Environment Agency

using science to create a better place

Freshwater biological indicators of pesticide contamination – an adaptation of the SPEAR approach for the UK

Science Report – SC030189/SR4

SCHO0508BOBX-E-P

ea/br/e/sci/v1

The Environment Agency is the leading public body protecting and improving the environment in England and Wales.

It's our job to make sure that air, land and water are looked after by everyone in today's society, so that tomorrow's generations inherit a cleaner, healthier world.

Our work includes tackling flooding and pollution incidents, reducing industry's impacts on the environment, cleaning up rivers, coastal waters and contaminated land, and improving wildlife habitats.

This report is the result of research commissioned and funded by the Environment Agency's Science Programme.

Published by:
Environment Agency, Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol, BS32 4UD
Tel: 01454 624400 Fax: 01454 624409
www.environment-agency.gov.uk

ISBN: 978-1-84432-903-8

Further copies of this report are available from:
The Environment Agency's National Customer Contact Centre by emailing:
enquiries@environment-agency.gov.uk
or by telephoning 08708 506506.

Author(s):
Beketov, M.A., Foit, K., Biggs, J.P., Sacchi, A., Schäfer, R.B., Schriever, C.A., Liess, M.

Dissemination Status:
Released to all regions
Publicly available

Keywords:
Pesticides, bioindicator, monitoring, SPEAR, macroinvertebrates, community,

Research Contractor:
UFZ – Helmholtz Centre for Environmental Research
Department of System Ecotoxicology
Permoserstrasse 15
04318 Leipzig
Germany

Environment Agency's Project Manager:
Claire Wells, Science Department

Science Project Number:
SC030189

Product Code:
SCHO0508BOBX-E-P

Science at the Environment Agency

Science underpins the work of the Environment Agency. It provides an up-to-date understanding of the world about us and helps us to develop monitoring tools and techniques to manage our environment as efficiently and effectively as possible.

The work of the Environment Agency's Science Department is a key ingredient in the partnership between research, policy and operations that enables the Environment Agency to protect and restore our environment.

The science programme focuses on five main areas of activity:

- **Setting the agenda**, by identifying where strategic science can inform our evidence-based policies, advisory and regulatory roles;
- **Funding science**, by supporting programmes, projects and people in response to long-term strategic needs, medium-term policy priorities and shorter-term operational requirements;
- **Managing science**, by ensuring that our programmes and projects are fit for purpose and executed according to international scientific standards;
- **Carrying out science**, by undertaking research – either by contracting it out to research organisations and consultancies or by doing it ourselves;
- **Delivering information, advice, tools and techniques**, by making appropriate products available to our policy and operations staff.

Steve Killeen

Steve Killeen

Head of Science

Executive summary

Monitoring for pesticides in surface waters can be problematic. These substances are generally diffuse and transient pollutants and are often present at low concentrations. Difficulties in monitoring for pesticides makes attributing any observed changes in a biological community to pesticide contamination complicated. An indicator that would allow us to diagnose pesticide contamination at a site would be very useful.

A review of current biological indicators shows the SPEAR approach to be one of the most promising bioindicator methods to detect pesticide contamination in lotic ecosystems (Schriever *et al*. 2008). The SPEAR approach has been used to show a link between levels of insecticide and fungicide exposure and stream macroinvertebrate community structure. As the SPEAR approach is based on species traits it is not restricted to use in one geographic area, and it has been successfully applied in different biogeographical regions in Europe. It was therefore proposed that the pesticide-specific SPEAR indicator should be revised for use in England and Wales for possible inclusion in routine Environment Agency monitoring.

The SPEAR concept is currently the only trait-based approach to identify species vulnerable to pesticide contamination. The approach is based on species sensitivity to organic toxicants and life-cycle traits responsible for potential exposure and recovery (generation time, migration ability, presence of aquatic stages during maximum pesticide exposure), and classifies the species into “species at risk” and “species not at risk” according to these traits. The existing SPEAR indicator is based on information on macroinvertebrates from mainland Europe. Although this may be directly applicable in the UK, adaptation and revision was desirable for a more efficient application of the method in this geographical region. In particular, the trait database required some limited revision and updating with UK species and ecological information from this region.

The existing SPEAR indicator is based on species-level data. Much of the macroinvertebrate data collected by the Environment Agency is at a family level. It was therefore necessary to develop a family-level SPEAR indicator and to establish whether the family-level tool remained sensitive enough to indicate pesticide exposure.

In summary, the aim of this study was to revise and update the SPEAR database for use in the UK and to compare the SPEAR indices based on species and family levels of taxonomic identification using data sets for other European regions (Finland, France, and Germany).

Revision of the database resulted in addition of 38 new taxa. For 125 taxa, UK-specific ecological information was included in the database as separate region-specific entries. For 54 taxa information on ecological traits was corrected, but not defined as UK-specific. Sixty-six out of all 152 families in the database were defined as families at risk for UK conditions. The updated database (Liess *et al*. 2008) now contains most of the UK stream macroinvertebrate taxa together with information about their respective ecological traits.

Statistical comparison of the SPEAR indexes based on family and species levels of taxonomic resolution has shown that the family-level index can be effectively used to detect pesticide contamination in streams. The effect of upstream recovery areas and levels of seasonal variability were similar for both the family and species level indexes. The predictive power of the family-level index is expected to be only slightly lower than that of the species-level index. Taking into account the time-consuming nature, cost and difficulties of species-level identification, the family-level index is a promising and cost-effective bioindicator tool for detecting pesticide contamination in streams.

Future application of the SPEAR approach in the UK requires validation in field investigations. A field survey programme, including assessment of exposure and effect, should be performed to validate the SPEAR approach for UK conditions.

Contents

List of tables and figures

1 Introduction

In order to understand whether the use of agricultural pesticides is having an impact on the aquatic ecology of the UK, we need to be able to link observed biological degradation at a site to pesticide exposure. The monitoring of pesticide exposure is problematic due to the large number of different compounds potentially present, their diffuse and transient nature, and the fact that they are often present at low concentrations. It would thus be useful to have a biological indicator that would indicate pesticide exposure, even if we were unable to monitor the pesticides themselves.

A previous review of current biological indicators for pesticide assessment (Schriever *et al*., 2008) recommended that a community based indicator could be used as a screening tool at the landscape level. It was envisioned that this tool could be used as a quick and cheap method of identifying areas where pesticide exposure may be adversely affecting the aquatic community. If pesticide impacts were indicated, then further work would be needed to identify the type of pesticide responsible.

Based on recommendations in Schriever *et al.* (2008), it was suggested that the SPEAR approach be revised and validated for UK conditions. The SPEAR index is the only method available at present that can link degradation of biology at a site to pesticide exposure and give an indication of the level of contamination (Schriever *et al.* 2008). The SPEAR approach has been successfully used in different biogeographical regions in Europe. Field studies in Germany, France, and Finland have shown a firm link between insecticide and fungicide exposure and stream macroinvertebrate community alterations expressed as pesticide-specific SPEAR indices (Liess and von der Ohe 2005; Schäfer *et al*. 2007; von der Ohe *et al*. 2007). In addition, this approach has been shown to be relatively independent of abiotic environmental factors other than pesticides (Liess and von der Ohe 2005; Schriever *et al*. 2007).

The SPEAR concept is currently the only trait-based approach to identify species vulnerable to pesticide contamination. This approach is based on species sensitivity to organic toxicants (von der Ohe and Liess 2004) and life-cycle traits. Certain traits, such as the length of the life cycle, the ability of the species to migrate, and whether the species is present in the water during periods of expected maximum pesticide exposure, can be used to estimate the potential for exposure and recovery. The species are classified into “species at risk” and “species not at risk” according to these characteristics (Liess and von der Ohe 2005).

The SPEAR bioindicator approach was expected to be applicable in the UK. However, it was necessary to revise and update the trait database (Liess *et al.* 2008) with UK species that were not already included and ecological information from this region, as life-cycle traits such as emergence time can vary depending on biogeographic region.

In addition, we investigated whether it was possible to create a SPEAR indicator based on family-level macroinvertebrate data that was still a sensitive indicator of pesticide exposure, as most of the macroinvertebrate data collected by the Environment Agency is at the family level. If such an indicator could be created, it would require no changes to current Environment Agency monitoring procedures.

