediately to the laboratory after a brief immersion in stream water to correct for handling losses. A disadvantage of wood sticks is their long deployment period (Fig. 2) that is owed to the much lower breakdown rate in comparison with leaves or cotton (Webster et al., 1999). In our study this lead to major losses of samples resulting in the recovery of only 9 samples after the deployment period (Table 2), thus decreasing the statistical power to detect relationships with environmental gradients.

Temperature loggers (Hobo Pendant, Onset, Pocasset, USA) with hourly temperature recording were deployed in concert with the bags and wood sticks. The temperature data was used for the calculation of the sum of degree days (dday) for the deployment period of leaves, cotton strips and wood sticks in order to standardise the breakdown rates for temperature. The breakdown rate k for each of the three substrates in a site i was calculated based on the exponential mass loss or tensile strength loss per dday:

$$k_i = \frac{-\ln\left(\frac{S_i(t)}{S_i(0)}\right)}{\sum_{j=1}^{t} \overline{T_i}(j)} \quad (2)$$

where S is the mass or tensile strength as a function of the deployment time, t being the total number of deployment days and T is the mean temperature for a day j. $S_i(t)$ was corrected for handling losses. In

Table 2
Environmental variables (see Table 1 for full variable names) with highest explanatory power for biotic response variables with % contribution in hierarchical partitioning, r^2 and Bayesian Information Criterion (BIC) for the best-fit model and sample size n.

Response variable	$mTU_{D.\ magna}$[a]	$mTU_{S.\ capri.}$[a,b]	EC (µS cm^{-1})[a]	Sand (%)	PO4[d] (mg/L)[a]	T (°C)	r^2	BIC	n
$k_{leaves\ invertebrates}$	40		60				0.67	−378	23
$k_{leaves\ microorganisms}$[c]		32 (35)	68 (65)				0.74 (0.56)	−380 (−384)	22 (23)
$k_{cotton\ invertebrates}$[c]	41 (52)				59 (48)		0.63 (0.38)	−433 (−436)	22 (23)
$k_{cotton\ microorganisms}$		47		53			0.44	−450	23
$k_{wood\ sticks}$									9
Functional microbial richness[c]	100						0.44 (0.30)	28 (38)	14 (15)
Ergosterol concentration[a,c]			100				0.74 (0.61)	1 (6)	14 (15)
GPP[c]						100	0.59 (0.41)	23 (68)	14 (16)
ER									16

[a] Variable log-transformed in linear model.
[b] *capri.* = *capricornutum.*
[c] Values in brackets give the result for inclusion of observations that exhibited unduly influence according to Cook's distance.
[d] PO4 = phosphate.

addition, $S_i(t)$ of leaves and cotton strips in coarse bags was corrected for losses due to microbial degradation and leaching in site i to determine the contribution of invertebrates to breakdown (for details see Benfield, 2007).

2.4. Estimation of fungal biomass and microbial carbon source use

We determined the fungal biomass and the richness of carbon source use by microorganisms (in the following called functional microbial richness) to allow for an attribution of potential effects on AOM breakdown to changes in the microbial community (Hieber and Gessner, 2002; Stefanowicz, 2006). Leaf-associated fungal biomass was estimated by measuring ergosterol, which is a component of the fungal cell membrane. This was done according to a method developed by Gessner and Schmitt (1996) using leaves from the second section of the fine bags. Briefly, ergosterol was extracted from the leaves in 10 mL alkaline methanol at 80 °C for 0.5 h and then purified by solid-phase extraction using 500 mg Sep-Pac® Vac RC tC18 cartridges (Waters, Eschborn, Germany). Separation of ergosterol was done on an Agilent 1200 Series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Waldbronn, Germany) equipped with a LiChrospher 100 RP18 column (CS-Chromatographie Service, Langerwehe, Germany). Subsequently, ergosterol was measured at a wavelength of 282 nm with an ultraviolet detector and quantified using a standard curve prepared with the respective chemical standard (Fluka, purity 97.8%).

The carbon source use of the microbial community was assessed using 96-well Biolog EcoPlates™ (Biolog, CA, USA) that consisted of triplicated 31 different carbon sources and water blanks (Appendix Table A.2) (Garland, 1996; Stefanowicz, 2006). For each site, 10 g of wet leaves, the cotton strip from the second section of the fine bags and one randomly selected wood stick were placed in a sterile 250 mL glass bottle containing 90 mL of the maximum recovery diluent CM0733 (Oxoid, Adelaide, Australia) and 10 g of glass beads. The bottle was placed on a rotary shaker and mixed at 400 rpm for 4 min. 100 μL samples of the supernatant were inoculated into each well of the Ecoplate™. Subsequently the plates were incubated at 20 °C for 72 h. Absorption was measured at 595 nm in a plate reader. For each site, the absorption was corrected by subtraction of the absorption from (1) the water blank and (2) the respective carbon source for control leaves, cotton strips and wood sticks that were not deployed. Carbon sources with a statistically significant higher absorption than the water blank of the respective plate in Dunnett's test were considered as being used by microorganisms. The p-values in Dunnet's test were adjusted for multiple testing according to a method developed by Benjamini and Hochberg (1995). The number of carbon sources used by microorganisms were summed per site and used as variable in data analysis.

2.5. Estimation of stream metabolism

Stream metabolism was estimated with the single-station open-channel method as outlined in Grace and Imberger (2006) and Young and Collier (2009). This method relies on the continuous measurement of dissolved oxygen (DO) concentrations over a minimum period of 24 h at a site. In our study, we used three D-Opto (Zebratech, Nelson, New Zealand) DO loggers that were circulated between the sampling sites from middle of January to end of February 2009 (Fig. 2). In each site, DO loggers were installed in the middle of the water column and DO was measured in 10 minute intervals for a period of at least 72 h. An Odyssey photosynthetically active radiation (PAR) recording system 64 k (Dataflow Systems, Christchurch, New Zealand) was attached to each DO logger and recorded PAR every 10 min. Sites were monitored for at least one cloudless day to minimise variability due to differences in light exposure. Calculation of GPP and ER from the DO concentrations was based on the R software package StreamMetabolism (Sefick, 2009). This package computes temperature corrected GPP and DO from the diel oxygen curve for the open station method and requires the re-aeration coefficient K as well as temperature and oxygen as input data. In the current version of this software package (Sefick, 2009), K was estimated from the empirical O'Connor Dobbins surface renewal method that relies on hydromorphological parameters (O'Connor and Dobbins, 1958). Given the uncertainties related to methods based on hydromorphological parameters (Aristegi et al., 2009), we also implemented the nighttime regression method to estimate K (Grace and Imberger, 2006; Owens, 1974) (see Appendix B). The nighttime regression did not yield statistically significant regression estimates ($p>0.05$) of K for 9 of the 16 sites that were monitored (see Section 2.1), which is higher than in another study that did not find significant regression estimates for only 3 out of 18 sites (Aristegi et al., 2009). This may be explained by the fact that 6 of the 9 sites where the nighttime regression method failed were low productivity sites ($GPP \approx 1$ mg O_2 L^{-1} d^{-1}), which presumably had insufficient variation in the oxygen deficit and change in oxygen concentration (Grace and Imberger, 2006). Indeed, the sites in our study with a GPP <2.4 mg O_2 L^{-1} d^{-1} did not yield a significant nighttime regression (Appendix Table A.1). However, since for sites with statistically significant nighttime regression the estimated reaeration coefficients of the nighttime regression method and the O'Connor Dobbins method were in reasonable agreement ($r^2 = 0.8$; Appendix Fig. A.1), the O'Connor Dobbins method was used to estimate K for all 16 sites.

