Environmental Toxicology and Chemistry, Vol. 24, No. 4, pp. 954–965, 2005

Printed in the USA
0730-7268/05 $12.00 + .00

ANALYZING EFFECTS OF PESTICIDES ON INVERTEBRATE COMMUNITIES IN STREAMS

MATTHIAS LIESS* and PETER CARSTEN VON DER OHE
UFZ—Centre for Environmental Research, Permoserstrasse 15, D-04318 Leipzig, Germany

(*Received* 1 *December* 2003; *Accepted* 25 *September* 2004)

Abstract—The aim of this investigation was to find patterns in aquatic invertebrate community composition that are related to the effects of pesticides. Investigations were carried out in 20 central European streams. 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 pesticides. They were classified as species at risk (SPEAR) and species not at risk (SPEnotAR). Ecological traits used to define these groups were sensitivity to toxicants, generation time, migration ability, and presence of aquatic stages during time of maximum pesticide application. Results showed that measured pesticide concentrations of 1 : 10 of the acute 48-h median lethal concentration (LC50) of *Daphnia magna* led to a short- and long-term reduction of abundance and number of SPEAR and a corresponding increase in SPEnotAR. Concentrations of 1 : 100 of the acute 48-h LC50 of *D. magna* correlated with a long-term change of community composition. However, number and abundance of SPEAR in disturbed stream sections are increased greatly when undisturbed stream sections are present in upstream reaches. This positive influence compensated for the negative effect of high concentrations of pesticides through recolonization. The results emphasize the importance of considering ecological traits and recolonization processes on the landscape level for ecotoxicological risk assessment.

Keywords—Field effect Pesticides Recovery Cumulative risk assessment

INTRODUCTION

The European Union uniform principles for the assessment of pesticides require that if the preliminary risk characterization indicates potential concerns, registration cannot be granted unless it can be demonstrated that ''under field conditions no unacceptable impact on the viability of exposed organisms occurs.'' To date, such assessments have been made by conducting higher-tier studies, which have included a range of laboratory and semifield experiments. Therefore, it still is not clear to what extent pesticides change population dynamics and community structures in the field. Recently, some studies have quantified pesticide exposure, adverse effects on aquatic life, and recovery of these invertebrate communities in the field. Mortality of six mayfly species in an Australian river was linked to endosulfan contamination due to runoff [1]. Other investigations also found a link between mortality of invertebrate species and insecticide concentrations in streams [2,3]. Several invertebrate species that declined in abundance due to pesticides were found to recover within a year [2]. Nevertheless, most existing studies lack sufficient numbers of investigations in various streams to evaluate the frequency of potentially harmful events in a specific region, evaluation of long-term effects on invertebrate communities, and quantification of the recovery of impacted communities due to recolonization from undisturbed stream sections. The inclusion of habitat quality may put the risks resulting from contamination in context with other stressors.

According to these open questions, the aim of the present investigation was to find patterns in community composition that were related to the effect of pesticides. It is challenging in field investigations to reveal the importance of a specific environmental factor, because other environmental factors may mask possible effects. Therefore, to tackle this problem a new approach that aims at reducing variability in community characterization is presented.

METHODS

Study area

The study area is located around Braunschweig, Lower Saxony, Germany. The dominant land use is agricultural (field 61%, forest 34%, pasture 5%; Fig. 1). The most common crops in the catchments are sugar beets, winter barley, and winter wheat. The investigation was carried out in an area where sites had a risk of runoff ranging from very low (level 0) to high (level 5), on a scale ranging from level 0 to level 6 (very high), defined for German agricultural areas [4].

Description of streams

Twenty sites, located on first-order streams, were selected to match the following requirements: All-year water flow; no dredging in the years before and during the investigation; no pollution from other than agricultural nonpoint sources; various pesticide loads, stemming from differences in the percentage of adjacent arable land. Nine streams were monitored for one year; six streams were monitored for two years; and five streams were monitored for three years (11 sites in 1998, 11 sites in 1999, and 14 sites in 2000). The streams investigated for three years spanned the entire range of measured toxicity. Data on streams that were investigated for several years were pooled to avoid temporal pseudoreplication. Physical and chemical standard parameters were measured monthly in April (period before the application of insecticides), May, and June. Oxygen, pH, and temperature were recorded with instruments made by WTW (Weilheim, Germany). Concentrations of nitrate, nitrite, ammonium, and phosphate were determined in

* To whom correspondence may be addressed (matthias.liess@ufz.de).

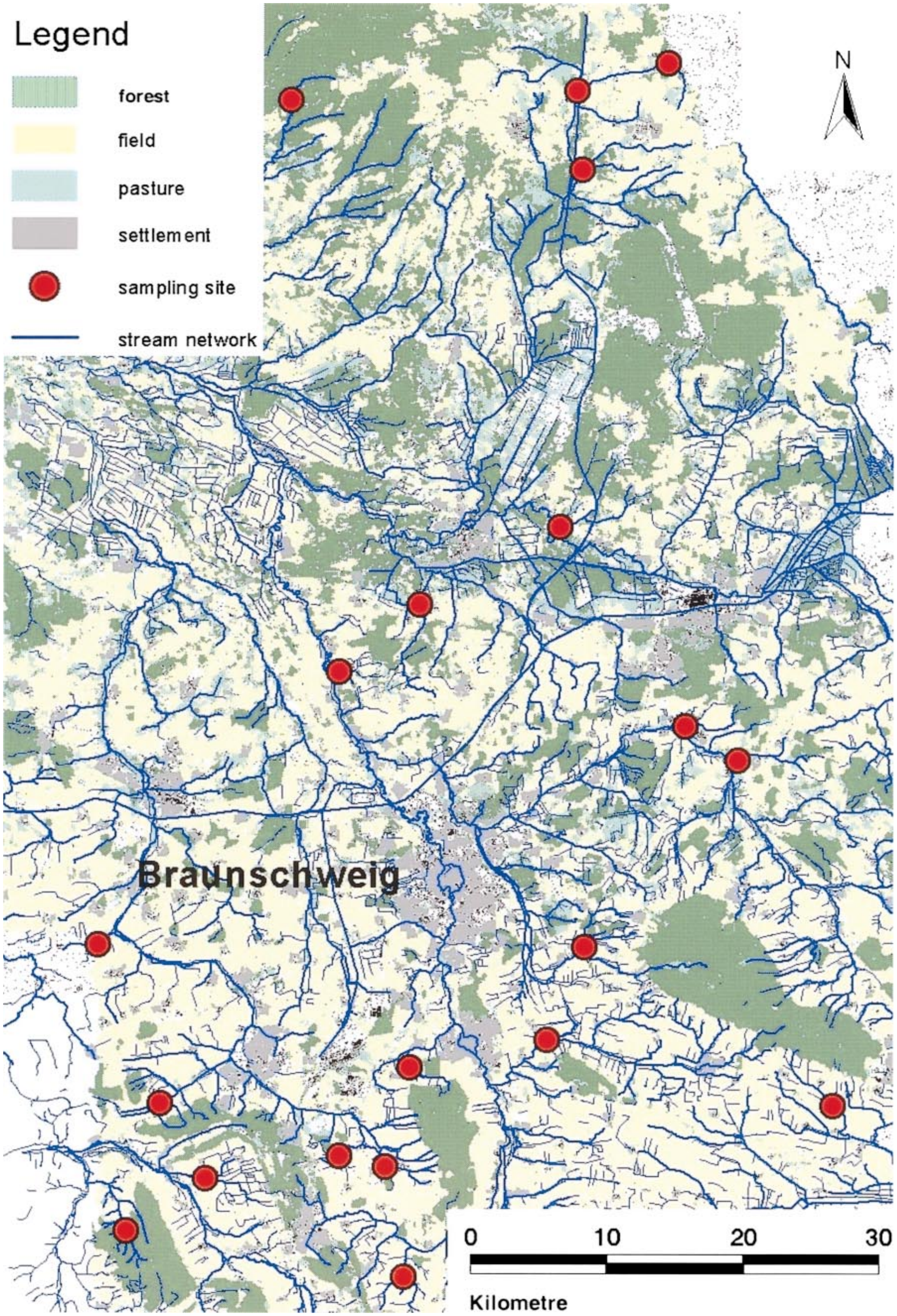

Fig. 1. Stream network, land-use pattern, and distribution of the sampling sites located around Braunschweig, Lower Saxony, Germany.