The aim of this study was to revise and update the SPEAR database and to compare SPEAR indices based on species and family levels of taxonomic identification using existing European data sets (Liess and von der Ohe 2005; Schäfer *et al*. 2007).

2 Revision of SPEAR database

The SPEAR database (Liess *et al*. 2008) contains information about ecological traits of macroinvertebrate taxa that is used for computation of the SPEAR indices. Currently, this database includes information on taxon-specific sensitivity to organic toxicants ($S_{organic}$, von der Ohe and Liess 2004), generation time, presence of aquatic stages in water during the maximum pesticide usage period, and migration abilities. These traits are used to define “species at risk” according to criteria defined in Liess and von der Ohe (2005), with the only exception that the parameter “presence of aquatic stages during maximum pesticide exposure” is now defined not only according to emergence time of merolimnic insects, but also by the duration of adult life-spans. A taxon is regarded as a “species at risk” if it has: (i) a $S_{organic}$ value above -0.36; (ii) a generation time equal to or above half a year; (iii) aquatic stages during May-June; and (iv) low migration abilities (for details see Liess and von der Ohe 2005). Defining a “species at risk” is performed automatically by the algorithm included in the database. Taxa defined as “species at risk” are used for the calculation of the pesticide-specific $SPEAR_{pesticides}$ indicator (details of the calculation method are given in Section 3).

In addition to ecological traits, the database contains references to the information sources used, and other information (type of species, geographical distribution, synonyms of species and genus names, body size of aquatic stages). Where region-specific differences were found for species, species-specific data sets are attributed to particular regions. Otherwise taxa are considered as universal and marked with “UNI” (for detailed explanations, see the database, Liess *et al*. 2008).

In order to adapt the SPEAR approach for use in England and Wales, the SPEAR database was revised and a plausibility check was performed by expert judgement. The species list was checked for completeness in representing the UK species list and existing ecological information was checked for validity for the UK region.

Revision of the database resulted in the addition of 38 new taxa (Table 2.1). For 125 taxa, UK-specific ecological information was included in the database as separate entries (coded with UK).In these UK-specific entries, information on generation time was changed for 47 taxa and for emergence time for 88 taxa (both parameters were changed for 10 taxa). For 54 taxa, information on ecological traits was updated based on additional information, but not defined as UK-specific (coded with UNI). Twelve new information sources were added.

In order to adapt the SPEAR database to the family level of taxonomic resolution, family-level SPEAR definitions (whether family at risk or not at risk) were derived for each species included in the database. These definitions were calculated according to the majority of the species comprising the family (above 50 per cent). Hence, all species from a particular family would have the same family-level SPEAR definition for a particular region, and any of them could be used for calculating the family-level $SPEAR_{pesticides}$ index. Family-level SPEAR definitions are shown in separate columns in the database for different regions, including the UK. As for species, defining the “families at risk” is performed automatically by the algorithm in the database. In addition, the complete list of families with their SPEAR definition for the UK was created on a separate page of the database (152 families). This list included 66 families at risk, 83 not at risk, and three which are not found in the UK (Table 2.2).

The updated database is now ready for use in the UK, as it contains the majority of the UK stream macroinvertebrate taxa together with information about their respective ecological traits.

Table 2.1: List of taxa newly included in the SPEAR database (Liess *et al*. 2008)

Order	Species
Odonata	*Aeshna isosceles*
	Coenagrion lunulatum
	Coenagrion mercuriale
	Libellula fulva
	Somatiochlora arctica
	Oxygastra curtisii *
Ephemeroptera	*Labiobaetis atrebatinus*
	Caenis pusilla
	Electrogena affinis
	Ephemera lineata
	Procloeon pennulatum
Plecoptera	*Brachyptera putata*
	Capnia vidua
	Chloroperla tripunctata
	Dinocras cephalotes
	Isogenusa nubecula
	Leuctra inermis
	Nemoura erratica
	Protonemura montana
	Protonemura praecox
	Rhabdiopteryx acuminata
Trichoptera	*Agapetus delicatulus*
	Agapetus ochripes
	Allotrichia pallicornis
	Beraea maurus
	Chimarra marginata
	Diplectrona felix
	Ecclisopteryx guttulata
	Glossosoma conformis
	Glossosoma intermedium
	Glossosoma boltoni
	Limnephilus coenosus
	Metalype fragilis
	Philopotamus montana
	Rhadicoleptus alpestris
	Rhyacophila munda
	Wormaldia mediana
	Wormaldia subnigra

* Extinct in UK, but potentially can reappear

Table 2.2: List of families included in the SPEAR database (Liess *et al*. 2008) with their SPEAR definition for UK (1 – at risk; 0 – not at risk; NP – not present in UK).

Family	SPEAR definition
Ameletidae	1
Argulidae	1
Athericidae	1
Atyidae	1
Baetidae	1
Balanidae	1
Beraeidae	1
Bosminidae	1
Brachycentridae	1
Caenidae	1
Calopterygidae	1
Capniidae	1
Ceratopogonidae	1
Chaoboridae	1
Chloroperlidae	1
Chydoridae	1
Coenagrionidae	1
Crangonyctidae	1
Culicidae	1
Daphniidae	1
Dixidae	1
Dolichopodidae	1
Ecnomidae	1
Empididae	1
Ephemerellidae	1
Ephemeridae	1
Glossosomatidae	1
Goeridae	1
Grapsidae	1
Heptageniidae	1
Hydracarina	1
Hydroptilidae	1
Lepidostomatidae	1
Leptoceridae	1
Leptodoridae	1
Leptophlebiidae	1
Leuctridae	1
Limnephilidae	1
Limoniidae	1
Molannidae	1
Mysidae	1
Nemouridae	1

Family	SPEAR definition
Niphargidae	1
Odontoceridae	1
Palaemonidae	1
Perlodidae	1
Philopotamidae	1
Phryganeidae	1
Polycentropodidae	1
Polymitarcyidae	1
Portunidae	1
Potamanthidae	1
Psychodidae	1
Psychomyiidae	1
Ptychopteridae	1
Pyralidae	1
Rhagionidae	1
Rhyacophilidae	1
Sciomyzidae	1
Sericostomatidae	1
Sialidae	1
Siphlonuridae	1
Sisyridae	1
Stratiomyidae	1
Syrphidae	1
Taeniopterygidae	1
Acroloxidae	0
Aelosomatidae	0
Aeshnidae	0
Ampharetidae	0
Ancylidae	0
Aphelocheiridae	0
Argyronetidae	0
Asellidae	0
Assimineidae	0
Astacidae	0
Bithyniidae	0
Chironomidae	0
Chrysomelidae	0
Corbiculidae	0
Cordulegasteridae	0
Corduliidae	0
Corixidae	0
Corophiidae	0
Crangonidae	0
Curculionoidea	0

Family	SPEAR definition
Dendrocoelidae	0
Dreissenidae	0
Dryopidae	0
Dryopoidea	0
Dugesiidae	0
Dytiscidae	0
Elmidae	0
Erpobdellidae	0
Gammaridae	0
Gerridae	0
Glossiphoniidae	0
Gomphidae	0
Gyrinidae	0
Haemopidae	0
Haliplidae	0
Haplotaxidae	0
Hebridae	0
Heteroceridae	0
Hydraenidae	0
Hydrobiidae	0
Hydrometridae	0
Hydrophilidae	0
Hydropsychidae	0
Hygrobiidae	0
Lestidae	0
Libellulidae	0
Limnichidae	0
Lumbricidae	0
Lumbriculidae	0
Lycosidae	0
Lymnaeidae	0
Mesovelidae	0
Mideopsidae	0
Muscidae	0
Naididae	0
Naucoridae	0
Nepidae	0
Nerededidae	0
Neritidae	0
Noteridae	0
Notonectidae	0
Oligochaeta	0
Perlidae	0
Physidae	0

Family	SPEAR definition
Piscicolidae	0
Pisidiidae	0
Planariidae	0
Planorbidae	0
Platycnemididae	0
Pleidae	0
Scirtidae	0
Simuliidae	0
Sperchonidae	0
Spionidae	0
Succineidae	0
Tabanidae	0
Tipulidae	0
Tubificidae	0
Turbellaria	0
Unionidae	0
Valvatidae	0
Veliidae	0
Viviparidae	0
Apataniidae	NP
Isonychiidae	NP
Stenopsychidae	NP

3 Link between exposure and effect expressed by SPEAR indices

Biomonitoring by the Environment Agency generally identifies macroinvertebrates to the family level. However, previous studies with the SPEAR bioindicator have been based to a great extent on species-level data (Liess and von der Ohe 2005; Schäfer *et al*. 2007). Therefore, prior to validation and possible routine use of SPEAR indices in UK, it was necessary to compare the SPEAR indices based on family- and species-level data, to establish whether family-level information could be used without significant loss of diagnostic capability.