2.6. Data analysis

Before analysis, the data for all variables was aggregated per site using the mean, as the sampling periods of the different methods differed (Fig. 2). Variables with a wide spread of values (maximum/minimum observation >100) or a very skewed distribution (checked visually) were log-transformed (Table 1). Linear models were used to examine the relationship between environmental variables and the response variables related to ecosystem functions, fungal biomass and functional microbial richness. We employed automatic model building to identify the environmental variables with the highest explanatory power for the respective response variable. Automatic model building started with the null model (no explanatory variable included) and used backward and forward entering variables with the Bayesian Information Criterion (BIC) as goodness-of-fit measure to identify the best-fit linear model. In addition, manual model building was used to check the results of the automatic modelling procedure (Sheather, 2009). Here, we started with models based on expert judgement and used the t-test for testing the significance of individual variables and the partial F-test for testing for significant differences during model simplification. However, automatic model building and manual model building lead to identical best-fit models.

Statistical models were checked for error assumptions (constant variance, non-correlation and normality of residuals) and unusual observations (leverage, outliers) (Sheather, 2009). A variable cluster analysis was conducted before modelling to identify pairs of abiotic variables with high intercorrelation (Pearson's $r>0.7$), where the variable with lower relevance for the respective response variable was omitted based on expert judgement. Hierarchical partitioning was used to determine the independent explanatory power of environmental variables in the best-fit models (Chevan and Sutherland, 1991; Grömping, 2006). To assess the relationship of the response variables with estimated pesticide toxicity and salinity, we built linear models for every response variable with each mTU and conductivity as explanatory variables. In addition, linear models with both explanatory variables and their interaction term were constructed to test for a potential interaction of pesticides and salinity.

Nonmetric Multidimensional Scaling (NMDS) (function metaMDS in the R package "vegan" (Oksanen et al., 2010)) was employed to

examine the similarity in microbial carbon source use between sites. The Sorensen index (Sorensen, 1948) was selected as similarity measure and a two-dimensional NMDS was started a maximum of 20 times with random configurations to find the global solution. The model with the lowest stress-value was regarded as the best-fit model. Since NMDS is an unconstrained ordination method, environmental variables were fitted afterwards (function envfit in the R package "vegan" (Oksanen et al., 2010)) in order to explore the relationship between gradients in microbial carbon source use and environmental variables. The fitted variables were selected based on the significant correlation ($p<0.05$) with the ordination that was assessed using 10,000 random permutations. All statistical computations and graphics were created with the open source software package R (www.r-project.org) using version 2.12.1 (for Mac OS X, 10.6.6) (R Development Core Team, 2011).

3. Results

3.1. Relationship between environmental variables and biotic endpoints

Of all the 32 environmental variables (Table 1), six mainly physicochemical variables such as the estimated pesticide toxicity ($mTU_{D.\ magna}$ and $mTU_{S.\ capricornutum}$), EC or phosphate concentrations were selected as best predictors for the different biotic endpoints (Appendix Table A.1), whereas habitat and geographical variables had minor explanatory power (see Table 2 for linear models, Fig. 3 for NMDS). Variation in leaf breakdown and the associated endpoint fungal biomass and functional microbial richness were best explained by the estimated pesticide toxicity and salinity in terms of EC (Table 2). For cotton strip breakdown, the % of sand in the habitat as well as nutrients in the form of phosphate concentrations exhibited strong explanatory power beside the estimated pesticide toxicity (Table 2). No reasonable linear model could be established for the breakdown of wood sticks in either manual or automatic model building. However, with only 9 sites due to losses of sticks at some sites, the statistical power was reduced (Appendix Table A.1). The physicochemical variables $mTU_{D.\ magna}$, EC, temperature and turbidity exhibited the closest correlation with the two-dimensional NMDS ordination for the carbon source use of microorganisms (Fig. 3). The ecosystem function of GPP displayed the highest correlation with temperature and no other variable was included in the best-fit model. For ER, no model was found with a good fit (Table 2).

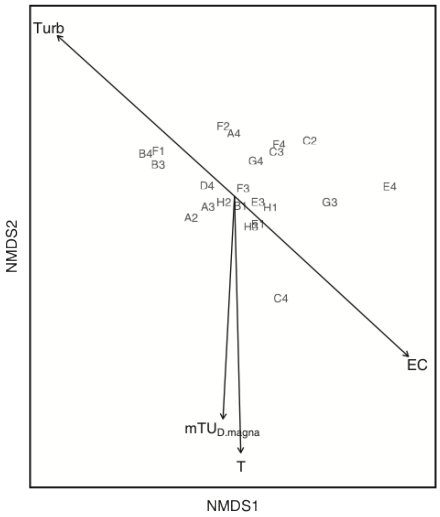

Fig. 3. Two-dimensional nonmetric multidimensional scaling for the carbon source use of microorganisms colonising AOM in the sampling sites with fitted environmental variables that exhibited a significant correlation with the gradients ($p<0.05$). The stress value was 9.5%. The r^2 after 10,000 permutations for log-transformed electrical conductivity (log EC), temperature (T), log-transformed maximum toxic units for *D. magna* ($mTU_{D.\ magna}$) and turbidity was 0.53 ($p=0.007$), 0.65 ($p=0.006$), 0.55 ($p=0.008$) and 0.49 ($p=0.02$), respectively. The substrates related to the carbon source codes are given in Appendix Table A.2. The following carbon sources are not displayed due to overlap (overlapping carbon source given in brackets): D2, D3 and H4 (B3); E2 and G1 (A3); G2 (A2); B2 (E3).

3.2. Influence of estimated pesticide toxicity and salinity on biological endpoints

The estimated pesticide toxicity and salinity as EC explained considerable parts (r^2 ranging from 0.13 to 0.48) of the variation in biotic variables related to the ecosystem function AOM breakdown except for wood stick breakdown (Appendix Table A.3). The leaf breakdown exhibited a stronger relationship (r^2 values between 0.04 and 0.44 greater for leaf breakdown than for cotton breakdown) with the $mTU_{D.\ magna}$, $mTU_{S.\ capricornutum}$ and EC than cotton strip breakdown (Fig. 4, Appendix Fig. A.2). Variables associated with AOM breakdown such as fungal biomass (ergosterol) and functional microbial richnessin most cases displayed a reasonable relationship (r^2 values between 0.08 and 0.74) with $mTU_{D.\ magna}$ and EC, respectively (Appendix Table A.3, Fig. A.3). Similarly, $mTU_{D.\ magna}$ and EC correlated significantly with the NMDS for the carbon source use of microorganisms colonising AOM (Fig. 3). Neither GPP nor ER displayed a linear or non-linear relationship with estimated pesticide toxicity or EC (Appendix Fig. A.4, Table A.3). For linear models that contained both mTU and EC, the inclusion of an interaction term for both variables was not statistically significant (all $p>0.35$).

4. Discussion

4.1. Predictors of AOM breakdown and associated biotic endpoints

The most important variables to explain the variation in the AOM breakdown and associated endpoints (fungal biomass, functional microbial richness and carbon source used by microorganisms) were estimated pesticide toxicity, salinity, percentage of sand in the habitat, phosphate, temperature and turbidity (Table 2, Fig. 3). The relevance of environmental factors such as temperature or nutrient concentrations for AOM breakdown rates is well established (Imberger et al., 2010; Tank et al., 2010; Webster and Benfield, 1986). Furthermore, sedimentation can affect the AOM breakdown (Blasius and Merritt, 2002; Imberger et al., 2010) and the variables' percentage of sand in the habitat and turbidity may represent a proxy for this stressor.