the field with colorimetric tests by Visicolor® (Macherey&Nagel, Düren, Germany). Detection limits were 5.0, 0.005, 0.025, and 0.25 mg/L, respectively. During April, May, and June 1998, the concentrations of suspended particles were estimated weekly at nine sites using sediment traps [5]. Stream width was measured at each location at mean water level. The biological oxygen demand was measured once during mid-March, because during this time oxygen depletion might occur due to field applications of liquid manure. However, application of liquid manure was not observed in any of the investigated catchments. We investigated in-stream structure and channel alteration of sites by visually estimating the percentage of various substrates and macrophytes in a 50-m reach within the stream on the base of the German classification system of morphological structure [6] (Table 1). Stream sections up- and downstream of the investigated sites that were bordered by forest or meadows at least 50 m wide on both sides were identified using geographic information system maps. The length of these stream sections and the distances from the investigated sites yielded estimates of the potential for in-stream recolonization by invertebrates. No correlation between any of the investigated parameters with the measured toxicity was observed. Hence, the description of sites in Table 1 is an average of all sites.

Quantification of pesticides

The study sites did not receive point-source inputs of pesticide residues. Therefore, we assumed that pesticide peak concentrations in the streams we studied were due to runoff-induced inputs via nonpoint sources, and that these inputs were the dominant source of exposure to pesticides [7]. We used two event-controlled runoff sampling systems to characterize the exposures. One system was an automated active sampler that measured conductivity and water level in the stream continuously. Samples of 500 ml were obtained every 8 min for 1 h (pooled) when runoff was indicated by a decline in conductivity of more than 10% within 10 min, or if water level increased by ≥5 cm [8]. The samples were prefiltered (0.2 μm) and pumped under high pressure (6 bar) on-site through a C18 column (Bakerbond, Baker, Hannover, Germany). The second system consisted of two passive samplers. Each of these was a 1-L bottle mounted in the stream. Rising water level triggered sampling, and the bottles then filled through a thin (5-mm diameter) glass tube, 10 cm in length. The glass tubes of the samplers were positioned 5 and 10 cm above medium water level. The water samples collected by these devices were prefiltered (0.2 μm) and processed by solid-phase extraction with C18 columns in the laboratory. Both sampling systems were checked every week from April to July, the period before and during the most toxic pesticides (insecticides) are used and detectable in this area [5,8].

Selection of analyzed pesticides was done on the base of use information provided by the local agricultural advisory board (Pflanzenschutzamt, Hamburg, Germany). Analyzed pesticides are listed in Table 1. Pesticide measurements were made with a gas chromatograph (GC)/electron capture detector (GC NP 5990, Series II; Hewlett-Packard, Avondale, PA, USA). The values were confirmed with GC/mass spectrometry (negative chemical ionization, a Varian 3400 GC [Walnut Creek, CA, USA]). The GC/mass spectrometry was fitted with an HP 7673 autosampler, which directly was capillary-coupled to the quadruple mass spectrometer SSQ 700 (Finnigan, Bremen, Germany), with a quantification limit of 0.05 μg/L. The results obtained from the two sampling systems did not differ significantly, so the data were pooled by sampling method. Data on the measured pesticide concentrations are summarized in Table 1 and 2.

Derivation of the index of toxicity

To compare the toxicity of pesticides present during runoff events in the different streams, toxic units (TU) were calculated from the measured concentrations [9]. The TU values for each compound were based on the acute (48-h) LC50 of *D. magna* (Eqn. 1, below; from Tomlin [10]):

$$\mathrm{TU}_{(D.\ magna)} = \log\,(C_i/\mathrm{LC50}_i) \qquad (1)$$

where $\mathrm{TU}_{(D.\ magna)}$ is the toxic unit of the pesticide *i*, with the highest $\mathrm{TU}_{(D.\ magna)}$ for one runoff event; C_i = the concentration (μg/L) of the respective pesticide *i*; and $\mathrm{LC50}_i$ = the corresponding LC50 (48 h) of *D. magna* exposed to substance *i* (μg/L).

A TU value of −5 was assigned for the two sites where no pesticides were found. For additional calculations, the highest calculated $\mathrm{TU}_{(D.\ magna)}$ based on the measurements of pesticides at one site was used. We also evaluated the possibility of adding all pesticides detected in one runoff event, and adding all pesticides detected in one year. We used $\mathrm{TU}_{(D.\ magna)}$ calculated with the highest $\mathrm{TU}_{(D.\ magna)}$ for one substance, because this was the most basic assumption. This method also yielded slightly higher correlations between TU values and community endpoints, compared to correlation values computed with TU values calculated using methods adding all pesticides detected in one runoff event or adding all pesticides detected in one year.

Macroinvertebrate sampling

Invertebrates were collected three times per year (April, May, June) from 1998 to 2000, with a Surber sampler (area, 0.062 m^2; 1-mm mesh). On each sampling date, four samples were taken randomly over a stream length of 50 m. Macroinvertebrates were sorted in white plastic tubs, preserved in 70% EtOH, and identified to species level except for dipterans, which were identified to family. Earlier investigations showed that the highest concentrations of the most toxic insecticides in the investigated area occurred during May and, to a lesser extent, in June [2,8]. Hence, our April surveys occurred before the main period of contamination and were regarded to represent possible long-term effects of pesticides from the previous year. Similarly, the May surveys occurred when pesticide levels were highest, so the May and June surveys represent possible short-term effects of pesticides from the same year.

Species at risk

Site-specific combinations of environmental factors resulted in a unique composition of species at each site, masking the effect of individual environmental factors. In our investigation, this difficulty was reduced by grouping species according to their sensitivity to pesticides [11] and their life-cycle traits that are known to influence recovery from toxicant effects [12,13]. The sensitivity to organic toxicants was assigned on the basis of the classification of [14] and its revision by [11]. Species with a value >-0.36 (median of sensitivity, from Von der Ohe et al. [11]) were regarded as sensitive. Three main life-cycle traits were used. These traits were generation time, migration ability, and presence of sensitive aquatic stages during the time of maximum exposure to pesticides. Gener-

Table 1. Mean ± standard deviation (SD), minimum, and maximum of environmental parameters at 20 sites in streams during April to July, 1998 to 2000