In order to compare the family- and species-level SPEAR indices, biomonitoring data sets from Germany (Liess and von der Ohe 2005), France, and Finland (Schäfer *et al*. 2007) were combined and analysed. These data sets included the results of extensive pesticide measurements, information on macroinvertebrate community composition (abundance of species), relevant landscape characteristics (presence of undisturbed upstream reaches), and sets of basic water quality parameters (for details see Liess and von der Ohe 2005; Schäfer *et al*. 2007).

The identified taxa were classified into "species at risk" (SPEAR) and "species not at risk" based on the database and the traits described in Section 2 (updated version of the database, 17.03.2008). The available data included region-specific ecological information compiled in the SPEAR database. After defining the "species at risk", the relative abundance of these taxa (hereafter $SPEAR_{pesticides}$) was computed for each site and date as follows:

$$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n} \log(x_i + 1) \cdot y}{\sum_{i=1}^{n} \log(x_i + 1)} \cdot 100$$

where n is the number of taxa, x_i is the abundance of the taxon i and y is one if taxon i is classified as SPEAR, otherwise zero. These calculations where performed for the lowest possible identified taxonomic levels (down to species level) to define $SPEAR(sp)_{pesticides}$ and for the families to define $SPEAR(fm)_{pesticides}$.

To compare the toxicity associated with the pesticide concentrations measured in the different sites, toxic units (TU) were computed from the maximum peak water concentrations measured at each site (Liess and von der Ohe 2005):

$$TU_{(D.\ magna)} = \max^{n}_{i=1}(\log(C_i / LC50_i))$$

where $TU_{(D.\ magna)}$ is the maximum number of toxic units of the n pesticides detected at the considered site, C_i is the concentration (µg/L) of pesticide i and $LC50_i$ is the 48-hour LC50 of pesticide i for *D. magna* (µg/L) as given in Tomlin (2001).

The sites investigated were characterised by TU values ranging from -0.42 to -5.0 (Figures 3.1 and 3.2). Most of the contaminated sites had from -0.42 to -3.0 TU. If no pesticide was found a TU value of -5.0 was assigned to that site, corresponding to the value found for unpolluted streams in the study by Liess and von der Ohe (2005). The

TU value -5.0 indicates the toxicity level that is of five orders of magnitude below the 48-hour LC50 of *D. magna*.

The relationship between the SPEAR indices and water toxicity was analysed by linear regression. Analysis of covariance (ANCOVA) was applied to check for significant differences in slope and intercept between the models for SPEAR(sp)$_{pesticides}$ and SPEAR(fm)$_{pesticides}$. A paired t-test was used to check for significant differences in values of these two indices, with the data points paired for each observation site. Two- and one-way ANOVAs were applied to test for significant differences between groups of sites characterised by low, medium and high contamination level (below -4, from -4 to -2, and above -2 respectively) with factors "taxonomic level" and "TU". These contamination levels were defined according to groupings of the sites' TU values (Figures 3.1 and 3.2). Two- and one-way ANOVAs were used subsequently with "model simplification" method. Dunnett post-hoc test was used to compare site-groups with medium and high contamination levels with sites of low contamination. Prior to analysis, the average values for the two sampling dates were calculated for all variables that were measured twice at each site, in order to avoid temporal pseudoreplication (samples collected during periods of maximum pesticide usage). The data set for the streams having upstream undisturbed reaches (recovery areas) was analysed separately from the set for streams without undisturbed reaches, as it had previously been shown that the presence of such reaches significantly influenced the correlation between pesticide exposure and observed effect (Liess and von der Ohe 2005; Schäfer *et al*. 2007; also see next section). The analyses were performed using STATISTICA® 7.1 for Windows (StatSoft, Tulsa, OK, USA).

3.1 Comparison of species- and family-level indices

All the correlations between the SPEAR indices and water toxicity were statistically significant, with higher values of Spearman's r^2 for the sites without upstream recovery areas ($p < 0.05$, Figures 3.1 and 3.2). Comparison of the correlations found for the species- and family-level SPEAR indices showed that these indices similarly correlate with water toxicity (Figures 3.1 and 3.2). No significant differences in slope were found between the linear regressions for SPEAR(sp)$_{pesticides}$ and SPEAR(fm)$_{pesticides}$ for sites both with and without upstream recovery areas ($p > 0.05$, ANCOVA), although in sites without recovery areas the slope of SPEAR(sp)$_{pesticides}$ was slightly steeper than that of and SPEAR(fm)$_{pesticides}$ (Figure 3.1). The r^2 values were only slightly higher for SPEAR(sp)$_{pesticides}$ than for SPEAR(fm)$_{pesticides}$ in sites both with and without recovery areas (Figures 3.1 and 3.2). Comparison of SPEAR(sp)$_{pesticides}$ and SPEAR(fm)$_{pesticides}$ values by paired t-test showed statistically significant differences between them for streams both with and without upstream recovery areas ($p < 0.05$, paired t-test), with SPEAR(fm)$_{pesticides}$ values being higher for the same sample. These results indicate that correlation patterns (slopes) derived for the family- and species-level SPEAR indices are similar, although the actual values of the family-level index are relatively higher than those of the species-level index.

Two-way ANOVA for the site groups with low, medium, and high levels of contamination with factors "taxonomic level" and "TU" showed insignificant effect of the former factor ($p > 0.05$), but significant effect of the latter ($p < 0.05$) in sites both with and without recovery areas. As the level of taxonomic resolution caused no significant effect, the following one-way ANOVA was performed with "TU" as the only factor. This statistical technique, followed by post-hoc Dunnett test, showed significant differences between sites of low contamination and those with both medium and high levels of pesticide contamination, in sites both with and without recovery areas (Figure 3.3).

These results suggest that the family-level SPEAR index can be used to detect pesticide contamination. The efficiency of this index is expected to be only slightly (non-significantly) lower that of the species-level index. However, the differences in values of SPEAR(sp)$_{pesticides}$ and SPEAR(fm)$_{pesticides}$, as well as possible differences in the slopes, should be considered in future validation and use of the approach in UK.