Much less is known on the importance of anthropogenic stressors in general and pesticides and salinity in particular on AOM processing. Although several field studies have investigated the general impact of agricultural land-use on leaf breakdown in streams (Hagen et al., 2006; Magbanua et al., 2010; Piscart et al., 2009; Schäfer et al., 2007), only one of these studies quantified pesticide exposure (Schäfer et al., 2007). Schäfer et al. (2007) reported a 2.5 fold reduction of leaf breakdown in streams subject to highest pesticide exposure and this effect size matches well with the reduction observed in the present study (Fig. 4). However, the three- to four-fold reduction in leaf breakdown in our study was equally attributed to estimated pesticide toxicity and salinity (Table 2, Fig. 4, Appendix Fig. A.2). We therefore suggest that the effects of salinity and pesticides were additive, as firstly, both stressors were not significantly correlated (Pearson's $r=0.23$, $p=0.28$, $n=23$) so that collinearity played no role, i.e. each stressor had an independent effect (Appendix Fig. A.6). Secondly, no

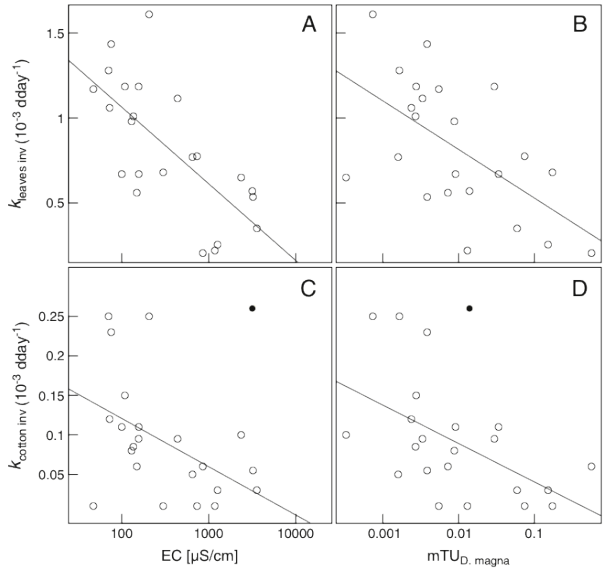

Fig. 4. Relationship of salinity (A, C) in terms of electrical conductivity (EC) and estimated pesticide toxicity (B, D) in terms of maximum toxic units for *D. magna* ($mTU_{D.\ magna}$) with the breakdown of leaves (k_{leaves})(A, B) and cotton strips (k_{cotton})(C, D) by invertebrates (inv) per degree day (dday). Log EC and log $mTU_{D.\ magna}$ explained 48% and 34% variation in $k_{leaves\ inv}$, respectively. For the variation in $k_{cotton\ inv}$, log EC and log $mTU_{D.\ magna}$ explained 23% and 30%, respectively (6% and 22% when including a point (filled dot) that unduly influenced the linear model according to Cook's distance).

statistically significant interaction ($p>0.35$) between the stressors was found, and this is in agreement with another study on joint effects of pesticides and salinity on macroinvertebrate communities in streams of southeast Australia (Schäfer et al., 2011a).

Both $mTU_{D.\ magna}$ and $mTU_{S.\ capricornutum}$ were included in linear models for AOM breakdown. Since the macroinvertebrate-related AOM breakdown showed a much stronger relationship to $mTU_{D.\ magna}$ than to $mTU_{S.\ capricornutum}$ (Appendix Table A.3, Fig. 4), this suggests direct effects of pesticides on macroinvertebrates that translated to effects on ecosystem functions. This hypothesis is also supported by a companion study that identified estimated pesticide toxicity as a dominant stressor for the structure of macroinvertebrate communities in the streams investigated here (Schäfer et al., 2011b). For microorganisms the situation is ambiguous. Firstly, there is no standard test species for microorganisms and hence no toxicity data was available to calculate microorganism-specific toxic units for all chemicals measured in the present study. Therefore, we used *D. magna* and *S. capricornutum* as surrogates, though their validity is uncertain. AOM breakdown by microorganisms showed a stronger relationship with $mTU_{S.\ capricornutum}$ than with $mTU_{D.\ magna}$ (Appendix Table A.3). This may either indicate direct effects on microorganisms, under the assumption that $mTU_{S.\ capricornutum}$ represents a valid surrogate, but could otherwise indicate indirect effects from alterations in primary producers that interact with the leaf-associated microbial communities (Franken et al., 2005). However, $mTU_{S.\ capricornutum}$ was not a major explanatory variable for the fungal biomass, functional microbial richness or the similarity in carbon source use of microorganisms (Appendix Table A.3, Fig. 3). By contrast, the functional microbial richness and the similarity in carbon source use responded to $mTU_{D.\ magna}$ (Fig. 3, Appendix Fig. A.3). In addition, the companion study did not find a link to potential effects on the density of different major groups of microorganisms (bacteria, flagellates, ciliates, amoebas, nematodes, and gastrotrichs) (Schäfer et al., 2011b), though this may not exclude changes in more sensitive microbial endpoints (Widenfalk et al., 2008). To sum up, it remains unclear, whether the reduction of the microbial breakdown is a direct effect from pesticide exposure or an indirect effect from the alteration of the community of primary producers (Franken et al., 2005).

We compared the performance of three different methods when used to determine the breakdown of AOM: leaf bags, cotton strips and wood sticks. Both leaf bags and cotton strips identified the explanatory variable estimated pesticide toxicity as of high importance, whereas wood sticks did not yield a reasonable relationship with any environmental variable (Appendix Fig. A.5). The wood stick breakdown rates were very similar to those reported in other studies, which also found no (Clapcott et al., 2010) or no clear (Young and Collier, 2009) relationship of wood stick breakdown with different stressor gradients.

Cotton strips have been employed as a substrate that is more standardised than leaves to determine AOM breakdown (Fritz et al., 2011; Tiegs et al., 2007). In agreement with the study of Tiegs et al. (2007) cotton strip and leaf breakdown in coarse bags were significantly correlated (invertebrates: Pearson's $r=0.58$, $p=0.004$, $n=23$; microorganisms: Pearson's $r=0.23$, $p=0.29$, $n=23$). In addition, they responded similarly to estimated pesticide toxicity (Fig. 4, Appendix Fig. A.2), albeit the response was weaker for cotton strips. However, while the variation in leaf breakdown and fungal biomass on leaves also correlated strongly with salinity in terms of EC, the cotton strip breakdown responded to percentage of sand in the habitat and phosphate concentrations (Table 2). In a study on cotton strip breakdown in 12 streams, cotton strips also responded strongest to sedimentation and phosphorus concentrations and not to specific land-use patterns (Imberger et al., 2010). Leaf breakdown may

represent the most sensitive indicator for effects of pesticides and salinity on the ecosystem function of AOM breakdown, while cotton strips may be more sensitive to gradients in nutrients or sedimentation.

The response of leaf breakdown to the logarithm of EC and mTU_{DM} was linear and therefore no obvious effect threshold was apparent (Fig. 4). Nevertheless, the leaf breakdown rate for sites with EC>1000 and mTU_{DM}>0.01 was reduced compared to sites with lower exposure (Fig. 4). Studies in southeast Australia on the response of the macroinvertebrate community structure to salinity and pesticides showed considerable community change when salinity levels exceed approximately 500–1000 µS/cm (Kefford et al., 2010a,b; Schäfer et al., 2011a). For pesticides, field and mesocosm studies in central European regions and southeast Australia reported adverse effects on the macroinvertebrate communities for mTU_{DM} exceeding between 0.001 and 0.01 (Beketov et al., 2008; Liess and von der Ohe, 2005; Schäfer et al., 2007, 2011b). These results indicate that salinity and pesticides trigger change in structural and functional endpoints at similar levels, hence suggesting that there is not much functional redundancy in the communities for these stressors (Rosenfeld, 2002). However, additional field studies are needed to confirm these results.