Parameter (units)	Mean	± SD	Min.	Max.
Physical				
Width (m)[a]	1.30	±0.44	0.50	2.50
Depth (m)[a]	0.16	±0.10	0.04	0.60
Current (m/s)[a]	0.17	±0.09	0.02	0.50
Temperature (°C)[a]	13.3	±3.0	3.5	19.5
Suspended particles (ml/week)[b]	161	±69	77	294
Catchment area (km^2)[c]	12.6	±4.9	6.0	20.4
Gradient of streams (%)	4.4	±1.9	2.1	8.1
Forest, length upstream (m)[c]	730	±800	0	3,300
Forest, distance upstream (m)[c]	2,640	±2,300	0	5,300[d]
Streambed substrate				
Cobble (%)[a]	2	±7	0	30
Gravel (%)[a]	5	±10	0	40
Sand (%)[a]	24	±37	0	100
Silt (%)[a]	55	±46	0	100
Streambed cover				
Allochton leaves (%)[a]	20	±28	0	100
Submersed plants (%)[a]	8	±11	0	50
Emerged plants (%)[a]	5	±9	0	65
Filamentous algae (%)[a]	1	±4	0	25
Water quality—standard				
Oxygen (mg/L)[a]	10.2	±2.2	3.4	13.8
pH[a]	7.9	±0.34	6.8	8.6
Nitrate (mg/L)[a]	3.4	±9.2	0.5	47.5
Nitrite (mg/L)[a]	0.15	±0.13	0.01	0.80
Ammonium (mg/L)[a]	0.07	±0.21	0.00	1.75
Ortho-phosphate (mg/L)[a]	0.19	±0.13	0.00	0.60
Insecticides				
Beta-cyfluthrin (μg/L)[a]	ND[e]			
Cypermethrin (μg/L)[a]	ND			
Es-fenvalerate (μg/L)[a]	ND			
Lambda-cyhalothrin (μg/L)[a]	ND			
Lindane (μg/L)[a]	0.25	±0.07	ND	0.3
Parathion-ethyl (μg/L)[a]	0.24	±0.12	ND	0.5
Pirimicarb (μg/L)[a]	ND			
Fungicides				
Kresoxim-methyl (μg/L)[f]	0.41	±0.49	ND	2.9
Epoxiconazol (μg/L)[f]	0.40	±0.69	ND	5.6
Azoxystrobin (μg/L)[f]	0.46	±1.31	ND	11.1
Propiconazol (μg/L)[f]	0.60	±0.34	ND	0.8
Fenpropimorph (μg/L)[f]	0.20	±0.14	ND	0.4
Tebuconazol (μg/L)[f]	0.56	±1.74	ND	9.1
Herbicides				
Bifenox (μg/L)[f]	0.27	±0.14	ND	0.5
Chloridazon (μg/L)[f]	5.63	±12.12	ND	33
Ethofumesat (μg/L)[f]	8.66	±23.35	ND	129
Isoproturon (μg/L)[f]	0.62	±0.67	ND	2.6
Metamitron (μg/L)[f]	1.18	±2.08	ND	9.3
Metribuzin (μg/L)[f]	0.27	±0.33	ND	1.2
Pendimethalin (μg/L)[f]	0.40	±0.00	ND	0.4
Prosulfocarb (μg/L)[f]	0.35	±0.39	ND	1.0
$TU_{(D.\ magna)}$[g]	−2.52	±1.46	−5.00	−0.70

[a] Measured monthly.
[b] Measured at nine sites.
[c] Measured once.
[d] Distances to forested stream sections greater than 6,000 m were not taken into account.
[e] ND = not detected.
[f] Measured event controlled.
[g] TU = toxic units.

ation time ≥ 0.5/year was regarded as potentially sensitive due to a slow recovery potential [12,13,15]. If no data on generation time could be found for a species, we assigned it the value for its closest relative. Low migration ability was presumed to reflect reduced potential for recolonization. Species regarded as being not at risk due to their better ability to migrate included *Gammarus pulex*, *Limnephilus lunatus*, and *Anabolia nervosa* [16]. Species for which adults emerged be-

Table 2. Short-term peak concentrations of pesticides in streams during runoff events. Only concentrations with a toxicity of $TU_{(D.\ magna)} > -2.5$ are shown. TU = toxic units; LC50 = median lethal concentration

Site	Date	Compound	$LC50_{D.\ magna}$	Concn. (μg/L)	$TU_{(D.\ magna)}$
1	03-05-98	Parathion-ethyl	2.5	0.5	−0.70
1	02-06-98	Parathion-ethyl	2.5	0.3	−0.92
1	14-07-98	Parathion-ethyl	2.5	0.3	−0.92
2	03-05-98	Parathion-ethyl	2.5	0.3	−0.92
3	24-05-00	Parathion-ethyl	2.5	0.3	−0.92
4	03-05-98	Parathion-ethyl	2.5	0.2	−1.10
1	23-05-00	Parathion-ethyl	2.5	0.2	−1.10
5	19-07-98	Parathion-ethyl	2.5	0.2	−1.10
2	03-05-98	Parathion-ethyl	2.5	0.2	−1.10
5	12-05-99	Azoxystrobin	259	11.1	−1.37
1	30-05-98	Parathion-ethyl	2.5	0.1	−1.40
6	24-05-00	Parathion-ethyl	2.5	0.05	−1.70
7	12-05-99	Kresoxim-methyl	168	2.9	−1.81
7	12-05-99	Azoxystrobin	259	3.8	−1.83
8	15-06-00	Azoxystrobin	259	3.8	−2.02
9	14-06-99	Ethofumesat	13,500	129	−2.02
10	15-06-00	Azoxystrobin	259	2.1	−2.09
7	05-07-99	Azoxystrobin	259	2.0	−2.11
2	12-05-99	Kresoxim-methyl	168	1.0	−2.27
7	03-06-98	Azoxystrobin	259	1.3	−2.30
1	30-05-98	Kresoxim-methyl	168	0.9	−2.32
2	02-06-98	Kresoxim-methyl	168	0.9	−2.32
2	27-05-98	Kresoxim-methyl	168	0.8	−2.37
7	03-06-98	Kresoxim-methyl	168	0.7	−2.42
7	14-06-98	Kresoxim-methyl	168	0.7	−2.42
7	27-05-98	Kresoxim-methyl	168	0.6	−2.49
1	23-05-98	Kresoxim-methyl	168	0.6	−2.49
2	24-06-98	Kresoxim-methyl	168	0.6	−2.49

fore May (before the time of maximum pesticide application) were regarded as insensitive, because exposure to aquatic stages would not occur. This categorization was made based on best professional judgment. The three traits were combined with the Boolean ''and'' in our literature searches. Only if all three traits were present did we consider a species to have a potential sensitivity to pesticides; the species was then regarded as a species at risk of being affected by pesticides. All life-cycle traits used as SPEAR attributes were obtained from the literature, including online sources (http://www.ent3.orst.edu/StreamEcology/database/streamlife.htm) [17–25]. All classifications followed the definitions listed in Table 3.

Community endpoints

The following endpoints for the invertebrate communities were calculated: Species number, or the mean number of species found on the three sampling dates during one year. The index of diversity and evenness is calculated according to Shannon-Wiener. The $SPEAR_{(number)}$, or the number of species at risk, is calculated according to the definition given above. Accordingly, the number of species not at risk is termed $SPEnotAR_{(number)}$. The abundance of species at risk, the $SPEAR_{(abundance)}$, is calculated as the sum of the log abundance of each species. The index Percent-$SPEAR_{(abundance)}$ is the ratio of the abundance of species at risk compared to the abundance of all species. Temporal change of community endpoints was assessed from April to May, and from April to June, based on the concept of SPEAR.

Statistical analyses

For multivariate linear regressions, data normality was determined with the Kolmogoroff-Smirnoff test. Where significant deviation from the assumption of normality occurred, the data were transformed. Equality of variances was verified using Levene's homogeneity-of-variance test. Environmental factors were not intercorrelated with $TU_{(D.\ magna)}$ (linear regression, $p > 0.05$). Statistical procedures used were: Stepwise entering of variables (criteria: Probability of F to enter ≤ 0.05, probability of F to remove ≥ 0.10), adjusted r^2. Differences between groups of sites with different $TU_{(D.\ magna)}$ were investigated using one-way analysis of variance (ANOVA). Dunnett's multiple comparison test was used to detect significant differences among means. Differences between slopes of regression lines of sites with and without forested stream sections (covariate) were identified using analysis of covariance. Multivariate linear regressions were carried out with SPSS® 11 for Macintosh® (Chicago, IL, USA). Both ANOVA and analysis of covariance were carried out with Prism® for Macintosh (GraphPad Software, San Diego, CA, USA).

RESULTS

Environmental conditions

During the investigation period (1.8 years per site), pesticides were detected in 125 runoff events at 18 of the 20 sites. Runoff events with a toxicity > -2.5 occurred between May and July; most of the contaminated-runoff events occurred in May (57%), followed by June (32%), and July (11%). In April, no runoff events with a TU above −3 were detected. The four pesticides contributing the most to the TUs ($TU_{[D.\ magna]}$) were parathion-ethyl, azoxystrobin, kresoxim-methyl, and ethofumesat (Table 2). The $TU_{(D.\ magna)}$ varied between sites, but 40% of the between-year variance among sites that were investigated more than one year was explained by the value measured in one year (linear regression of $TU_{(D.\ magna)}$ of $year_n$ with $year_{n+1}$; $r^2 = 0.40$, $p < 0.01$).