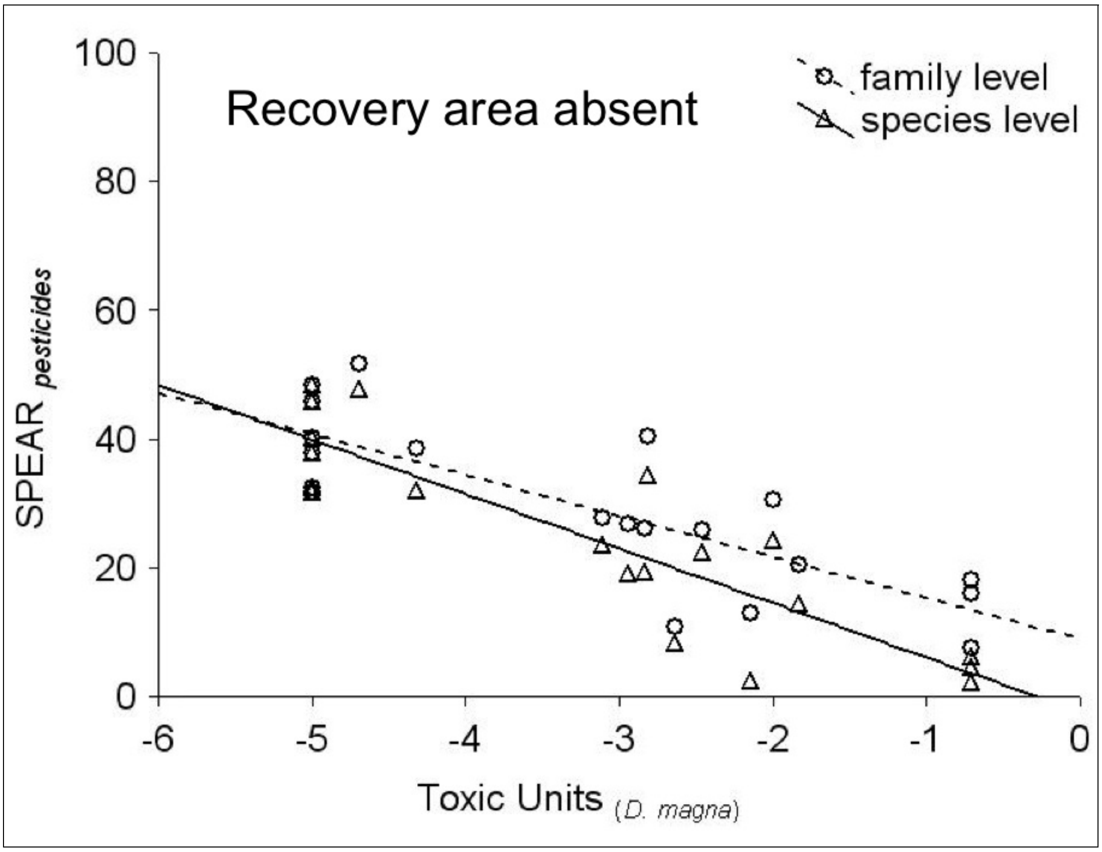

Figure 3.1: Linear regressions for SPEAR indices based on family (r^2 =0.66, $p<0.001$) and species (r^2=0.79, $p<0.001$) levels of taxonomic resolution and water toxicity expressed as Toxic Units $_{(D.\ magna)}$ for sites without upstream recovery areas. The intercepts are significantly different ($p < 0.05$); the slopes are not different ($p > 0.05$, ANCOVA).

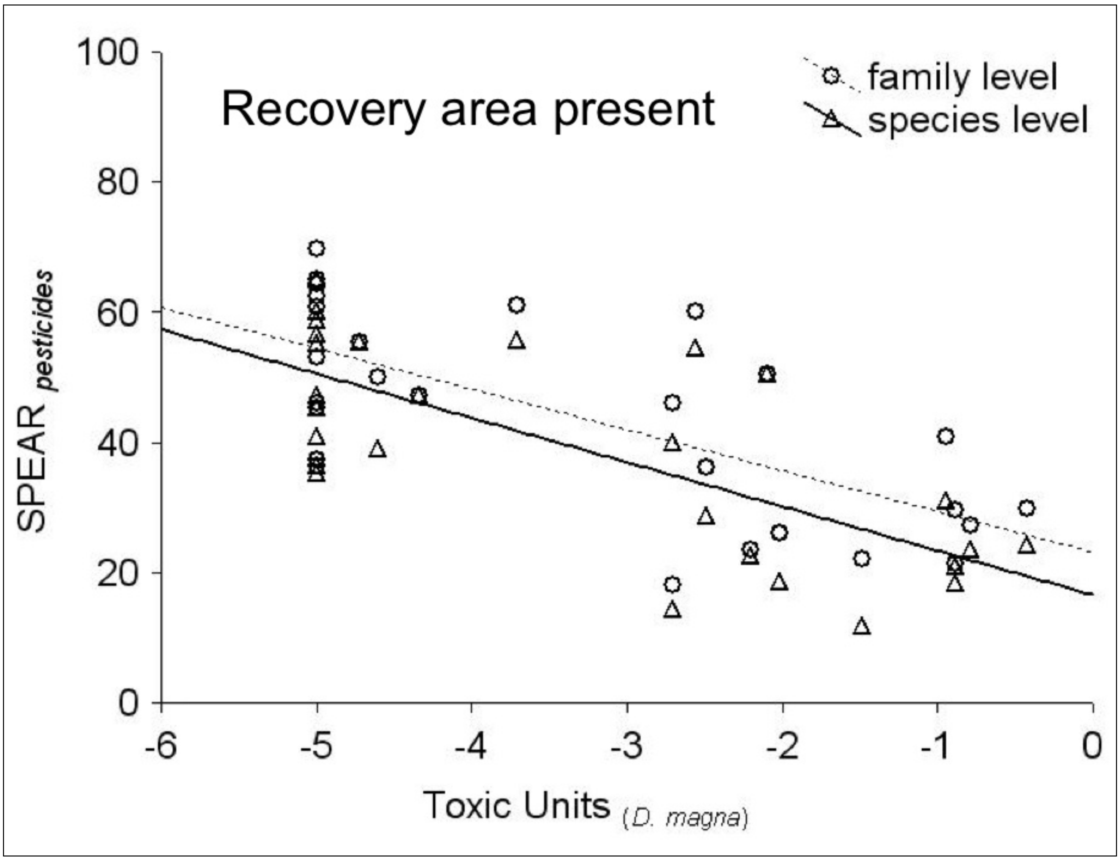

Figure 3.2: Linear regressions for SPEAR indices based on family (r^2=0.47, p<0.001) and species (r^2=0.53, p<0.001) levels of taxonomic resolution and water toxicity expressed as Toxic Units $_{(D.\ magna)}$ for sites with upstream recovery areas. The intercepts and slopes are not significantly different (p > 0.05, ANCOVA).

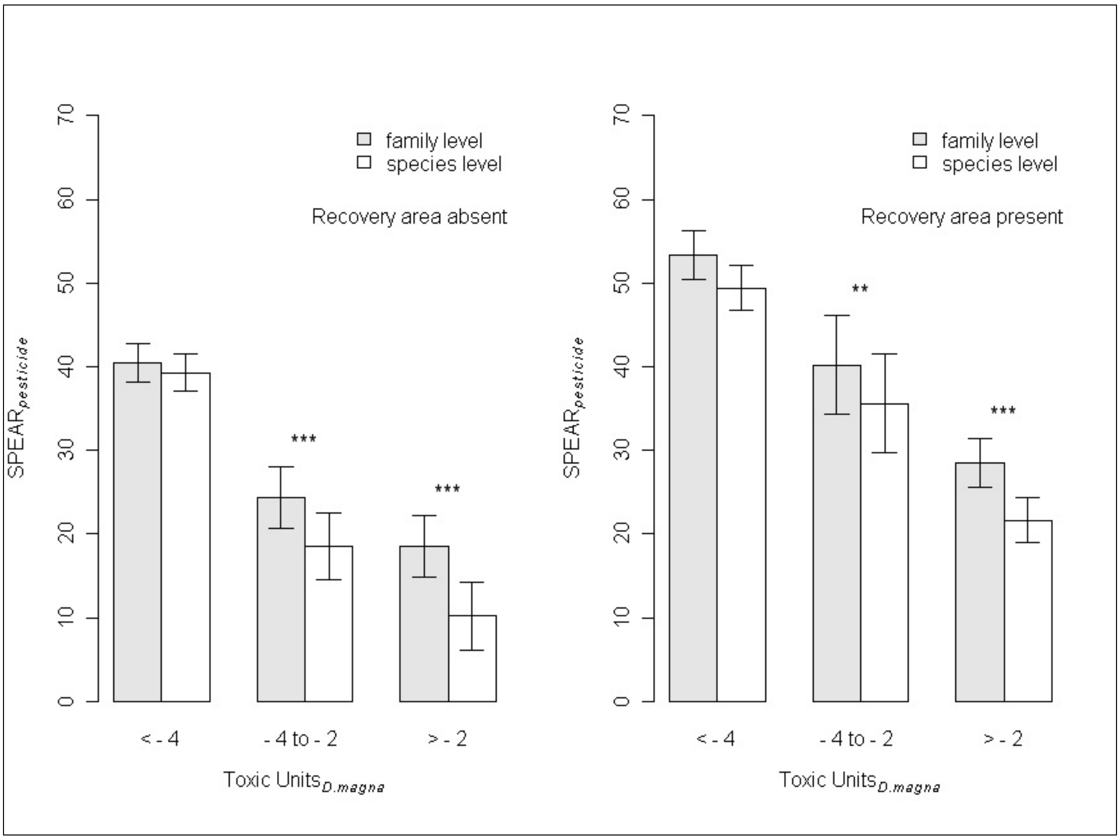

Figure 3.3: Comparison of SPEAR indices based on family and species levels of taxonomic resolution for sites with low, medium, and high pesticide contamination (< -4, from -4 to -2, and > -2 respectively). Sites with and without recovery areas are analysed separately. Asterisks indicate significant differences from the site group with low contamination level (** $p < 0.01$, *** $p < 0.001$, ANOVA, Dunnett post-hoc test).