4.2. Relationship of stream metabolism with estimated pesticide toxicity and salinity

Stream metabolism comprises the ecosystem functions of GPP and ER and was determined in this study to elucidate potential effects of pesticides and salinity. More specifically, we hypothesised that an increase in estimated pesticide toxicity for primary producers in terms of $mTU_{S.\ capricornutum}$ would result in a decreasing GPP. The values for GPP of our streams ranged from 1 to 12 mg O_2 L^{-1} d^{-1}, except for one outlier with a value of 44 mg O_2 L^{-1} d^{-1} (Appendix Fig. A.4, Table A.1). These values are well within the range given for streams in Victoria, Australia (0.2–50 mg O_2 L^{-1} d^{-1}) (Grace and Imberger, 2006). Furthermore, the values for ER (3–101 mg O_2 L^{-1} d^{-1}) (Table 1) also corresponded well to those (8–100 mg O_2 L^{-1} d^{-1}) reported in the same publication. In the present study temperature was a main predictor for GPP (Table 2) and the same holds for a modelling study of Marcarelli et al. (2010) on ecosystem metabolism in a fifth-order river. However, the ecosystem functions of GPP and ER showed no relationship to estimated pesticide toxicity or to the salinity gradient (Appendix Fig. A.4).

To our knowledge, no other study has investigated the effects of pesticides and salinity on stream metabolism. Nevertheless, four studies have examined the effect of agricultural land-use on stream metabolism (Bernot et al., 2010; Gücker et al., 2009; McTammany et al., 2007; Young and Collier, 2009). Three of which reported an increase of GPP in agricultural streams that ranged from twofold to sixfold in comparison with reference streams (Bernot et al., 2010; Gücker et al., 2009; McTammany et al., 2007). By contrast, there was no statistically significant relationship between a land-use gradient that included agriculture and GPP in a study on 15 streams in New Zealand (Young and Collier, 2009). Regarding the general impact of stressors on GPP, a study on 213 sites proposed thresholds for non-impacted, lightly impacted and highly impacted sites at GPP<3.5, 3.5<GPP<7 and GPP>7, respectively (Young et al., 2008). When classifying the sites of our study according to these classes, the resulting groups were not statistically significantly different in their $mTU_{S.\ capricornutum}$ (ANOVA with F-test, $p=0.57$, $n=16$) or salinity in terms of EC (ANOVA with F-test, $p=0.19$, $n=16$). Overall, pesticides and salinity did not exhibit a major influence on GPP or ER in our study, despite measured levels of $mTU_{S.\ capricornutum}$ that would lead to acute mortality of the green algae *S. capricornutum* in the laboratory (Table 1). This may be explained firstly by a pollution-induced shift in the community of primary producers that did not compromise the ecosystem functions of GPP and ER due to functional redundancy (Rosenfeld, 2002). Future studies should examine this hypothesis by determining the community tolerance along a gradient of $mTU_{S.\ capricornutum}$, where a positive correlation between the community tolerance and $mTU_{S.\ capricornutum}$ would be expected according to the pollution-induced community tolerance concept (Blanck and Dahl, 1996; Blanck and Wangberg, 1988). Second, (1) the natural variability in GPP and ER due to groundwater inputs or hyporheic flows (Hall and Tank, 2005) and (2) uncertainty associated with the measurement (e.g. stream metabolism measured not simultaneously in all sites) and calculation (e.g. different methods available, see Aristegi et al. (2009)) of GPP and ER may have prevented identification of the effects of stressors. A third explanation would be that contrasting effects of different stressors in agricultural streams cancelled each other out (Clapcott et al., 2010). For example, the inhibition of GPP by pesticides may be compensated by stimulation of primary producers by nutrients. Further studies are required to examine the validity of these three explanations.

4.3. Relevance of the observed effects for ecosystem services

A first step for an ecological risk assessment based on ecosystem services is the identification of the relevant ecosystem services for a certain environmental compartment (e.g. freshwaters, soil), which is followed by the derivation of suitable and measurable endpoints in the second step (see Nienstedt et al., 2012). Based on the Millennium Ecosystem Assessment (MEA, 2005), Harrison et al. (2010) compiled an extended list of ecosystem services relevant for freshwater ecosystems. According to this list, freshwater ecosystems play a key role in the provision of food, energy, water and genetic resources and in the regulation of water flow and water purification. Finally, they deliver several cultural services such as education, recreation, aesthetic values and sense of place (Harrison et al., 2010). The ecosystem functions investigated in our study are central for the delivery of energy, in terms of food, to freshwater ecosystems. A reduction of energy processing, for example by impediment of AOM breakdown, will primarily translate to a lower carrying capacity i.e. less biomass in the system, and therefore affect the provision of food to humans e.g. fish (Wipfli, 2005; Wipfli and Baxter, 2010). In addition, the ecosystem service of water purification may also be affected if the biomass of species involved in this ecosystem service decreases, whereas effects on other of the abovementioned services are dependent on whether an alteration of the composition of the freshwater community occurs. However, effects of pesticides and salinity on ecosystem functions were only shown for AOM breakdown in the present study and this energy source is mainly important for first- to fifth-order streams (Vannote et al., 1980; Webster, 2007). Future studies should elucidate whether especially herbicides have any effect on the GPP or whether they are masked by stimulatory effects of nutrients. Furthermore, to quantify the effects on ecosystem services on the landscape or regional level (as for example Rutgers et al., 2012 for soil ecosystem services) data on the spatial extent of pesticides and salinity effects is required. For pesticides, the spatial and temporal dynamic of effects on ecosystem functions such as AOM breakdown in the field is largely unknown which is owed to the episodic (days) exposure (Schäfer et al., 2011c). By contrast, for salinity the exposure is relatively constant over time scales from days to months (McNeil and Cox, 2007) and the spatial extent can be delineated much easier. Moreover, the trophic linkages between macroinvertebrates and fish should be further explored to enable quantification of the dietary relevance of macroinvertebrate biomass and consequently the effects of their reduction (Wipfli and Baxter, 2010). Beside further field studies, ecological modelling may prove useful here, to extrapolate results to higher levels of ecological and spatial organisation and to guide the operationalisation of ecosystem services in research projects (see Galic et al., 2012). Finally, previous studies have demonstrated that landscape patterns such as undisturbed upstream sections can alleviate episodic disturbances on

macroinvertebrate communities (Hatakeyama and Yokoyama, 1997; Liess and von der Ohe, 2005; Schäfer et al., 2007; von der Ohe et al., 2009). Whether undisturbed upstream sections also prove beneficial for AOM breakdown in impacted stream reaches is therefore a question that may, among other measures, foster environmental risk assessment.

Supplementary materials related to this article can be found online at doi:10.1016/j.scitotenv.2011.05.063.

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14. Synoptic Discussion and Conclusions

The current thesis features several publications on the impact of toxicants on freshwater ecosystems. This chapter includes the main results, a synoptic discussion and conclusions.

Time-integrative passive sampling of toxicants

As outlined in the introduction, time-integrative passive sampling can be used for the the continuous characterisation of the exposure to toxicants. This thesis comprises three publications (I, II and VI) dealing with different aspects of passive sampling including a comparison to standard water monitoring methods in terms of grab water and sediment sampling. In order to compute Time-Weighted Average (TWA) concentrations from the mass of a substance in the receiving phase of a passive sampler after field deployment, substance-specific sampling rates are required, which are usually determined in laboratory experiments (Gunold et al., 2008; Macleod et al., 2007; Morin et al., 2012; Tran et al., 2007). Publication I demonstrates that a 10-day biofouling of the Empore disk receiving phase of the Chemcatcher passive sampler during field deployment reduced the sampling rate approximately fourfold. This would lead to an underestimation of the exposure to toxicants when TWA concentrations were calculated using uncorrected sampling rates determined in the laboratory. To reduce biofouling of the Empore disk, the deployment time could be shortened. For example if the sampling is targeted at episodic exposures of toxicants such as pesticides, the passive samplers could be deployed shortly before expected exposure events. Moreover, protection of the receiving phase from sunlight has been demonstrated to reduce the fouling of the Empore disk (Vermeirssen et al., 2008). Thus, the samplers should be deployed in shaded stretches of waterways or a container could be constructed that shields the passive samplers from sunlight during field deployment. Another option to cope with biofouling is the use of a diffusion-limiting membrane. Diffusion-limiting membranes from Low-Density Polyethylene (LDPE) or polyethersulfone (PES) are resistant to biofouling and consequently protect the receiving phase from fouling (Harman et al., 2009; Stephens et al., 2009; Vrana et al., 2006). In addition, diffusion-limiting membranes increase the possible sampling time in the linear uptake regime (Stephens et

al., 2009), though this comes at the cost of a lower sampling rate. Publication I shows that short-term exposures of 24 hours are not captured in the receiving phase when using a diffusion-limiting PES membrane. This is very likely due to the lag phase of the substances passing through the PES membrane, which is in agreement with other studies (Vermeirssen et al., 2012; Vermeirssen et al., 2009). Co-extraction of the diffusion-limiting membrane with the receiving phase could be an option when such a membrane is employed, though the water-PES partitioning behaviour needs to be known for the correct calculation of TWA concentrations (Vermeirssen et al., 2012).