The morphological diversity of the streams was poor because streambeds contained a large percentage of fine substrates and the amount of plants or organic debris was slight in most cases. According to the German classification system for assessing stream morphology, the sites were classified as strong and heavily modified [6]. Based on the morphological and standard water quality parameters, the sites in the area of Braunschweig were typical of those for small lowland streams [26]. The physical and chemical characteristics of the streams are summarized in Table 1.

Correlating environmental parameters and community descriptors

The measure for toxic stress of pesticides $TU_{(D.\ magna)}$ best described the variance of community descriptors related to SPEAR (Table 4). In general, the number and abundance of SPEAR correlated negatively with $TU_{(D.\ magna)}$. In contrast, the average number and abundance of species not at risk (SPEnotAR) did not correlate with toxic stress. Other parameters contributing to the variability of SPEAR are length of forested stream sections, type of substrate, and coverage with submersed plants (Table 4).

The two subdivisions of the SPEAR parameter, the sensitivity of species to pesticides and life-cycle traits influencing the recovery potential (generation time, migration ability), contributed about equally to the correlation of abundance of SPEAR with toxic stress. On average, 57% ($p < 0.01$) of the explained variance was accounted for by species sensitivity, and 44% ($p < 0.01$) of the explained variance was accounted for by life-cycle traits related to recovery potential.

Threshold of community response to toxic stress

To determine the $TU_{(D.\ magna)}$ at which a change in community structure became apparent, the most-sensitive endpoint was used ($SPEAR_{[abundance]}$—June), compared to the abundance of all species in June (Table 4). This ratio indicated a significant change in community structure compared to sites with a low $TU_{(D.\ magna)}$ (i.e., <-4), relative to sites in the range of -3 to -2 TU and higher (Fig. 2; ANOVA, Dunnett's multiple comparison test, $p < 0.01$). The total number and the total abundance of SPEAR in April showed a dependence on $TU_{(D.\ magna)}$ also, but to a lesser extent (ANOVA, Dunnett's multiple comparison test). Then, significant differences between sites with a TU <-4 compared to sites with a TU in the range of -3 to -2, -2 to -1, and -1 to 0 were indicated by *p*-values of < 0.01, < 0.05, and < 0.01, respectively.

The sensitivity of species and the life-cycle traits contributed about equally to the reduced proportion of SPEAR at toxic stress. The number of sensitive species and the number of species with a low recovery potential according their life-cycle traits were reduced substantially at $TU_{(D.\ magna)}$ from -3 and higher (ANOVA; Dunnett's multiple comparison test, $p < 0.01$).

Temporal changes in community structure

The abundance of SPEAR decreased from April until May at sites with values of $TU_{(D.\ magna)}$ exceeding -2 to -1, compared to sites where $TU_{(D.\ magna)}$ values were below -4 (Fig. 3). Furthermore, an increase in abundance of SPEnotAR occurred from April until June at sites where $TU_{(D.\ magna)}$-values were greater than -3 to -2, compared to the sites where $TU_{(D.\ magna)}$-values were below -4 (Fig. 4).

Contribution of uncontaminated stream sections to recovery

The presence of forested stream sections >200 m in length and <4,000 m upstream of the investigated sites had a strong influence on the intercept and slope of the correlation between $TU_{(D.\ magna)}$ and SPEAR in April. When forested stream sections were present, the numbers of SPEAR tended to be greater. At the same time, the reduction of SPEAR was increasing

The $TU_{(D.\ magna)}$ was greater than at sites without forested stream sections (analysis of covariance, $p < 0.05$). However, the positive influence of forested stream sections upstream of the investigated sites compensated the negative effect of high $TU_{(D.\ magna)}$ on SPEAR. Indeed, sites with TU >-2 and forested stream sections contained a number and abundance of SPEAR similar to those at sites with TU < -3 without forested stream sections (Fig. 5). The described differences of sites with and without forested stream sections were apparent only in April, the time period before the highest toxic units have been measured. The differences of sites with and without forested stream sections were not detectable in June, the time period directly after the highest toxic units have been measured.

DISCUSSION

Finding patterns in community composition

The aim of the present investigation was to find patterns in community composition that are related to the effect of pesticides. However, as is well known, it is very difficult to determine the importance of a specific environmental factor in field investigations that involve different sites. Each site has a unique combination of environmental factors and, thus, a unique composition of species. This situation obscures the effect of any particular environmental factor. In the present investigation, we reduced this problem by grouping species according to their sensitivity to pesticides [11] and life-cycle traits known to influence recovery from toxicant stress [12,13]. The approach of grouping species at risk has the advantage of reducing the variability of the site-specific community characterization, and increasing the ability to detect the effect of pesticides on community composition at low toxic units (TU -2 to -3), values that are equivalent to <1/100 of the 48-h $LC50_{(D.\ magna)}$ (Table 4, Fig. 2). However, the SPEAR approach also has some disadvantages. For example, because species-level data are aggregated according to sensitivity and life-cycle traits related to recovery, the effect of a pesticide cannot be assigned to any particular species or taxon.

Temporal changes in community structure: Reduction in SPEAR

Sites characterized by high TUs (between -1 and 0 based on the 48-h $LC50_{[D.\ magna]}$) showed a 75% reduction of SPEAR from April to May, when the highest concentrations of pesticides were measured (Fig. 3). Other investigations of streams in agricultural areas also reported that pesticides from surface runoff can cause acute mortality of benthic invertebrates when they reach the range of the 48-h $LC50_{(D.\ magna)}$. For example, mortality of the amphipod *G. pulex* occurred at 26.8 μg/L Carbofuran, a level that is 2.5 times lower than the 48-h $LC50_{(D.\ magna)}$ [27]. Other examples include the amphipod *G. pulex* and the caddisfly *L. lunatus*, which suffered mortality at 6.0 μg/L Parathion-ethyl (3.3 times above the 48-h $LC50_{[D.\ magna]}$) [2]. Similarly, the dipteran *Chironomus* spp. had greater mortality at 0.7 μg/L Azinphos-methyl (3.6 times be-

Table 3. Classification of invertebrate species at risk of being affected by pesticides (SPEAR). E = month of emergence, G = generation time, S = sensitivity according to [11], SPEAR = species at risk (1), SPEnotAR = species not at risk (0)