3.2 Importance of upstream undisturbed reaches as recovery areas

As mentioned previously, the presence of upstream undisturbed reaches (recovery areas) has been shown to significantly affect correlations between pesticide exposure and SPEAR indices (Liess and von der Ohe 2005; Schäfer *et al*. 2007; Schriever *et al*. 2007). The mechanisms underlying this effect are thought to be due to downstream drift of sensitive aquatic taxa from undisturbed upstream reaches to the contaminated stream sections. Downstream drift is well known for many stream invertebrates and can be initiated by natural and anthropogenic factors (Waters 1972; Brittain and Eikeland 1988; Beketov and Liess 2008a).

Previous studies have shown significant effects of upstream recovery areas on SPEAR indices based on the species level of taxonomic resolution. Similar effects were expected for family-level indices, but had not previously been investigated.

In order to evaluate the effect of upstream recovery areas on the $SPEAR(fm)_{pesticides}$ index, we compared linear regressions for this index and water toxicity computed separately for the sites with and without recovery areas using ANCOVA. The same comparison was performed for the species-level index $SPEAR(sp)_{pesticides}$ values.

Comparisons of the correlations derived for the sites with and without upstream recovery areas showed that both $SPEAR(fm)_{pesticides}$ and $SPEAR(sp)_{pesticides}$ intercepts were significantly different ($p < 0.05$, ANCOVA), but the slopes were not significantly different between these two types of sites ($p > 0.05$, ANCOVA, Figures 3.4 and 3.5). This suggests that although the presence of recovery areas significantly increases the values of both $SPEAR(fm)_{pesticides}$ and $SPEAR(sp)_{pesticides}$ compared to sites with the same TU but without upstream recovery areas, it does not influence the correlation patterns between the indices and water toxicity imposed by pesticides. Consequently, the effect of the recovery areas on the family-level index was similar to the effect on the species-level index. All this suggests that for both the species- and family-level SPEAR indices, the presence of upstream recovery areas should be taken into account in monitoring programmes. Pesticide effects in streams with and without such recovery areas should be analysed separately.

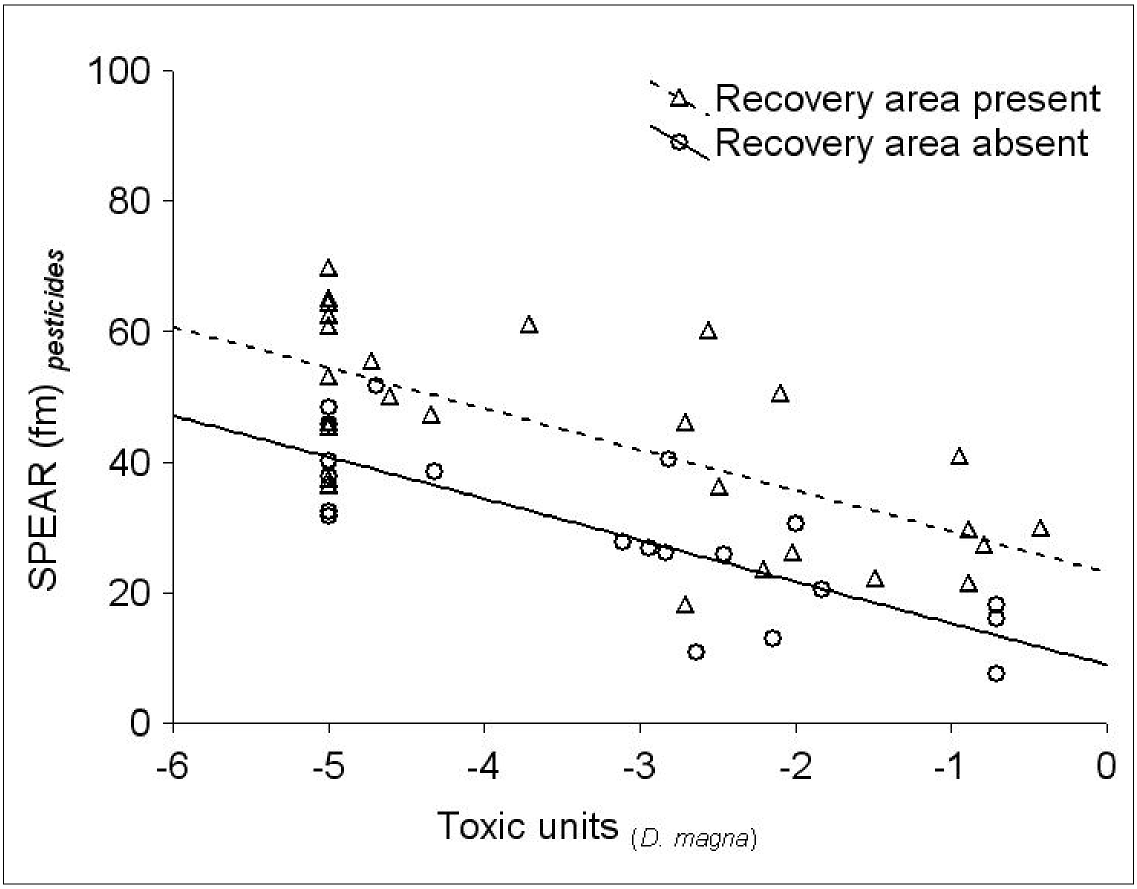

Figure 3.4: Linear regressions for the SPEAR(fm)$_{pesticides}$ index and water toxicity expressed as Toxic Units $_{(D.\ magna)}$ for the sites with (r^2=0.47, p <0.001) and without (r^2=0.66, p <0.001) upstream recovery areas. The intercepts are significantly different ($p < 0.05$); the slopes are not different ($p > 0.05$, ANCOVA).