Generally, not only biofouling but also hydrodynamics and temperature influence the sampling rates of passive samplers (Soderstrom et al., 2009). To account for all of these influences during field exposure, the so-called Performance Reference Compound (PRC) approach has been developed (Huckins et al., 2002). PRCs are usually deuterated analogues of target compounds spiked into the receiving phase before field deployment. Subsequently, the dissipation of these compounds in the field is monitored. Under isotropic exchange kinetics (i.e. uptake rate equals dissipation rate) PRCs can be used to correct laboratory sampling rates for field conditions (Huckins et al., 2002). The PRC approach has been successfully employed in several studies with passive sampling devices for rather hydrophobic substances (Allan et al., 2009; Booij et al., 2002; Huckins et al., 2002; Komarova et al., 2009). Unfortunately, the development of a PRC approach for hydrophilic compounds is problematic because for most potential PRCs the dissipation rate is different from their uptake rate (Gunold et al., 2008; Mills et al., 2007; Shaw et al., 2009; Stephens et al., 2009). Nevertheless, recent studies suggest that a few hydrophilic deuterated compounds including atrazine-desisopropyl (DIA-d5), carbendazim-d4, diclofenac-d4 and ibuprofen-d3 may be suitable as PRCs (Camilleri et al., 2012; Mazzella et al., 2010). Future studies should elucidate whether these PRCs are reliable for polar passive sampling in the field as this would allow to correct sampling rates for the influence of biofouling and other field conditions. However, based on a novel approach employing receiving phases of two different thicknesses to determine the kinetic regime (cf. Bartkow et al., 2004; Muller et al., 2001), publication II demonstrates that for episodic exposures the PRC approach can be misleading. This is because PRCs are continuously dissipated from the receiving phase immediately after deployment and thus may indicate equilibrium regime after recovery of the sampler. By contrast, the receiving phase may still be in the integrative uptake regime for the target

substance if the episodic exposure event occurs late during deployment time. Moreover, the novel approach with receiving phases of different thicknesses revealed that the dissipation rates were underestimated by the PRC approach. Nevertheless, prediction of the kinetic status of the polydimethylsiloxane (PDMS) passive samplers by the novel approach exhibited high variation and higher replication as well as multiple thicknesses should be employed in future studies. To sum up, the approach to use receiving phases of different thicknesses to derive uptake kinetics may represent a valuable method for field deployments but is limited to samplers where receiving phases of different thicknesses are readily available such as PDMS. For polar passive samplers relying on Empore disks (e.g. Chemcatcher (Greenwood et al., 2007; Schäfer et al., 2008)) or on sorbents between membranes (e.g. Polar organic chemical integrative sampler [POCIS] (Alvarez et al., 2004; Morin et al., 2012)) this approach may be difficult to implement.

The third study on passive sampling (publication VI) used a TRIMethylpentane Passive Sampler (TRIMPS) (Hyne et al., 2004; Leonard et al., 2002) for the monitoring of 97 pesticides in 24 streams and compared its performance to conventional monitoring methods encompassing grab water and sediment sampling. Sediment sampling detected 48 different pesticides, followed by TRIMPS passive samplers with 34 pesticides. Grab water sampling detected 27 of the 97 pesticides. More importantly, TRIMPS contributed considerably to the estimation of the maximum ecotoxicity per sampling site and when combined with sediment-derived ecotoxicity explained 67% of the variance in the $SPEAR_{pesticides}$ index. However, when exclusively based on TRIMPS data the ecotoxicity estimation yielded no good relationship with $SPEAR_{pesticides}$. Similarly, the results from grab water sampling showed negligible explanatory power for $SPEAR_{pesticides}$ alone or when added to the estimation from the other sampling methods. While it is well known that grab water sampling is likely to miss relevant pesticide exposure (Mortimer et al., 2007), ecotoxicity derived from passive samplers alone yielded a reasonably good relationship with $SPEAR_{pesticides}$ in another study (Schäfer et al., 2008). This may be due to differences in the exposure paths and in the chemical characteristics of the most ecotoxicologically relevant substances between the studies. In detail, passive samplers do primarily extract the water-soluble fraction of substances (Allan et al., 2007; Chen et al., 2007; Gourlay-France et al., 2008; Haftka et al., 2010; Pablo and Hyne, 2009), while a considerable fraction of the total mass of hydrophobic and ionic substances can be adsorbed or bound to dissolved organic matter (DOM) and sediment particles.

Although in most cases the water-soluble fraction determines bioavailability and consequently ecotoxicological effects (Chen et al., 2007; Droge et al., 2008; Gourlay et al., 2005; Green et al., 1993; Hoke et al., 1994; Perron et al., 2012; Rico-Rico et al., 2009), the adsorbed or particle-bound fraction can be important for aquatic organisms that ingest particles or are in direct contact with the sediment phase (Lauridsen et al., 2006; Liber et al., 2011; Selck et al., 1998; Verrhiest et al., 2001). In addition, DOM has been demonstrated to increase the uptake of hydrophobic toxicants (Chiou et al., 1986), whereas passive samplers exhibited no clear response to different levels of DOM in two studies (Charlestra et al., 2012; Li et al., 2011). Moreover, the toxicant concentrations in the water column, which are sampled by passive samplers, may differ from the pore-water concentrations in the sediment layer (Droge et al., 2008; Liber et al., 2011), which has also been shown for the TRIMPS used in the South-East Australian study (publication VI) (Pablo and Hyne, 2009). Thus, in cases where hydrophobic or ionic substances as well as particle- or sediment-borne pesticide exposure are ecotoxicologically relevant, passive samplers may underestimate the ecotoxicity. In the latter case, passive sampling may still provide relevant information (cf. publication VI), but should be used in concert with suspended particle- and/or sediment sampling for an appropriate assessment of the ecotoxicological risk. Overall, the studies on passive sampling in this thesis show that it can be a valuable tool to determine continuous and episodic exposure to toxicants, but development is still needed regarding sampling rates under field conditions and, depending on the characteristics of the target analytes, should be complemented by conventional or event-driven sampling methods.

Trait-based approaches for ecological risk assessment

Several publications revolve around the issue of trait-based ecological risk assessment (cf. Baird et al., 2008). Publication III presents an overview on several studies where the trait-based $SPEAR_{pesticides}$ index has been used to establish a relationship between macroinvertebrate community structure and estimated pesticide toxicity. It is argued that the empirical relationship between the abundance of the pesticide-sensitive taxa in terms of $SPEAR_{pesticides}$ and the estimated pesticide toxicity derived from field sites can be extrapolated to the continental scale using predicted pesticide runoff (cf. Schriever and Liess, 2007). Based on this extrapolation, the ecological risk from pesticides was predicted as very high for > 90% of the streams located in 19% of the European area.

