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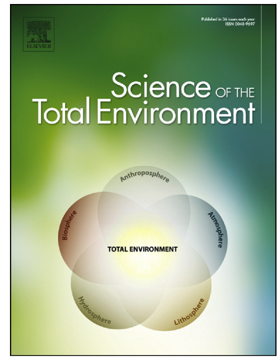

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Species occurrence relates to pesticide gradient in streams

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Keywords

Macroinvertebrates; Monitoring; Pesticides; Substrate preference; Oxygen; Ecological traits

Abstract

Freshwater communities are threatened worldwide, with pesticides being one of the main stressors for vulnerable invertebrates. Whereas the effects of pesticides on communities can be quantified by trait-based bioindicators such as $SPEAR_{pesticides}$, single species responses remain largely unknown.

We used the bioindicator $SPEAR_{pesticides}$ to predict the toxic pressure from pesticides in 6942 macroinvertebrate samples from 4147 sites during the period 2004 to 2013, obtained by environmental authorities in Germany, and classified all samples according to their magnitude of pesticide pressure. Along this gradient of pesticide pressure, we quantified the occurrence of 139 macroinvertebrate species.

We identified 71 species characterized by decreasing occurrence with increasing pesticide pressure. These ‘decreasing species’, mainly insects, occurred at a frequency of 19.7% at sites with reference conditions and decreased to 1.7% at sites with the highest pesticide pressure. We further determined 55 ‘nonspecific species’ with no strong response as well as 13 ‘increasing species’, mainly Gastropoda, Oligochaeta and Diptera, which showed an increase of frequency from 1.8% at sites with reference conditions to 11.4% at sites with the highest pesticide pressure. Based on the change in frequency we determined the pesticide vulnerability of single species, expressed as pesticide associated response (PARe).

Furthermore, a trait analysis revealed that species occurrence may additionally depend on oxygen demand and, to a lesser extent on substrate preference, whereas no significant effect of feeding and respiration type could be found.

Our results provide the first extensive pesticide vulnerability ranking for single macroinvertebrate species based on empirical large-scale field data.

1 Introduction

All over the world pesticides have a major impact on freshwater communities. In Germany more than 48,000 t of pesticides were sold in 2017 (BVL, 2018). A toxicological relevant proportion of these substances enter freshwater streams worldwide (Malaj et al., 2014), affecting freshwater communities and leading to a reduction in regional aquatic biodiversity (Beketov et al., 2013), changes in ecosystem functions such as leaf litter breakdown (Schäfer et al., 2012) and an altered community structure dominated by less sensitive species (Liess and von der Ohe, 2005; Schäfer et al., 2012; Knillmann et al., 2018).

At the community level, the effects of pesticides in the field can be identified by biological indicators such as $SPEAR_{pesticides}$ (SPEcies At Risk, Liess and von der Ohe, 2005). This indicator uses species traits including (i) the physiological sensitivity towards pesticides based on laboratory studies, (ii) the generation time, (iii) the exposure probability and (iv) the dependence on refuge areas with the ability to migrate from them. According to these traits each species is predicted to be 'at risk' (referred to as SPEAR) or 'not at risk' (referred to as NotSPEAR) from pesticide pressure. Finally, $SPEAR_{pesticides}$ reflects the pesticide impact on the community structure by the relative abundance of species 'at risk'. $SPEAR_{pesticides}$ has been shown to successfully indicate the effects of pesticides on freshwater communities in different geographical regions such as Germany (Schäfer et al., 2007; von der Ohe et al., 2007; Liess et al., 2008; Schäfer et al., 2012; Orlinskiy et al., 2015; Münze et al., 2017), elsewhere in Europe (Schäfer et al., 2007; von der Ohe et al., 2007; Liess et al., 2008; Schäfer et al., 2012; Rasmussen et al., 2013) and other regions in the world (Schäfer et al., 2011; Schäfer et al., 2012; Chiu et al., 2016; Hunt et al., 2017). However, due to lack of data, the response of single species over a gradient of pesticide pressure has never been identified under field conditions. Instead species-specific responses to pesticides are usually derived from laboratory toxicity tests conducted under standard test conditions. Rankings of laboratory-based sensitivity to pesticides have been compiled for example by Von der Ohe and Liess (2004) or Rubach et al. (2010). However, the transferability of laboratory-based sensitivity to pesticides to the vulnerability of species under field conditions is prone to error.

In particular, additional environmental stressors are known to increase the vulnerability of species in the field and change their response to pesticides (Liess et al., 2016; Liess et al., 2019). This pertains to non-chemical stressors such as competition, changed food availability, temperature regime (Heugens et al., 2001; Liess and von der Ohe, 2005; Stampfli et al., 2011; Foit et al., 2012; Knillmann et al., 2012; Dolciotti et al., 2014) or additional chemical stressors (Nørgaard and Cedergreen, 2010) even though net effects of stressors may be antagonistic depending on the endpoint considered (Jackson et al., 2016). Because of this complex interaction between different environmental stressors, this paper empirically identifies the vulnerability of single species to pesticides in the field. Therefore, we used German-wide macroinvertebrate monitoring data to quantify single species' responses towards pesticide pressure. Due to lack of exposure data, we used the indicator $SPEAR_{pesticides}$ to predict the degree of pesticide pressure. Finally, we aimed to identify potential correlations between the species responses to pesticides and ecological traits and related the species' response to pesticides with their saprobic value, substrate preference, feeding type and respiration.

2 Methods

2.1 Study area and spatial analysis

For our analysis, we selected a total of 4147 macroinvertebrate sampling sites comprising 6942 samples in nine German federal states (see Fig. 1).