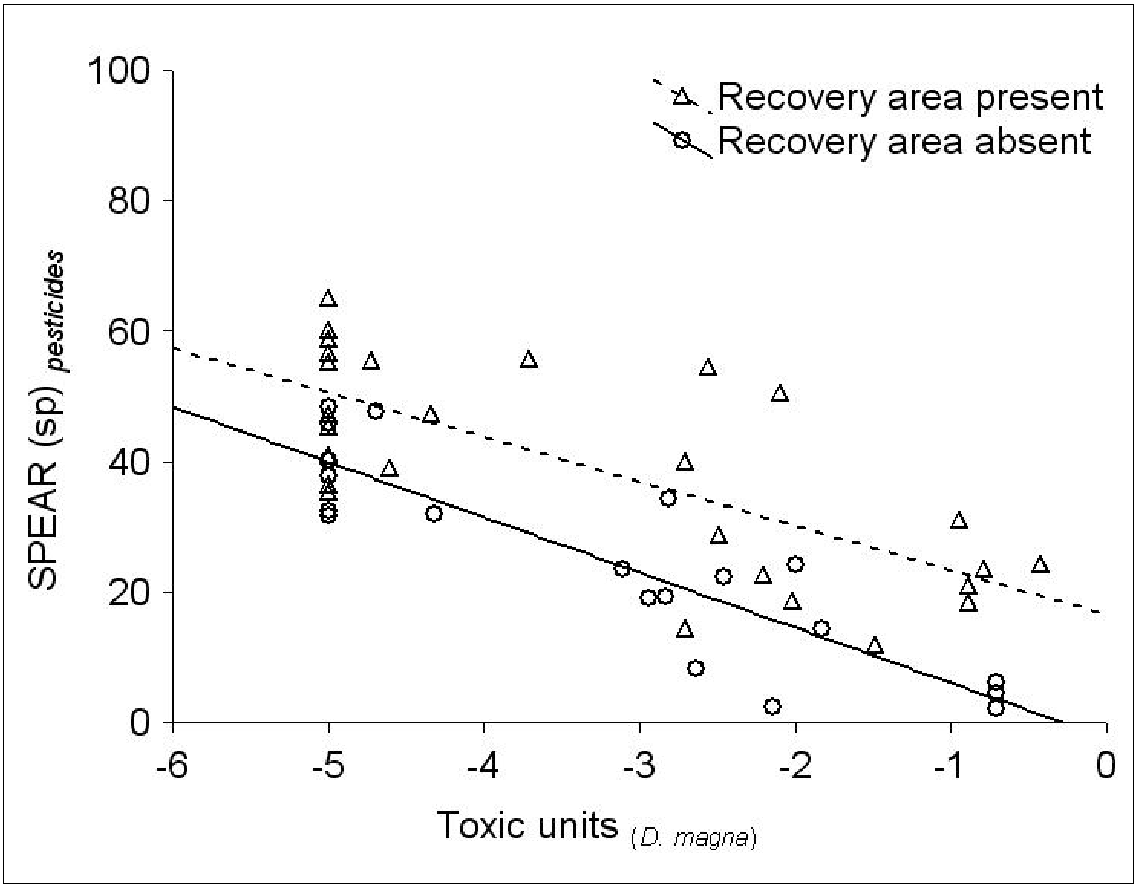

Figure 3.5: Linear regressions for the SPEAR(sp)$_{pesticides}$ index and water toxicity expressed as Toxic Units $_{(D.\ magna)}$ for the sites with (r^2=0.53, p <0.001) and without (r^2=0.79, p <0.001) upstream recovery areas. The intercepts are significantly different ($p < 0.05$); the slopes are not different ($p > 0.05$, ANCOVA).

3.3 Seasonal variability in SPEAR indices

Seasonal variability in the structure and dynamics of communities is well known for lotic aquatic habitats (Allan 1995). Pesticide contamination is also known to significantly vary during the year, with highest concentrations detected in the spring period of maximum pesticide, and especially insecticide, use (Liess and von der Ohe 2005). Given that communities recover after pesticide exposure, the influence of sampling time on SPEAR indices is expected to be significant. The strength of the observed effect may decrease over time after the maximum pesticide input.

In order to assess seasonal difference in the dependencies of SPEAR indices on water toxicity, three time periods were compared using the data set for Germany (Liess and von der Ohe 2005): April (before contamination), May and June (during and shortly after contamination), and July (after contamination). SPEAR indices based on both family and species taxonomic levels were computed for these time periods, and linear correlations of these indices with water toxicity (maximum TU detected during the entire observational period) were compared between the time periods using ANCOVA separately for the sites with and without upstream recovery areas and for the two levels of taxonomic resolution. In addition, ANCOVAs were performed to compare SPEAR(fm)$_{pesticides}$ and SPEAR(sp)$_{pesticides}$ indices separately for each sampling period. This temporal analysis was performed for the German data set only as those from Finland and France only comprised data before and during pesticide exposure, and not after this time period.

The relationships between the SPEAR indices and water toxicity for the three different sampling periods are shown in Figures 3.6 to 3.9. All the correlations derived were statistically significant ($p < 0.05$) except those for both the SPEAR(fm)$_{pesticides}$ and SPEAR(sp)$_{pesticides}$ in April for sites without upstream recovery areas (Figures 3.6 and 3.8). The absence of significant correlation is likely due to the relatively low number of samples (eight sites). No significant difference was found between the slopes or intercepts for all the time periods, levels of taxonomic resolution, and types of sites in terms of presence/absence of upstream recovery areas (Figures 3.6 to 3.9).

Although no statistically significant differences in slope were found for the data analysed, there is an obvious tendency for the correlations derived for April to be less steep than those in later observation periods (Figures 3.6 to 3.9). It is possible that a significant difference confirming this tendency could be found with a larger number of sampling sites. Future application of SPEAR$_{pesticides}$ indices should consider these temporal trends, and sampling programmes to monitor effect and recovery should be planned accordingly (for example, for strongest effect in May-June, and for maximum possible recovery in April).

Comparison of relationships between SPEAR indices and water toxicity performed separately for each sampling period showed no significant differences between these indices for any of the periods ($p > 0.05$, ANCOVA). This suggests that there is no difference between the seasonal variability of the family-level SPEAR index and that of the species-level index.

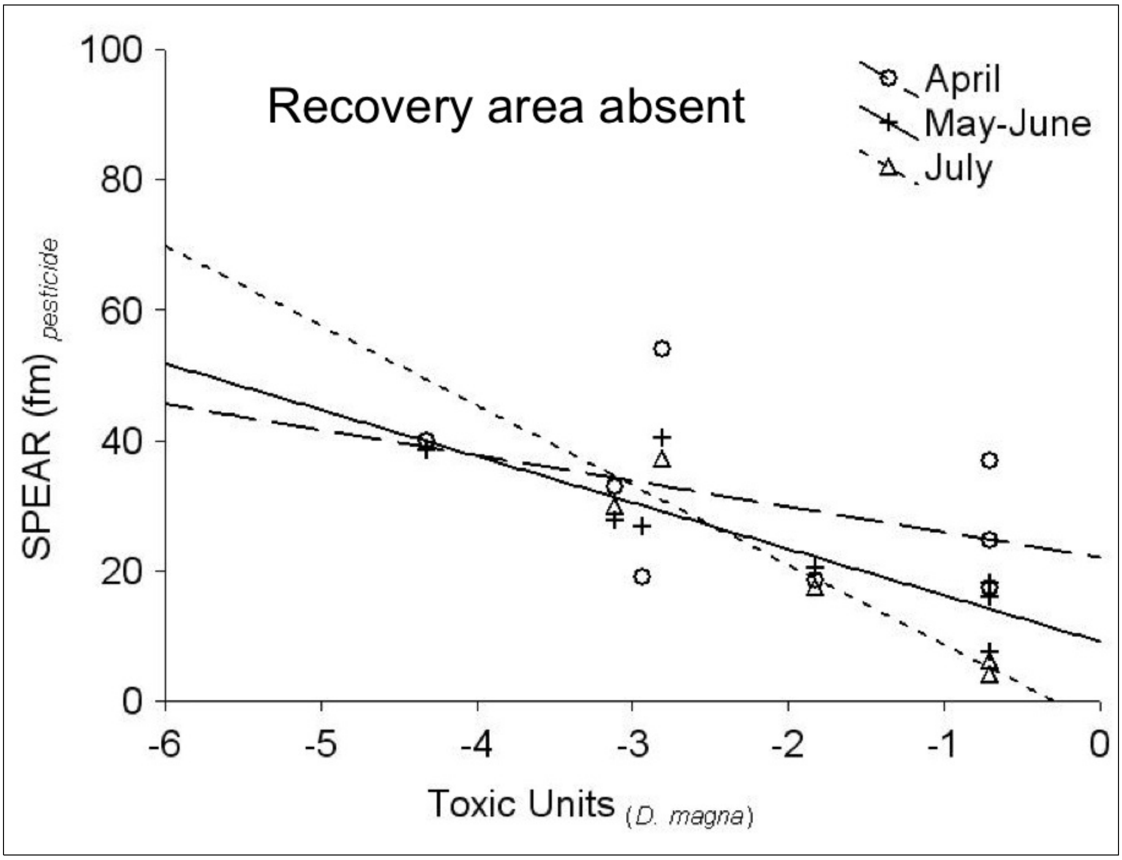

Figure 3.6: Linear regressions for the SPEAR(fm)$_{pesticides}$ index and water toxicity expressed as Toxic Units $_{(D.\ magna)}$ for different time periods: April (r^2=0.17, $p >$ 0.05), May-June (r^2=0.75, $p <$0.01), and July (r^2=0.92, $p <$0.01). Data are for sites without upstream recovery areas. The intercepts and slopes are not significantly different (p > 0.05, ANCOVA).