Hence, pesticides would present a very relevant stressor for freshwater ecosystems on the European level. This prediction could be tested in future studies, if European-level biomonitoring and pesticide exposure data was available. Moreover, publication III shows that commonly used biotic indices such as the % of EPT taxa (Plafkin et al., 1989) had a low selectivity and responded only to a minor extent to the pesticide toxicity estimated from concentrations in several agricultural streams in regions of France, Germany and Finland. By contrast, $SPEAR_{pesticides}$ exhibited a high and exclusive response to the estimated pesticide toxicity. Thus, based on this publication (III) it can be concluded that $SPEAR_{pesticides}$ is the most appropriate index for the detection of pesticide stress at least for biomonitoring data from agricultural streams. The index was also applied for the analysis of biomonitoring data (publication IV) and field studies (VI) in South-East Australian streams. Since the trait information required for the adaptation of $SPEAR_{pesticides}$ for this region was much scarcer than for Europe, an increase in the variation of the index was expected. Especially the trait physiological sensitivity, which is an essential element for this index, has been constructed based on acute toxicity data from predominantly European and North American taxa (von der Ohe and Liess, 2004). Nevertheless, the relationship between estimated pesticide toxicity and $SPEAR_{pesticides}$ was as high as in other field studies in terms of the explained variance (see publications III and VII for details). In fact, publication VII demonstrates that the trait composition of macroinvertebrate communities from different global regions (Europe, Siberia, South-East Australia) exhibits a very similar response to estimated pesticide toxicity. This is in agreement with studies on the convergence of traits from fish communities over continents for hydraulic and geomorphological environmental gradients (Lamouroux et al., 2002) and along the river continuum (Ibanez et al., 2009). Another study showed convergence of traits from fish communities across large spatial scales in response to anthropogenic disturbance in terms of urbanisation (Cunico et al., 2011). Thus, the relationship between pesticides and the trait pattern of macroinvertebrate communities may converge globally allowing for large-scale trait-based ecological risk assessment.

An obvious prerequisite for trait-based ecological risk assessment is access to trait databases. Several trait databases for macroinvertebrates have been developed for Europe (Liess and von der Ohe, 2005; Schäfer et al., 2007; Schmidt-Kloiber et al., 2006; Statzner et al., 2007; Usseglio-Polatera et al., 2000; Verberk et al., 2008) and

North America (Beche and Resh, 2007; Vieira et al., 2006). Except for databases for New Zealand (Doledec et al., 2006; NIWA, 2012) and Bolivia (Tomanova et al., 2007; Tomanova and Usseglio-Polatera, 2007) no such databases exist for Australia, Asia and other regions in the Southern hemisphere, presumably because of a lack of autecological data. In publication IV a first trait database consisting of nine traits for taxa from South-East Australia is presented. The traits were selected regarding (1) the adaptation of $SPEAR_{pesticides}$ and (2) *a priori* hypotheses which traits would respond to salinisation, one of the most important environmental problems in Australia (Cañedo-Argüelles et al., 2012; Williams, 1987). Subsequently, a trait-based index for salinity was developed following the SPEAR approach, including an examination of the relevance of the individual traits. The resulting $SPEAR_{salinity}$ index was applied to biomonitoring data from streams of two South-East Australian states. Between 38% to 50% of variance in the index was explained by salinity depending on the state and whether the biomonitoring data originated from riffle or pool sections. When compared to existing biotic indices including species richness and a Salinity Index (SI) that was developed using a machine learning method and claimed to be stressor-specific (Horrigan et al., 2005), $SPEAR_{salinity}$ exhibited the highest selectivity by exclusively responding to salinity. By contrast, all other indices responded to 3 or more further environmental variables. A recent study reported that statistical ecological models derived with machine-learning methods tended to score poorly when transferred to another biogeographical region (Wenger and Olden, 2012). This may explain the unexpected low selectivity of SI, which has been developed using artificial neural networks for biomonitoring data of North-East Australia. A recent study reported that statistical ecological models derived with machine-learning methods tended to score poorly when transferred to another biogeographical region (Wenger and Olden, 2012). Overall, this study suggests that ecological hypotheses and mechanistic knowledge should be considered in the development of trait-based indices. Publication IV outlines a conceptual model how this could be done. Briefly, the conceptual model proposes that the traits for the indices should be selected depending on the mode of action of the stressor of interest and the disturbance regime *sensu* Lake (2000). The validity of this conceptual model should be scrutinised in future studies.

Apart from stressor-specific indices to detect effects of toxicants, an efficient and protective management of freshwater resources with respect to chemicals requires

knowledge on effect thresholds. Publication VII describes the derivation of thresholds for the effects of pesticides on freshwater communities and ecosystem functions based on a meta-analysis of field studies. The derived threshold for effects on the pesticide-sensitive taxa in the macroinvertebrate community was a factor of 10 to 100 lower than thresholds determined in a review of mesocosm studies (Van Wijngaarden et al., 2005) and the safety factor of the first tier in the authorisation of pesticides (EEC, 1991). Several uncertainties with respect to the derived thresholds are discussed and it is concluded that the results are relatively robust. This suggests that the first tier safety factor is not protective for communities in the field and that field thresholds are in fact lower than those previously derived from mesocosm studies. Several reasons may explain the discrepancy between the field- and mesocosm-based assessment. First, pooled data from different field studies were re-analysed in the study in this thesis, which increases the statistical power to detect effects, whereas the review of mesocosm studies did not re-analyse pooled mesocosm data (Van Wijngaarden et al., 2005). Furthermore, mesocosm studies rarely consider repeated exposures and the joint effects of different stressors both of which can increase the effects of pesticides in the field (Belden et al., 2007; Heugens et al., 2001; Holmstrup et al., 2010; Liess and Beketov, 2011). Finally, sublethal long-term effects on merolimnic insects or slow-reproducing taxa may not be detected in mesocosm studies (Schulz and Liess, 2000), which rarely exceed a study period of a few months. Indeed, publication V reports that the abundance fraction of slow-reproducing taxa was only 10 to 24% in two mesocom studies for which raw data was available, compared to 40 to 80% in field communities from 15 reference sites in streams. Moreover, publication V describes a long-term mesocosm study with a community representative for field conditions, which was established over 16 months before contamination with a neonicotinoid insecticide and included an abundance fraction of 50% slow-reproducing species. This study demonstrates that in all treatments sensitive taxa with a slow reproduction did not recover within the 7 months of the study. Given the repeated exposure and additional stressors in the field situation, this could explain the discrepancy between the effects in mesocosms and the field, since over longer time periods sensitive slow-reproducing species might completely disappear from the communities. In addition, this mesocosm study highlights that considering species traits (e.g. physiological sensitivity, generation time) may facilitate the detection of effects on the community level (cf. Liess and Beketov, 2011; Liess and Beketov, 2012; but see also Van den Brink and Ter Braak, 2012).

Overall, the trait-related studies in this thesis foster the development of trait-based ecological risk assessment and the derivation of effect thresholds may represent a first step towards global boundaries for chemical pollution that are currently lacking (Rockstrom et al., 2009).

Statistical data analysis approaches to identify effects of toxicants

With the exception of publication IV, most studies described so far in this thesis relied on field or mesocosm investigations. While such studies are indispensable for a process-based understanding and can be tailored to answer specific research questions, large biomonitoring or chemical measurement data sets allow for the testing of hypotheses with a high statistical power as well as for large-scale or long-term analyses (Friberg, 2010). In publication VIII, the ecological risks for freshwater organisms is assessed for a large monitoring dataset encompassing 331 organic toxicants measured over 11 years in 7 sites in the largest rivers of North Germany. This was done comparing the exposure to effect concentrations from acute toxicity tests with standard test species of different trophic levels i.e. algae, invertebrates and fish. One major obstacle was the lack of acute toxicity data for 30% to 70% of the substances depending on the standard test species. Therefore, a novel Read-Across method for the quantitative prediction of effect concentrations was employed (Schüürmann et al., 2011). The study demonstrates that organic toxicants and especially pesticides can reach concentration levels that may cause acute toxic effects in primary producers and invertebrates. Although the estimated toxicity for fish remained largely below levels envisaging acute effects, chronic and indirect effects through reductions in prey populations could occur (Wipfli, 2005; Wipfli and Baxter, 2010). One important further aspect is that the characterisation of the chemical exposure in this study relied on grab-water sampling, which has been shown to underestimate the exposure (see publication VI in this thesis). Hence, the real maximum concentrations and consequently the real maximum toxicity was most likely higher than assessed in publication VIII. Only a slight temporal decrease in ecotoxicity was detected. Interestingly, priority pollutants played only a minor role for the highest estimated toxicity in the sites. Given that the chemical monitoring in the European Union is specifically targeted at priority pollutants (EC, 2000), ecological risks from ecotoxicologically more important substances may go unnoticed. Moreover, this study highlights that organic toxicants including pesticides may not only be an important stressor in small agricultural streams but also in large river systems. The spatial extent

of the problem, however, needs further investigation and since respective exposure data has recently become publicly available (European Environment Agency, 2012), this could be done in a follow up analysis for the European scale.