Major taxa	Species	E (month)	G (year)	S	SPEAR
Annelida					
Hirudinea					
Erpobdellidae		NA[a]	0.25	−0.41	0
Glossiphoniidae		NA	0.25	−0.60	0
Hirudidae		NA	0.25	−0.60	0
Piscicolidae		NA	0.25	−0.60	0
Oligochaeta					
Lumbricidae		NA	0.25	−1.10	0
Lumbriculidae		NA	0.25	−1.40	0
Naididae		NA	0.25	−1.10	0
Tubificidae		NA	0.25	−0.93	0
Crustacea					
Amphipoda					
Corophiidae		NA	0.25	+0.17	0
Gammaridae[b]		NA	0.75	+0.04	0
Cladocera					
Daphniidae	*Daphnia* sp.	NA	0.25	+0.20	0
Decapoda					
Astacidae	*Orconectes limosus*	NA	2	−0.57	0
Atyidae	*Atyaephyra desmaresti*	NA	1	−0.08	1
Grapsidae	*Eriocheir sinensis*	NA	1	−0.08	1
Isopoda					
Asellidae	*Asellus aquaticus*	NA	0.75	−0.17	1
	Proasellus coxalis	NA	0.75	−0.56	0
Insecta					
Coleoptera					
Donaciidae		5	1	−1.15	0
Dryopidae		NA	1	−1.15	0
Dytiscidae		6–7	0.5–2	−0.81	0
	Agabus sp.	3	1–2	−0.81	0
Elmidae		8	1–2	−1.15	0
Gyrinidae		3–6	1	−1.15	0
Haliplidae		4–5	1–2	−1.83	0
	Brychius elevatus	3	2	−1.83	0
Helodidae		8–9	1	−1.15	0
Hydraenidae		3–6	1	−1.15	0
Hydrophilidae		3–7	1	−0.89	0
Noteridae		4–7	1	−1.15	0
Scirtidae	*Scirtes* sp.	8	1	−1.15	0
Diptera					
Ceratopogonidae		NA	0.25	−0.35	0
Chaoboridae		NA	0.26	−0.35	0
Chironomidae		3	0.25	−0.39	0
Culicidae		NA	0.25	−0.29	0
Dixidae		NA	0.25	−0.35	1
Dolichopodidae	*Poecilobothrus* sp.	NA	1	−0.35	1
Limoniidae		NA	1–2	−0.35	1
Muscidae	*Limnophora* sp.	NA	1	−0.35	1
Psychodidae		NA	0.25	−0.35	0
Ptychopteridae	*Ptychoptera* sp.	5	1	−0.35	1
Heteroptera					
Corixidae		NA	1	−0.29	1
	Corixa punctata	3	1	−0.31	0
	Sigara sp.	NA	1	−0.24	1
Gerridae		2	0.25	−0.56	0
Hydrometridae	*Hydrometra stagnorum*	6	1	−0.56	0
Naucoridae	*Ilyocoris cimicoides*	NA	1	−0.56	0
Nepidae		NA	1	−0.56	0
Notonectidae		NA	1	−0.82	0
Pleidae		NA	1	−0.56	0
Veliidae	*Velia caprai*	6	1	−0.56	0
Megaloptera					
Sialidae		NA	2	NA	1
Plecoptera					
Capniidae	*Capnia bifrons*	2	1	+0.38	0
Chloroperlidae		5	1	+0.38	1
	Isoptena serricornis	2	1	+0.38	0
	Siphonoperla sp.	2	1	+0.38	0
Leuctridae		4–9	1	+0.38	1
	Leuctra hipopus	3	1	+0.38	0
	L. niveola	2	1	+0.38	0
	L. proma	2	1	+0.38	0
Nemouridae		4	1	+0.25	1
	Nemoura sp.	4	1	+0.20	1
Perlodidae		5	1–2	+0.38	1
	Perlodes microcephalus	2	2	+0.38	0
Taeniopterygidae		1–3	1	+0.38	0
	Brachyptera risi	3	1	+0.38	0
Trichoptera					
Beraeidae		5	1	−0.06	1
Brachycentridae	*Brachycentrus subnubilus*	3	1	−0.06	0
Ecnomidae	*Ecnomus tenellus*	7	1	−0.06	1
Glossosomatidae	*Agapetus fuscipes*	5	0.5	−0.06	1
Goeridae		5	1	−0.06	1
Hydropsychidae		4–8	1	−1.03	0
Hydroptilidae		4–8	0.5–1	−0.06	1
	Hydroptila vectis	2	0.5–1	−0.06	0
Lepidostomatidae		8	1	−0.06	1
Leptoceridae		5–8	0.5–1	−0.06	1
Limnephilidae		4–10	1	−0.06	1
	Anabolia nervosa[b]	7	1	−0.06	0
	Limnephilus lunatus[b]	4	1	−0.06	0
	Micropterna sp.	4	1	−0.06	0
Molannidae	*Molanna angustata*	8	1	−0.06	1
Odontoceridae	*Odontocerum albicorne*	5	1	−0.06	1
Philopotamidae	*Wormaldia occipitalis*	6	1	−0.06	1
Phryganeidae		4–6	1	−0.06	1
	Oligostomis reticulata	3	1	−0.06	0
	Oligotricha striata	3	1	−0.06	0
Polycentropodidae		5–8	1–2	−0.06	1

Table 3. Continued

Major taxa	Species	E (month)	G (year)	S	SPEAR
Diptera					
		NA	1	−0.35	1
Rhagionidae		NA	0.25	−0.35	0
Simuliidae	*Simulium* sp.	NA	0.25	−0.46	0
Stratiomyiidae	*Stictotarsus duodecimpus-*	NA	1	−0.35	1
Syrphidae	*tulatus*	3	1	−0.35	0
Tabanidae		NA	1	−0.35	1
Tanypodinae		NA	0.25	−0.35	0
Tipulidae		NA	1	−0.35	1
Ephemeroptera					
Baetidae		2–5	0.5–1	+0.02	0
	Baetis rhodani	2	0.25	+0.02	0
	Centroptilum luteolum	5	0.25	−0.25	0
	Cloeon dipterum	4	0.5	−0.32	1
	Procloeon sp.	5	1	−0.25	1
Caenidae		5–6	0.5–1	−0.30	1
Ephemerellidae		4–6	1	−0.30	1
Ephemeridae		5–7	2–3	−0.30	1
Heptageniidae		4–7	1	−0.30	1
	Heptagenia sulphurea	3	0.5–1	−0.30	0
Leptophlebiidae		4–5	1	−0.30	1
Siphlonuridae		5	1	−0.30	1
Odonata					
	Aeshna cyanea	6	1–3	−0.96	0
Aeshnidae		6	1	−0.96	0
Calopterygidae		5	2	−0.36	1
Coenagrionidae		4–6	1–2	−0.24	1
Cordulegasteri-dae	*Cordulegaster boltoni*	5	2	−0.96	0
Corduliidae		5	1–2	−0.96	0
Gomphidae		5	1–2	−0.96	0
Lestidae		5	1	−0.68	0
Libellulidae		5	1–2	−1.53	0
Platycnemidi-dae	*Platycnemis pennipes*	5	1	−0.96	0

Major taxa	Species	E (month)	G (year)	S	SPEAR
Psychomyiidae		5–6	1	−0.06	1
Rhyacophilidae		4–8	1–2	−0.06	1
Sericostomatidae		4–7	1–3	−0.06	1
Uenoidae		5	1	−0.06	1
Mollusca					
Basommatophora					
Acroloxidae		NA	0.25	−1.16	0
Ancylidae	*Ancylus fluviatilis*	3	0.25	−1.16	0
Lymnaeidae		NA	0.25	−0.48	0
	Lymnaea stagnalis	NA	0.25	−0.68	0
Physidae		NA	0.25	−1.64	0
	Physa acuta	NA	0.25	−1.88	0
Planorbidae		NA	0.25	−2.20	0
Eulamellibranchia					
Dreissenidae		NA	0.25	−2.50	0
Sphaeriidae		NA	0.25	−2.50	0
Unionidae		NA	0.25	−2.50	0
Prosobranchia					
Bithyniidae		NA	0.25	−1.82	0
Hydrobiidae		NA	0.25	−1.82	0
Valvatidae		NA	0.25	−1.82	0
Viviparidae	*Viviparus contectus*	5	0.25	−1.50	0
Plathelminthes					
Turbellaria					
Dendrocoelidae	*Dendrocoelum lacteum*	NA	0.25	−0.43	0
Dugesiidae		NA	0.25	−0.47	0
Planariidae		NA	0.25	−0.43	0

[a] NA = not applicable.
[b] Not at risk due to migration ability.