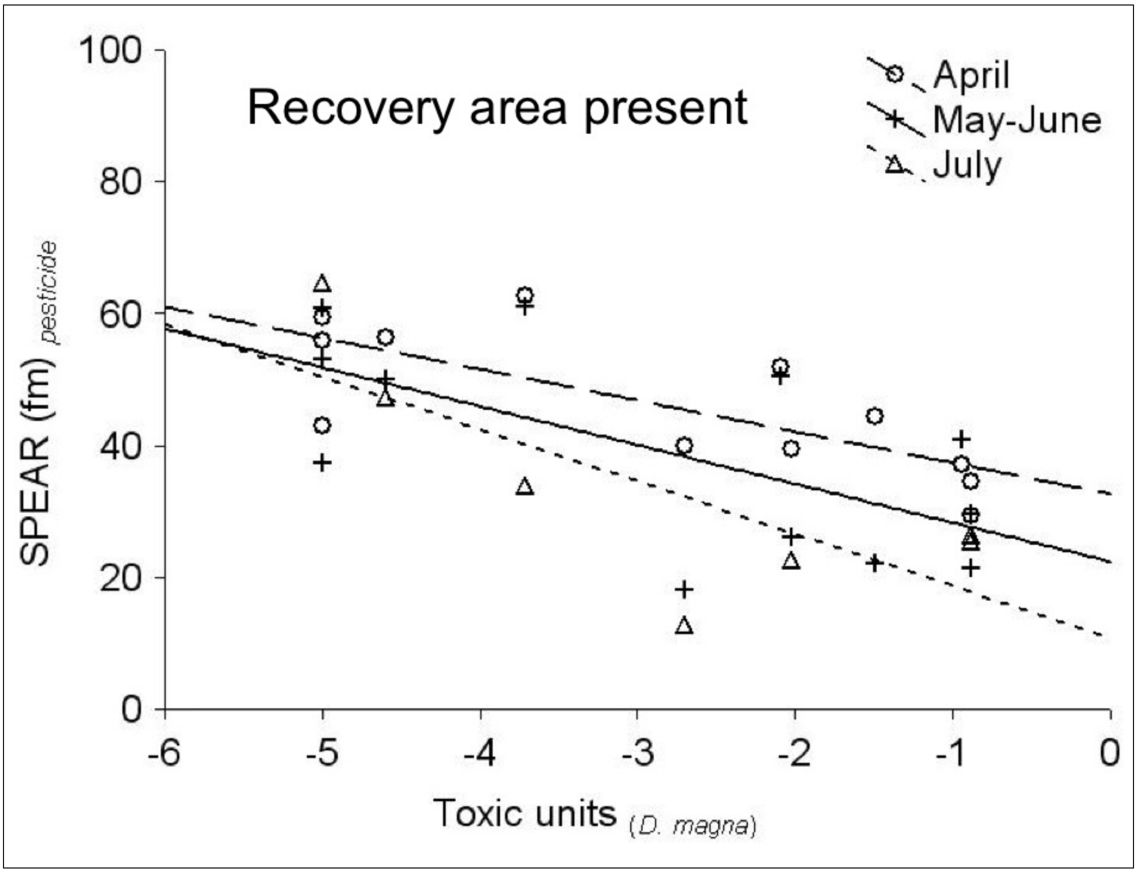

Figure 3.7: Linear regressions for the SPEAR(fm)$_{pesticides}$ index and water toxicity expressed as Toxic Units $_{(D.\ magna)}$ for different time periods: April (r^2=0.56, $p <$ 0.01), May-June (r^2=0.41, $p <0.05$), and July (r^2=0.58, $p <0.05$). Data are for sites with upstream recovery areas. The intercepts and slopes are not significantly different ($p > 0.05$, ANCOVA).

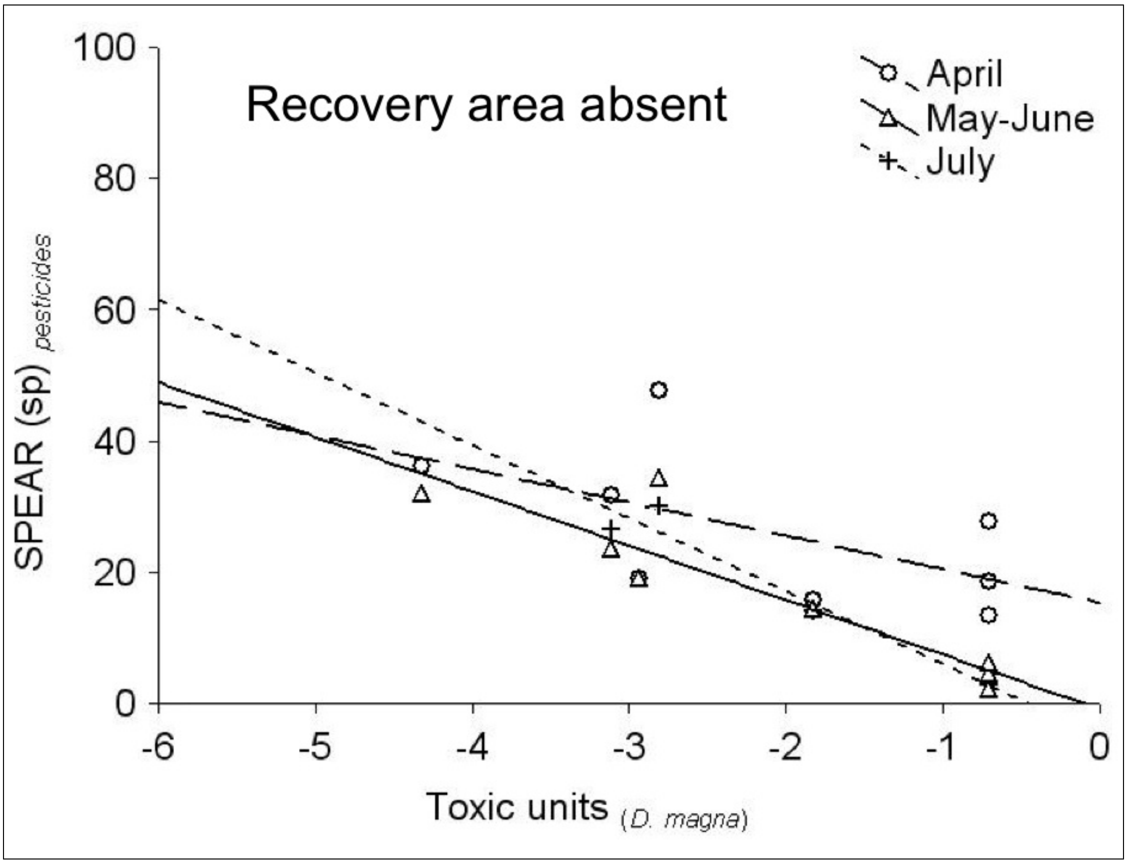

Figure 3.8: Linear regressions for the SPEAR(sp)$_{pesticides}$ index and water toxicity expressed as Toxic Units $_{(D.\ magna)}$ for different time periods: April (r^2=0.34, p > 0.05), May-June (r^2=0.83, p <0.001), and July (r^2=0.96, p <0.01). Data are for sites without upstream recovery areas. The intercepts and slopes are not significantly different (p > 0.05, ANCOVA).