If biomonitoring data complementary to chemical exposure information is available, effects on populations or communities can be estimated directly, instead of relying on acute toxicity data. Here, different endpoints can be inspected. While the trait-based approaches outlined in this thesis (publications II – VII) focused on changes in the trait community structure, changes in the taxonomical composition are relevant if biodiversity measures are of interest. In this context, a recent European Union Directive requires that no adverse effects on biodiversity should result from the use of pesticides (EEC, 2009). However, surprisingly little is known regarding whether, to which extent, and at which concentrations toxicants cause species loss (Beketov and Liess, 2012), tough they are frequently claimed to be an important driver for biodiversity loss (Gessner et al., 2010; MEA, 2005; Vorosmarty et al., 2010). Publication IX presents a novel statistical method to derive thresholds and quantify community change based on large community data sets with associated toxicity data, though this method could be applied to a wide range of stressors. An important peculiarity of this method is that it requires an ordinal grouping of the stressor of interest and that it quantifies community change, which includes both species loss and species gain. In case that all influential environmental factors for the biota under scrutiny have been recorded, the method can establish a causal link between the stressor of interest and community change. This is done by pooling data over spatial and/or temporal scales in order to remove community change due to natural variation and other influential factors. However, the results of this procedure are only reliable for large data sets (> 300 samples recommended), where the collinearity of the stressor of interest with other influential factors is low and these factors exhibit similar ranges for each level of the stressor. The study illustrates three case examples for stream macroinvertebrate communities and different toxicants comprising modelled pesticide exposure, salinity and heavy metals. The results were largely in agreement with those of previous analyses of these data sets, which focused on different biotic endpoints. Further development of this method could include the implementation of the Indicator Value index (Indval) algorithm (De Caceres and Legendre, 2009; De Caceres et al., 2010) in order to identify the species responsible for community change. Overall, given that more and more large data sets become available

for environmental research (Drew, 2011), this method may foster the derivation of boundaries for community change as well as support the conservation of pristine communities for a wide range of stressors.

The last study of this thesis using a statistical approach to identify effects of toxicants on communities is publication X. This publication describes the use of distance-based Redundancy Analysis (db-RDA) (Legendre and Anderson, 1999; McArdle and Anderson, 2001) to examine the interaction of two toxicants, pesticides and salinity, on macroinvertebrate communities in 24 sites in South-East Australia. Db-RDA was used in the study because it did not require certain assumptions of Redundancy Analysis that were not met. Although both toxicants exhibited a statistically significant effect on the community composition, no evidence for an interaction of pesticides and salinity was found. The results are in line with publication IV, where no interaction effect between predicted pesticide exposure and salinity was found for the trait composition of the communities. However, interactions between salinity and pesticide toxicity have been reported for single species acute toxicity tests (Hall and Anderson, 1995). This suggests that potential interaction effects are difficult to detect on the community level, but may occur for specific populations. The percentage of explained community variation by individual environmental variables was rather low. Besides toxicants, mainly variables related to the substrate and hydraulics exhibited explanatory power for the community composition. The low influence of individual variables explains, why on the taxonomic level the specific effects of toxicants are difficult to detect and trait-based approaches seem more suitable for ecological risk assessment (cf. Liess and Beketov, 2011). However, while the sheer number of environmental variables influencing the community composition reduces the relevance of individual variables (Allan and Castillo, 2007), this must not be interpreted as if the influence of individual variables such as hydraulics, substrate or toxicants would not be important. A very interesting finding of the study was that both toxicants seemed to act on different taxonomic levels. Salinity affected the community composition on a higher taxonomic level, i.e. phylogeny was more important for the effects of salinity. This may be due to the fact that macroinvertebrates have been exposed to salinity over evolutionary time scales (Williams, 1987), whereas organic pesticides and related substances were only introduced in the last centenary (Carson, 1962). In this context, a recent study of Guenard et al. (2011) showed that phylogeny can be used to predict effects of toxicants

on taxa. Given the public availability of phylogenetic information via GenBank (www.ncbi.nlm.nih.gov/genbank/), the relationship between phylogeny and toxicity may represent an avenue of future research.

Effects of toxicants on ecosystem functions

So far the publications in this thesis had their primary focus on the effects of toxicants on the taxonomic or trait composition of invertebrate communities. Publications XI and XII deal with the influence of toxicants on ecosystem functions and services. Effects on ecosystem functions and services highlight that the environmental impact of toxicants is not only of ethical concern. Given that ecosystem services represent economic value (Costanza et al., 1997), effects of toxicants on ecosystem functions and related services lead also to economic losses. Although the Millennium Ecosystem Assessment (MEA, 2005) ranks toxicants such as pesticides, salinity and heavy metals among the most important stressors for freshwater ecosystems, there is a paucity of field investigations on the effects of toxicants on ecosystem functions. In this context, a recent study argued that ecosystem functions such as leaf breakdown, stream metabolism or nutrient spiralling should be included in the assessment of ecosystem health (Woodward et al., 2012). Publication XI follows a similar leading thought and gives an overview on different articles (1) on how ecosystem functions, services and biodiversity could be integrated into the ecological risk assessment as well as (2) on case studies on the effects of different stressors on these endpoints. One of these case studies represents publication XII that describes a field study in 24 sites on the effects of pesticides and salinity on the ecosystem functions of allochthonous organic matter breakdown and stream metabolism. Three measures of allochthonous organic matter breakdown were employed encompassing leafs, cotton strips and wood, of which leaf breakdown exhibited the strongest response to toxicants. Salinity and pesticides reduced the leaf breakdown rate to a similar extent, but no interaction effect was found. This is in agreement with the studies described in publications IV and X that reported effects of pesticides and/or salinity on the invertebrate community but did not find interaction effects. No reasonable relationship between estimated pesticide toxicity or salinity and the ecosystem functions of gross primary production or ecosystem respiration could be established. This was surprising because the estimated pesticide toxicity observed in the field study exceeded thresholds where acute toxic effects were found in mesocosm studies (Brock et al., 2000). Several reasons that may explain this finding are discussed

in publication XII. Briefly, these reasons include too high natural variability, measurement-related uncertainties, functional redundancy and pollution-induced community tolerance (Blanck and Dahl, 1996). To sum up, the study shows that the current levels of salinity and pesticides in streams and rivers are potent to impede ecosystem functions that are crucial for the freshwater ecosystem. This raises the question of a threshold for effects on ecosystem functions. Publication VII includes a joint analysis of the three field studies that have investigated the effects of pesticides on leaf breakdown, and aims at deriving such a threshold. There was a linear relationship between the abundance fraction of pesticide-sensitive species in the invertebrate communities and the leaf breakdown rate. Thus, based on this analysis no effect threshold could be derived. Furthermore, for one of the three field studies no reasonable relationship between the leaf breakdown rate and pesticide-sensitive species or the estimated pesticide toxicity could be established. Whether this indicates that the effects of toxicants on ecosystem functions depends on the identity of species in the regional species pool, remains open to further investigations.