Table 4. Coefficients of multiple determination (r^2) and standardized partial regression coefficients (beta; $p < 0.05$*, $p < 0.01$**, optimized model) for correlations between environmental parameters and community metrics for 20 sites in streams during April to June, 1998 to 2000.[a] All parameters not shown, but described in the *Methods* section, were minimally important in terms of explaining investigated community endpoints

				Environmental parameter				
	r^2 (Sum)	*df*	*F*	Toxic unit (m)	Forest length (%)	Hard substrate (%)	Sand (%)	Submerse plants (%)
Species number—average	0.49	17	10.0	−0.45*	+0.44*	—	—	—
Diversity—average	0.28	18	8.2	—	+0.56	—	—	—
$SPEAR_{(number)}$—average[b]	0.72	17	25.2	−0.72**	+0.28*	—	—	—
$SPEAR_{(number)}$—April	0.81	15	18.0	−0.50**	+0.28*	+0.32*	—	+0.33**
$SPEAR_{(number)}$—June	0.49	18	19.0	−0.72**	—	—	—	—
$SPEAR_{(abund)}$—average[c]	0.60	18	29.5	−0.79**	—	—	—	—
$SPEAR_{(abund)}$—April	0.69	16	15.2	−0.53**	—	+0.43**	—	+0.30*
$SPEAR_{(abund)}$—June	0.53	18	22.6	−0.75**	—	—	—	—
% $SPEAR_{(abund)}$—average	0.62	18	31.6	−0.80**	—	—	—	—
% $SPEAR_{(abund)}$—April	0.57	17	13.5	−0.56**	+0.37*	—	—	—
% $SPEAR_{(abund)}$—June	0.60	18	29.1	−0.79**	—	—	—	—
$SPEnotAR_{(abund)}$—average[d]	0.26	18	7.6	—	—	—	−0.55**	—
$SPEnotAR_{(abund)}$—June/April	0.46	17	17.3	+0.70**	—	—	—	—

[a] Included in the table are parameters that account for at least 25% of the explained variance for the most relevant parameter, and adding at least 7.5% of explained variance for all successive parameters.
[b] SPEAR = species at risk.
[c] $SPEAR_{abund}$ = species at risk abundance.
[d] SPEnotAR = species not at risk.

low the 48-h $LC50_{[D.\ magna]}$) [28]. The present investigation also revealed a 60% reduction in SPEAR from April to May, when TUs were between −1 and −2. At that time, pesticide concentrations were about ten times lower than the 48-h $LC50_{(D.\ magna)}$ (Fig. 3). This finding may be explained by the fact that long-term effects of non-narcotic substances can occur at concentrations that are ten times lower than concentrations needed to cause acute (48-h) effects [29].

No indication was found in the present investigation that parameters other than pesticides (e.g., hydrodynamic stress, water quality parameters, etc.) might be responsible for the observed short-term reduction of sensitive species. In agricultural areas, hydrodynamic stress in streams due to increased current velocity and suspended particles during runoff events can occur frequently throughout the year [8]. Hence, this stressor probably is not responsible for the short-term reduction of individuals that occurred only during May. Furthermore, in another investigation involving year-long sampling, the only significant reduction in invertebrate taxonomic richness and abundance was found in May, when the highest pesticide concentrations were measured [2]. Concentrations of nitrite and ammonia high enough to cause toxicity also would not be restricted to May. Rather, maximum concentrations of these constituents should occur either near the beginning of the growing season (due to application of fertilizer or manure from animals), or during late summer (due to elevated temperature and low water levels) [30,31]. The emergence of SPEAR during May probably does not contribute much to the

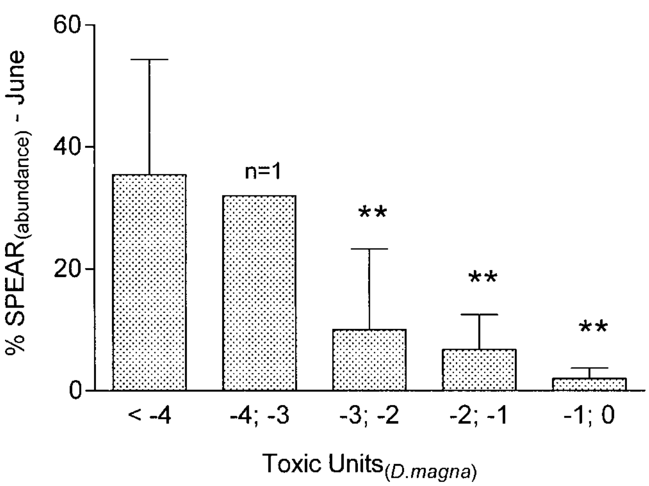

Fig. 2. Relation between toxic units$_{(Daphnia\ magna)}$ and the benthic invertebrate community structure expressed as percentage of the abundance of species at risk in June (percent $SPEAR_{(abundance)}$—June). Asterisks indicate significant differences from the sites with low $TU_{(D.\ magna)}$ (i.e., those below −4; analysis of variance, Dunnett's multiple comparison test, **$p < 0.01$). Error bars show standard deviations.

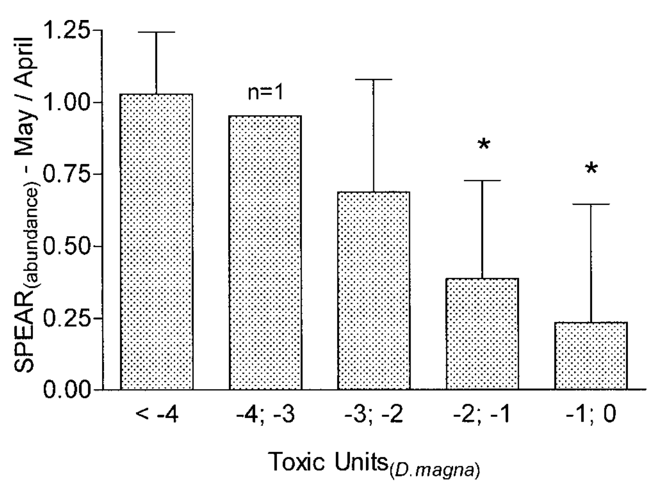

Fig. 3. Decrease of abundance of species at risk (SPEAR), from April to May. Asterisks indicate significant differences from the sites with low toxic unit$_{(D.\ magna)}$ (i.e., those below −4; analysis of variance, Dunnett's multiple comparison test, *$p < 0.05$). Error bars show standard deviations.

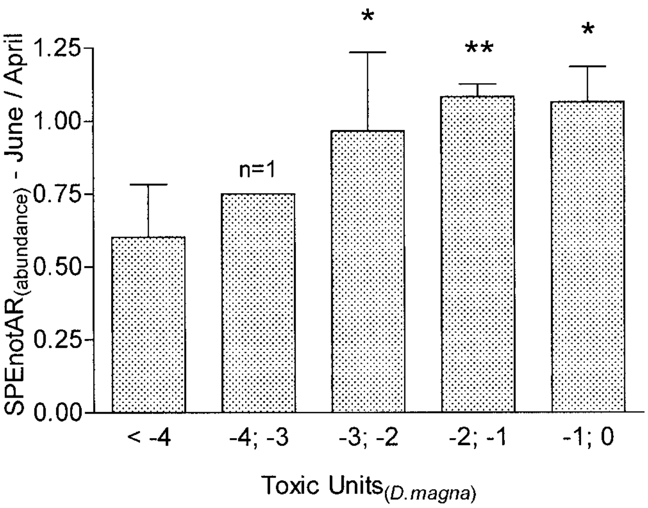

Fig. 4. Increase of abundance in species not at risk (SPEnotAR), from April to June. Asterisks indicate significant differences from the sites with low toxic units$_{(D.\ magna)}$ (i.e., below −4; analysis of variance, Dunnett's multiple comparison test, *$p < 0.05$, **$p < 0.01$). Error bars show standard deviations.

reduction of abundance at sites where TU levels are high, as abundance of SPEAR in stream benthic invertebrate communities at sites where TU levels were low did not change during May. Based on these considerations, the authors suggest that the short-term changes in SPEAR during May are best attributed to pesticides. If so, acute and chronic laboratory toxicity data for *D. magna* can provide an indication of the magnitude of concentrations at which pesticides cause a reduction in sensitive indigenous invertebrates.