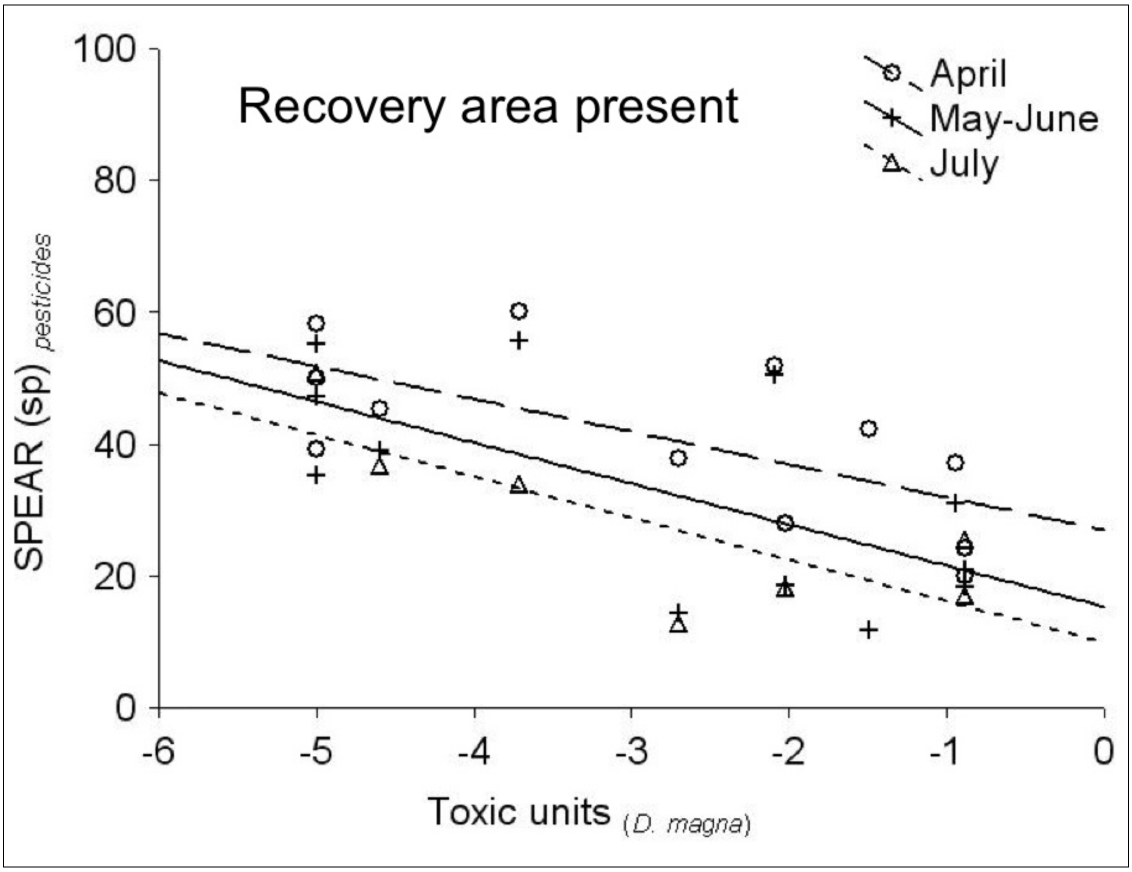

Figure 3.9: Linear regressions for the SPEAR(sp)*pesticides* index and water toxicity expressed as Toxic Units *(D. magna)* for different time periods: April (r^2=0.44, p < 0.05), May-June (r^2=0.43, p <0.05), and July (r^2=0.62, p <0.05). Data are for sites with upstream recovery areas. The intercepts and slopes are not significantly different (p > 0.05, ANCOVA).

4 Conclusions

The updated SPEAR database is ready for use in the UK, as it contains most of the UK stream macroinvertebrate taxa together with information about their respective ecological traits.

Comparison of the pesticide-specific SPEAR indices based on family and species levels of taxonomic resolution (SPEAR(fm)$_{pesticides}$ and SPEAR(sp)$_{pesticides}$ respectively) has shown that the family-level index can be used to detect pesticide contamination in streams. In particular, it has been shown to relate to levels of insecticides and fungicides. The efficiency of this index is expected to be only slightly lower than that of the species-level index. Taking into account the time-consuming nature, cost and difficulties of species-level identification, the SPEAR(fm)$_{pesticides}$ index is a promising and cost-effective bioindicator tool for detecting pesticide contamination in streams.

Use of the SPEAR approach in the UK is now possible, assuming that exposure-effect relationships in UK streams do not differ greatly to other investigated areas in Europe (Finland, France and Germany). However, future application of the SPEAR approach will benefit from validation in field investigations in the UK. A sampling programme including biological (macroinvertebrates) and chemical (pesticides and other contaminants) sampling and covering various streams with different levels of anthropogenic disturbance should be undertaken to increase the level of confidence in the SPEAR-based assessment of pesticide effects. The success of such a field validation will depend on selection of appropriate streams, the right time period for investigation and good sampling methods to obtain a realistic measure of short-term pesticide contamination (see Liess *et al*. 1996; 1999; Liess and von der Ohe 2005; Schäfer *et al*. 2007).

Future biomonitoring programmes may consider applying other stressor-specific SPEAR-bioindicators for various types of contaminants. For example, recent investigations in Western Siberia demonstrated the applicability of a modified SPEAR approach for organic toxicants such as petrochemicals and synthetic surfactants in lotic habitats of this region. The index SPEAR$_{organic}$ was developed for these contaminants and applied across a large gradient of longitudinal environmental factors (Beketov and Liess 2008b). This example shows the potential for creating further bioindicator tools for different stressors using this approach.

References

ALLAN, JD. 1995. *Stream ecology*. Dordrecht: Kluwer Academic Publishers.

BEKETOV, MA AND LIESS, M. 2008a. Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. *Archives of Environmental Contamination and Toxicology*, 55, in press.

BEKETOV, MA AND LIESS, M. 2008b. An indicator for effects of organic toxicants on lotic macroinvertebrates: independence of an extended gradient of natural environmental factors. *Environmental Pollution*, in review.

BRITTAIN, JE AND EIKELAND, TJ. 1988. Invertebrate drift – A review. *Hydrobiologia*, 166, 77-93.

LIESS, M, BROWN, C, DOHMEN, P, DUQUESNE, S, HEIMBACH, F, KREUGER, J, LAGADIC, L, REINERT, W, MAUND, S, STRELOKE, M AND TARAZONA, J. 2005. *Effects of Pesticides in the Field* – EPIF, SETAC Press, Brussels, Belgium.

LIESS, M & VON DER OHE, PC. 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry*, 24, 954-965.

LIESS, M, VON DER OHE, PC, SCHRIEVER, CA, SCHÄFER, RB AND BEKETOV, MA. 2008. *SPEAR database*. Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany. (http://www.ufz.de/index.php?en=14348)

LIESS, M, SCHULZ, R AND NEUMANN, M. 1996. A method for monitoring pesticides bound to suspended particles in small streams. *Chemosphere*, 32, 1963-1969.

LIESS, M, SCHULZ, R, LIESS, MHD, ROTHER, B AND KREUZIG, R. 1999. Determination of insecticide contamination in agricultural headwater streams. *Water Research*, 33, 239-247.

SCHÄFER, R B, CAQUET, T, SIIMES, K, MUELLER, R, LAGADIC, L AND LIESS, M. 2007. Effects of pesticides on community structure and ecosystem functions in agricultural headwater streams of three biogeographical regions in Europe. *Science of the Total Environment*, 382, 272-285.

SCHRIEVER, CA, CALLAGHAN, A., BIGGS, JP, AND LIESS, M. 2008. *Freshwater Biological Indicators of Pesticide Contamination*. Science Report. SC030189/SR3. Environment Agency, UK.

SCHRIEVER, CA, HANSLER-BALL, M, HOLMES, C, MAUND, S AND LIESS, M. 2007. Agricultural intensity and landscape structure: influences on the macroinvertebrate assemblages of small streams in northern Germany. *Environmental Toxicology and Chemistry*, 26, 346-357.

TOMLIN, CDS. 2001. *The e-Pesticide Manual* (Twelfth Edition) on CD. Version 2.1. The British Crop Protection Council, UK.

VON DER OHE, PC AND LIESS, M. 2004. Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. *Environmental Toxicology and Chemistry* 23, 150-156.

VON DER OHE, PC, PRÜß, A, SCHÄFER, RB, LIESS, M, DE DECKERE, E AND BRACK, W. 2004. Water quality indices across Europe - a comparison of the good ecological status of five river basins. *Journal of Environmental Monitoring*, 9, 970-978.

WATERS, TF. 1972. The drift of stream insects. *Annual Review of Entomology*, 17, 253-272.

We are The Environment Agency. It's our job to look after your environment and make it **a better place** – for you, and for future generations.

Your environment is the air you breathe, the water you drink and the ground you walk on. Working with business, Government and society as a whole, we are making your environment cleaner and healthier.

The Environment Agency. Out there, making your environment a better place.

Published by:

Environment Agency
Rio House
Waterside Drive, Aztec West
Almondsbury, Bristol BS32 4UD
Tel: 0870 8506506
Email: enquiries@environment-agency.gov.uk
www.environment-agency.gov.uk