Future research challenges

In 1996, Carpenter stated: " The contribution of ecology to environmental problem solving depends heavily on appropriately scaled field studies. […] Academic ecologists may avoid these scales in order to attain the rigorous experimental control possible in microcosms." (Carpenter, 1996). This statement holds still true for current ecotoxicology, where almost all studies are conducted in small-scale experimental systems (Beketov and Liess, 2012) and their relevance for the field is often unclear. While it can be argued that the understanding of small-scale processes can enable the extrapolation to higher levels of biological organisation, ecotoxicology is very distant to such a "grand unifying theory" (see Schäfer et al., 2011). Recent studies nurture concerns that on the exposure and effects side central aspects of the real-word situation are not well represented in the models and test systems employed in ecological risk assessment (see publication VII and Knäbel et al., 2012). This thesis aims at contributing to a more realistic ecological risk assessment of toxicants and mainly features studies that were conducted in the field or in a mesocosm with field-representative communities. The studies demonstrate that toxicants adversely affect freshwater ecosystems, including their functions and services. However, the spatial and temporal extent of these effects remains largely unknown, though some model

predictions are available (Schriever and Liess, 2007). Given the high costs involved in chemical monitoring and analysis, a large scale study on the effects of toxicants that would allow for evaluating the spatial extent (e.g. 100 sampling sites (cf. Woodward et al., 2012) is difficult to realise. Therefore, the examination of the spatial extent of toxicant effects remains a challenge and data analyses as in publication VIII seem a promising option. Nevertheless, governmental data also have their limitations as they often originate from grab water sampling, which is likely to underestimate the toxic exposure (see above). Thus, the coupling of such data analyses with modelling (see publication III) may be most appropriate. The temporal dimension of toxicant effects can be investigated easier. For example, a study with a high frequency of toxicant sampling and bioassessment could be conducted in a few streams or rivers on the regional scale. However, in order to extrapolate such results, the influence of landscape patterns such as forested upstream sections on the effect dynamics need to be elucidated. Finally, the link from toxicant effects on ecosystem functions to ecosystem services remains an open challenge. Only a few ecosystem functions have been regarded so far (Burkhard et al., 2009) and even those are understudied. Nevertheless, a long-term aim of ecological risk assessment should be the quantification of the losses in ecosystem services due to toxic chemicals. Overall, this thesis contributes to achieve this aim through the development of novel methods, mechanistic understanding of processes and indication of the relevance of toxicants for ecosystem health.

15. Summary

This habilitation thesis deals with the effects of toxicants on freshwater ecosystems and considers different toxicant classes (pesticides, organic toxicants, salinity) and biotic endpoints (taxonomic community structure, trait community structure, ecosystem functions). The thesis comprises 12 peer-reviewed international publications on these topics. All of the related studies rely on mesocosm or field investigations, or the analysis of field biomonitoring or chemical monitoring data. Publications I and II are devoted to passive sampling of a neonicotinoid insecticide and polycyclic aromatic hydrocarbons (PAHs), respectively. They show that biofouling and a diffusion-limiting membrane can reduce the sampling rate of the pulsed insecticide exposure and that receiving phases of different thicknesses can be used to assess the kinetic regime during field deployment of passive samplers. Publications III to VI mainly focus on trait-based approaches to reveal toxicant effects on invertebrates in streams. An overview on the framework and several applications of a trait-based approach to detect effects of pesticides ($SPEAR_{pesticides}$ index) are given in publication III. Publication IV describes the development of a trait database for South-East Australian stream invertebrates and its successful application in the adaptation of $SPEAR_{pesticides}$ as well as the development of a salinity index. Moreover, a conceptual model for the future development of trait-based biomonitoring indices is proposed. Publication V reports a mesocom study on the effects of a neonicotinoid insecticide on field-realistic invertebrate communities. The insecticide had long-term effects on the invertebrate communities, which were only detected when grouping the taxa according to their life-history traits. A comprehensive field study employing different pesticide sampling methods including passive sampling and biomonitoring of the invertebrate and microbial communities is presented in publication VI. The study did not find pesticide-induced changes in the microbial communities, but detected adverse effects of current-use pesticides on the invertebrate communities using the trait-based $SPEAR_{pesticides}$ index. This index is also applied in a meta-analysis on thresholds for the effects of pesticides on invertebrate communities in publication VII. It is shown that there is a similar dose-response relationship between $SPEAR_{pesticides}$ and pesticide toxicity over different biogeographical regions and continents. In addition, the thresholds for effects of pesticides are lower than derived

from most mesocosm studies and than considered in regulatory pesticide risk assessment. The publications VIII to X use statistical data analysis approaches to examine effects of toxicants in freshwater ecosystems. Using governmental monitoring data on 331 organic toxicants monitored monthly in 4 rivers over 11 years, publication VIII finds that organic toxicants frequently occurred in concentrations envisaging acute toxic effects on invertebrates and algae even in large rivers. Insecticides and herbicides were the chemical groups mainly contributing to the ecotoxicological risk. Publication IX introduces a novel statistical method based on a similarity index to estimate thresholds for the effects of toxicants or other stressors on ecological communities. The application of the method for deriving thresholds for salinity, heavy metals and pesticides in streams is presented in three case studies. Publication X tackles the question of interactive effects between different toxicants using data from a field study on stream invertebrates in 24 sites of South-East Australia. Both salinity and pesticides exhibited statistically significant effects on the invertebrate communities, but no interaction between the stressors was found. Moreover, salinity acted on a higher taxonomical level than pesticides suggesting evolutionary adaptation of stream invertebrates compared to pesticide stress. Publications XI and XII concentrate on the effects of toxicants on biodiversity, ecosystem functions and ecosystem services, with publication XI summarising different studies related to the ecological risk assessment for these endpoints. A field study on the effects of pesticides and salinity on the ecosystem functions of allochthonous organic matter decomposition, gross primary production and ecosystem respiration is presented in publication XII. Both pesticides and salinity reduced the breakdown of allochthonous organic matter, whereas no effects on the other ecosystem functions were detected. A chapter following these publications synoptically discusses all studies of this habilitation thesis and draws general conclusions. It is stressed that in order to advance the understanding of effects of toxicants on freshwater ecosystems more ecological realism is needed in ecotoxicological approaches and that the spatiotemporal extent of toxicant effects needs more scrutiny.

16. References

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Lebenslauf

Persönliche Daten

Ralf Bernhard Schäfer
geboren am 8.5.1977 in Kassel
verheiratet, 1 Kind

Bildung und Positionen

seit 10/2010	Junior-Professor für Quantitative Landsschaftsökologie, Universität Koblenz-Landau, Deutschland
06/2008 – 05/2010	Postdoc am Royal Melbourne Institute of Technology, Melbourne, Australien mit einem DFG-Forschungsstipendium.
2/2008	Promotion (Dr. rer. nat.) in Umweltwissenschaften, Leuphana Universität Lüneburg, Deutschland.
6/2004 - 5/2008	Wissenschaftlicher Mitarbeiter und Doktorand am Helmholt-Zentrum für Umweltforschung in Leipzig (UFZ) im Department Systemökotoxikologie mit einem Promotionsstipendium der Studienstiftung des deutschen Volkes e.V.
04/2003 – 03/2004	Zweitstudium im Magisterstudiengang Statistik, Soziologie und Philosophie an der Universität Kassel (Zwischenprüfung in Soziologie mit 1,0 bestanden)
10/1998 – 03/2003	Diplomstudiengang Umweltwissenschaften an der Leuphana Universität Lüneburg (Gesamtnote 1,1)
10/1997 – 09/1998	Diplomstudiengang Marine Umweltwissenschaften an der Universität Oldenburg
08/1996 - 10/1997	Zivildienst im Haus der Jugend, Kassel
08/1993 – 06/1996	Abitur an der Jacob-Grimm-Schule Kassel (Gesamtnote 1,3)
09/1987 – 06/1993	Heinrich-Schütz-Schule Kassel, Gymnasialzweig
08/1983 – 07/1987	Grundschule am Warteberg, Kassel

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Ralf B. Schäfer
Landau, 14.10.2012