Temporal changes in community structure: Alteration of SPEnotAR and long-term changes

Sites characterized by low TUs (below −3, based on the 48-h LC50$_{[D.\ magna]}$) showed a 60% reduction of SPEnotAR between April and June (Fig. 4). This reduction was not observed

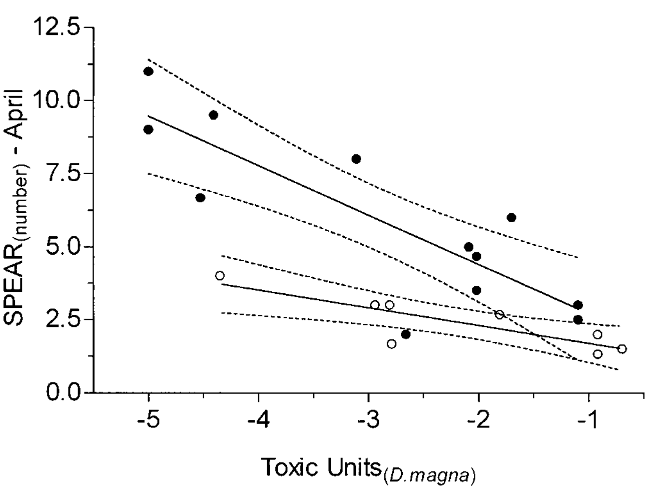

Fig. 5. Relation between toxic units$_{(Daphnia\ magna)}$ and the number of species at risk in April (SPEAR$_{(number)}$—April). Sites are differentiated on the presence of forested stream sections closer than 4,000 m upstream of the study site (filled circles; linear regression, $r^2 = 0.70$, $p < 0.01$) or absence of such sites (open circles; linear regression, $r^2 = 0.70$, $p = 0.01$). Confidence bands show the 95% confidence limit for the respective means. The slopes of the two regression lines differ (analysis of covariance, $p < 0.05$).

at sites where TU values were high (above the range of −3 and −2). This pattern could result from an indirect positive effect of pesticides on SPEnotAR due to negative effects of pesticides on sensitive species. Such negative effects might occur within the range of lethal effects (TU$_{[D.\ magna]}$ − 2 to 0) and sublethal effects (TU$_{[D.\ magna]}$ −3 to −2) [32]. We did not investigate sublethal effects in this study. However, previous investigations support the idea that short-term exposure to concentrations that are more than 100 times lower than concentration causing acute mortality can cause long-term effects. For example, development of the caddisfly *L. lunatus* was delayed in outdoor microcosms several months after a 1-h exposure to Fenvalerate at 1:1000 of the acute LC50 [33]. Similarly, an increase in mortality, a decrease in adult weight, and a delay in development of the caddisfly *L. lunatus* were observed in outdoor microcosms several months after a 1-h exposure to Fenvalerate at concentrations that were 1:100, 1:1000, and 1:10,000 of the acute LC50 [34]. Finally, mortality of Chironomidae and *Hyalella azteca* increased in littoral enclosures several weeks after short-term exposure to Es-Fenvalerate at a concentration that was 1:100 of the acute LC50 [35]. Such investigations show that low concentrations of pesticides (TU$_{[D.\ magna]}$ −3 to −2) may affect the taxonomic structure of benthic invertebrate communities, as indicated by the proportion of SPEAR in the current study.

Factors other than pesticides did not appear to be responsible for the observed long-term reduction of SPEAR. Hydrodynamic stress accompanying runoff, like other recurrent stressors, might favor species that have a high recovery potential due to a short generation time and good migration ability. So, the long-term reduction in SPEAR partly might be due to hydrodynamic stress. However, the affected taxa include those with a low recovery potential and species that are sensitive to toxicants, and no evidence suggests that the latter group especially is vulnerable to hydrodynamic stress. Thus, pesticides, rather than hydrodynamic stress, seem more likely to account for the observed reduction in sensitive species, even though they were present at low concentrations. The same logic applies to the rare events of dredging (not observed in the years before and during the investigation). Suspended particles also probably can be ruled out as causal factors. The results of experiments indicate that, compared to pesticides, suspended particles may not be very important to benthic invertebrates that dominate streams in agricultural areas. For example, the addition of suspended particles to experimental stream ecosystems in amounts up to 1.7 g/L did not alter the abundance or number of species of benthic invertebrates, or the rate of aquatic insect emergence, or the rate of leaf-litter decomposition [36]. The long-term survival of the caddisfly *L. lunatus* in outdoor test systems was not reduced by suspended particles (3 g/L), compared to controls [34,37]. The 3 g/L concentration of suspended particles in the cited investigations can be regarded as relevant to agricultural streams [8]. Finally, nitrite and ammonia probably cannot account for the change in SPEAR, because the concentrations of these constituents were lower than those that can be tolerated by most invertebrates. For example, *G. pulex* is relatively sensitive to ammonia, nitrite, and other types of organic pollution [38,39], but was abundant in our study, especially at sites with pesticide concentrations were highest. In contrast, *Asellus aquaticus* is more resistant than *G. pulex* to hypoxia and to un-ionized ammonia [39–41], but is classified as sensitive to pesticides in the present investigation.

Based on these considerations, the observed changes in SPEAR may be attributed most parsimoniously to the effect of pesticides. Factors other than pesticides clearly can influence benthic invertebrate community structure (and probably SPEAR), too, but we found no evidence that this occurred at the sites we studied. However, because the levels of contamination may have been quantified insufficiently, it remains uncertain at which concentration these changes occur.

Contribution of uncontaminated stream sections to recovery

In June, the correlation between community composition (SPEAR) and TUs was stronger than it was in April (Table 4). However, numbers of benthic invertebrate SPEAR in June were not altered significantly by the amount of forested stream sections. In contrast, the length of forested stream sections upstream of the investigated site did relate significantly to the number and proportion of SPEAR in April. The positive effect of upstream-forested stream sections on SPEAR at the downstream sites was not due to lower concentrations of contaminants at the downstream sites, because the correlation between contamination at the investigated sites and length of forested stream sections was not significant. Their relatively large distance from the study sites (several km, for many of the forested stream sections) may explain the lack of a measurable influence on physical and chemical parameters measured at the sites. On the basis of this result, we suggest that the positive effect of forested stream sections on SPEAR at downstream sites can be attributed to in-stream recolonization by invertebrates from the undisturbed stream sections where diversity is greater. This hypothesis is supported by the fact that forested stream sections had a positive effect on SPEAR only in April, 10 months after the time when pesticide concentrations were greatest. The positive effect of forested stream sections was not apparent in June, immediately after the period when pesticide concentrations were highest. Any water quality parameter that would have been ameliorated by forested stream sections should have exerted a positive influence in June, as well. In contrast, a positive effect of recolonization from forested stream sections likely is greatest in April, when more time for recolonization was available.

This hypothesis is supported by the fact, that in lotic habitats, passive dispersal of invertebrates by water currents or downstream drift can displace from 1% to 2% of benthic stream organisms. Drift is the most common means of transport for many stream invertebrates [42]. Various species of invertebrates emigrating from undisturbed stream sections can travel several km, by drifting, within a few months [43]. However, dispersal ability is species-dependent, and a more detailed evaluation would be needed to determine the particular requirements of species with very low dispersal abilities [44].

Cumulative risk

Including proximity and amount of uncontaminated stream sections in our habitat-quality assessments helped reveal the effects of pesticides. However, they also allowed us to put risks due to contamination into context with other stressors. The levels of biological impairment observed at sites with high concentrations of pesticides and good habitat quality (indexed as undisturbed upstream sections) were similar to those at sites where pesticide concentrations were low but habitat quality was poor. For the streams we studied, habitat quality seemed about as important as toxicity, expressed as TU. Thus, landscape and land-use information may increase predictability in the assessment of risk due to pesticides. For streams, we suggest that the geographical unit of assessment should be extended to include the recovery potential of the landscape associated with undisturbed upstream sections.

Acknowledgement—This work was presented and discussed as an oral presentation at the Effects of Pesticide in the Field workshop (EPIF, Le Croisic, France, 21.10.03–24.10.03, organized by the European Union and SETAC Europe). The authors are indebted to the participants, especially to V. Forbes, S. Maund, M. Streloke, and R. Sibly for valuable discussions. We are grateful to N. Berenzen, H. Schulz, and J. Wogram for their invaluable contribution to the fieldwork. We thank anonymous reviewers for very helpful comments on the manuscript.

REFERENCES

1. Leonard AW, Hyne RV, Lim RP, Pablo F, Van den Brink PJ. 2000. Riverine endosulfan concentrations in the Namoi River, Australia: Link to cotton field runoff and macroinvertebrate population densities. *Environ Toxicol Chem* 19:1540–1551.
2. Liess M, Schulz R. 1999. Linking insecticide contamination and population response in an agricultural stream. *Environ Toxicol Chem* 18:1948–1955.
3. Schulz R, Thiere G, Dabrowski JM. 2002. A combined microcosm and field approach to evaluate the aquatic toxicity of azinphosmethyl to stream communities. *Environ Toxicol Chem* 21:2172–2178.
4. Bach M, Huber A, Frede H-G, Mohaupt V, Zullei-Seibert N. 2000. Schätzung der Einträge von Pflanzenschutzmitteln aus der Landwirtschaft in die Oberflächengewässer Deutschlands—Berichte. Umwelt Bundes Amt, Berlin, Germany.
5. Liess M, Schulz R, Neumann M. 1996. A method for monitoring pesticides bound to suspended particles in small streams. *Chemosphere* 32:1963–1969.
6. Landesumweltamt Nordrhein-Westfalen. 1999. Gewässerstrukturgüte in Nordrhein-Westfalen. Essen, Germany.
7. Dabrowski JM, Schulz R. 2003. Predicted and measured levels of azinphosmethyl in the Lourens River, South Africa: Comparison of runoff and spray drift. *Environ Toxicol Chem* 22:494–500.
8. Liess M, Schulz R, Liess MH-D, Rother B, Kreuzig R. 1999. Determination of insecticide contamination in agricultural headwater streams. *Water Res* 33:239–247.
9. Peterson DR. 1994. Calculating the aquatic toxicity of hydrocarbon mixtures. *Chemosphere* 29:2493–2506.
10. Tomlin CDS. 2000. *The Pesticide Manual, a World Compendium.* Crop Protection Publications, Farnham, Surrey, UK.
11. Von der Ohe P, Liess M. 2004. Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. *Environ Toxicol Chem* 23:150–156.
12. Sherratt TN, Roberts G, Williams P, Whitfield M, Biggs J, Shillabeer N, Maund SJ. 1999. A life-history approach to predicting the recovery of aquatic invertebrate populations after exposure to xenobiotic chemicals. *Environ Toxicol Chem* 18:2512–2518.
13. Stark JD, Banks JE, Vargas R. 2004. How risky is risk assessment: The role that life-history strategies play in susceptibility of species to stress. *Proc Nat Acad Sci USA* 101:732–736.
14. Wogram J, Liess M. 2001. Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna*. *Bull Environ Contam Toxicol* 67:360–367.
15. Van Urk G, Kerkum F, Van Leeuwen CJ. 1993. Insects and insecticides in the lower Rhine. *Water Res* 27:205–213.
16. Williams D, Williams NE. 1993. The upstream downstream movement paradox of lotic invertebrates—quantitative evidence from a Welsh mountain stream. *Freshw Biol* 30:199–218.
17. Reusch H. 1985. *Zur Kenntnis der Köcherfliegenfauna des Niedersächsischen Tieflandes.* Naturschutz und Landschaftspflege in Niedersachsen, Hannover, Germany.
18. Studemann D, Landolt P, Satori M, Hefti D, Tomka I. 1992. Ephemeroptera—insecta helvetica. Fauna, 9. Schweizerische Entomologische Gesellschaft, Genf, Switzerland.
19. Hynes HBN. 1977. *A Key to the Adults and Nymphs of the British Stoneflies* (*Plecoptera*) *with notes on their Ecology and Distri-*

bution. Scientific Publication 17; 90 S. Freshwater Biological Association, Ambleside, UK.
20. Heneghan PA. 2000. The Applied Stream Ecology Class Database. Corvallis, OR, USA.
21. Wallace ID, Wallace B, Philipson GN. 1990. *A Key to the Case-Bearing Caddis Larvae of Britain and Ireland.* The Freshwater Biological Association, Cumbria, UK.
22. Tobias W, Tobias D. 1981. *Trichoptera Germanica—Bestimmungstafeln für die deutschen Köcherfliegen.* Senkenbergische Naturforschende Gesellschaft, Frankfurt am Main, Germany.
23. Glöer P, Meier-Brook C, Ostermann O. 1986. *Süsswassermollusken—Ein Bestimmungsschlüssel für die Bundesrepublik Deutschland.* Deutscher Jugendbund für Naturbeobachtung, Hamburg, Germany.
24. Nilsson A. 1996. *Aquatic Insects of North Europe—A Taxonomic Handbook.* Apollo Books, Stenstrup, Denmark.
25. Klausnitzer B. 1984. *Käfer im und am Wasser.* A. Ziemsen Verlag, Wittenberg, Germany.
26. Faasch H. 1997. *Gewässergütebericht—Ergänzungen* 1997. Bezirksregierung Braunschweig, Braunschweig, Germany.
27. Matthiesen P, Sheahan D, Harrison R, Kirby M, Rycroft R, Turnbull A, Volkner C, Williams R. 1995. Use of a *Gammarus pulex* bioassay to measure the effects of transient carbofuran runoff from farmland. *Ecotoxicol Environ Saf* 30:111–119.
28. Schulz R, Peall SKC, Hugo C, Krause V. 2001. Concentration, load, and toxicity of spraydrift-borne Azinphos-methyl at the inlet and outlet of a constructed wetland. *Ecological Engineering* 18: 241–247.
29. Roex EWM, Van Gestel CAM, Van Wezel AP, Van Straalen NM. 2000. Ratios between acute aquatic toxicity and effects on population growth rates in relation to toxicant mode of action. *Environ Toxicol Chem* 19:685–693.
30. Kladivko EJ, Vanscoyoc GE, Monke EJ, Oates KM, Pask W. 1991. Pesticide and nutrient movement into subsurface tile drains on a silt loam soil in Indiana. *J Environ Qual* 20:264–271.
31. Chokmani K, Gallichand J. 1997. Use of indicators for assessing the diffused pollution potential on two agricultural watersheds. *Can Agric Eng* 39:113–122.
32. Fleeger JW, Carman KR, Nisbet RM. 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci Total Environ* 317:207–233.
33. Liess M, Schulz R. 1996. Chronic effects of short-term contamination with the pyrethroid insecticide fenvalerate on the caddisfly *Limnephilus lunatus*. *Hydrobiologia* 324:99–106.
34. Liess M. 2002. Population response to toxicants is altered by intraspecific interaction. *Environ Toxicol Chem* 21:138–142.
35. Lozano SJ, Ohalloran SL, Sargent KW, Brazner JC. 1992. Effects of esfenvalerate on aquatic organisms in littoral enclosures. *Environ Toxicol Chem* 11:35–47.
36. Fairchild JF, Boyle T, English TR, Rabeni C. 1987. Effects of sediment and contaminated sediment on structural and functional components of experimental stream ecosystems. *Water Air Soil Pollut* 36:271–293.
37. Schulz R, Liess M. 2001. Runoff simulation with particle-bound fenvalerate in multispecies stream microcosms: Importance of biological interactions. *Environ Toxicol Chem* 20:757–762.
38. Berenzen N, Schulz R, Liess M. 2001. Effects of chronic ammonium and nitrite contamination on the macroinvertebrate community in running water microcosms. *Water Res* 35:3478–3482.
39. Friedrich G. 1990. Eine Revision des Saprobiensystems. *Zeitschrift für Wasser- und Abwasserforschung* 23:141–152.
40. Maltby L. 1995. Sensitivity of the crustaceans *Gammarus pulex* (L.) and *Asellus aquaticus* (L.) to short-term exposure to hypoxia and un-ionized ammonia: Observations and possible mechanisms. *Water Res* 29:781–787.
41. Whitehurst IT. 1991. The Gammarus–Asellus Ratio as an index of organic pollution. *Water Res* 25:333–340.
42. Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. *Annu Rev Ecol Syst* 32:159–181.
43. Hatakeyama S, Yokoyama N. 1997. Correlation between overall pesticide effects monitored by shrimp mortality test and change in macrobenthic fauna in a river. *Ecotoxicol Environ Saf* 36:148–161.
44. Purse BV, Hopkins GW, Day KJ, Thompson DJ. 2003. Dispersal characteristics and management of a rare damselfly. *J Appl Ecol* 40:716–728.