Pesticide pressure at the respective sites was calculated with $SPEAR_{pesticides}$ (see section 2.3). This indicator is calibrated and validated for small freshwater streams without extreme morphological degradation (Bunzel et al., 2014). Hence, in order to prevent any confounding factors regarding the prediction of pesticide pressure, we excluded the following sampling sites within the site selection process:

- Sites in the low mountain ranges and the Alps, so only those sampling sites at elevations < 500 m were considered. Elevation levels were taken from the ASTER Global Digital Elevation Model v2.
- Sites with a structural quality class of 6 (strongly changed) and 7 (completely changed). Information on the structural quality class was provided by the respective official authorities (see section 2.2).
- Sites within brackish water bodies, indicated by respective landscape types in the structural quality shapefiles defined as 'marshland waters and backwater' and 'brackish water influenced inflow of the Baltic Sea' in the federal states of Mecklenburg-Vorpommern and Schleswig-Holstein as well as 'coastal marshlands' in Niedersachsen.
- Sites with a distance from the stream source of > 50 km. For this, available information on the flow direction and the geometry of the water courses were combined (ATKIS DLM25, GeoBasis-DE/BKG (2010).

Spatial analysis was conducted with ArcGIS (Version 10.3).

2.2 Macroinvertebrate data

Macroinvertebrate data from 2004 to 2013 was provided by the official authorities of the respective German federal states comprising of Bayerisches Landesamt für Umwelt (LfU, Augsburg), Hessisches Landesamt für Naturschutz, Umwelt und Geologie (HLNUG, Wiesbaden), Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig-Holstein (LLUR, Flintbek), Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV, Recklinghausen), Landesamt für Umwelt, Naturschutz und Geologie Mecklenburg-Vorpommern (LUNG, Güstrow), Landesbetrieb für Hochwasserschutz und Wasserwirtschaft Sachsen-Anhalt (LHW, Magdeburg), Niedersächsischer Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz (NLWKN, Norden), Sächsisches Landesamt für Umwelt, Landwirtschaft und Geologie (LfULG, Dresden) and Thüringer Landesanstalt für Umwelt und Geologie (TLUG, Jena).

Data was collected by the authorities according to the Water Framework Directive (European Commission, 2000) or by using similar sampling procedures.

To reduce the effect of seasonal variations of the species present in a sample, we furthermore only included samples taken during the main season of insecticide application (April to June).

To reduce errors during sampling and identification, we excluded the following entries from the dataset:

- Insects in the stage of pupae or imago (in case of emerging adults)
- Species declared as 'uncertain' at the time of identification by the respective authority
- Samples with a low number of species, namely less than five aggregated species according to $SPEAR_{pesticides}$ (For the calculation of $SPEAR_{pesticides}$, the level of taxonomic determination in the raw data is standardized by means of species aggregation. Information on species aggregation was derived from the website www.systemecology.eu/indicate, version 1.0.0, June 2019).

After these selections, the dataset included 1733 taxa on species, genus, family and order level. In a final step we reduced our dataset on 1163 taxa only on species and sub-species level to account for the known variability of pesticide effects between species of the same genus or family (Wiberg-Larsen et al., 2016).

2.3 Prediction of pesticide pressure

Whereas the monitoring data within the WFD offers biological data in excellent quality, the availability of associated chemical measurements indicating ecological relevant pesticide exposure is limited. In current government monitoring, biological and chemical study sites are often poorly synchronized in space and time (Brinke et al., 2015). In addition, chemical measurements often underestimate the pesticide load when using grab samples from routine monitoring programs, whereas event-driven samples would be preferable (Liess et al., 1999; LUWG, 2011). Moreover, routine monitoring programs often only include a limited number of pesticides (Moschet et al., 2014). To overcome this lack of ecologically relevant data of pesticide exposure, we predicted the pesticide pressure for each macroinvertebrate sample using the indicator $SPEAR_{pesticides}$ (Liess and von der Ohe, 2005), updated by Knillmann et al. (2018). The indicator determines the relative abundance of species at risk towards pesticides within a community whereby the vulnerability of a species is defined by ecological traits. $SPEAR_{pesticides}$ is calculated as follows:

$$\mathrm{SPEAR_j} = \frac{\sum_{i=1}^{n} \log(4x_i+1)\cdot y}{\sum_{i=1}^{n} \log(4x_i+1)} \qquad (1)$$

where n is the total number of species in a sample j, x_i is the abundance of species i, and y is set to 1 for SPEAR (species at risk) and set to 0 for NotSPEAR (species not at risk). Abundance data was log(4×+1)-transformed to decrease the influence of populations with mass developments (Knillmann et al., 2018). In a second step, the values of $SPEAR_{pesticides}$ are normalized to reference conditions as follows:

$$\mathrm{SPEAR_{pesticides}} = \frac{SPEAR_j}{SPEAR_{reference}} \qquad (2)$$

where $SPEAR_j$ is the indicator value of a sample j, and $SPEAR_{reference}$ refers to the mean indicator value assumed under reference conditions with no or negligible pesticide pressure. The value of $SPEAR_{reference}$ is set to 0.27 based on the evaluation of German monitoring data. For more details on the calculation see Liess and von der Ohe (2005), Liess et al. (2008), Knillmann et al. (2018) and the website www.systemecology.eu/indicate (version 1.0.0, June 2019).

In a next step we converted the values of $SPEAR_{pesticides}$ into field toxicities of pesticides applying the concept of Toxic Units (TU). The conversion is based on the linear relationship between $SPEAR_{pesticides}$ and the TU obtained by Knillmann et al. (2018). Predicted field toxicities were then divided into five classes (see Table 1) according to Beketov et al. (2013) and Knillmann et al. (2018). The assignment of classes also reduced the variance of frequency over pesticide pressure, which improved the statistical determination of potential trends. Each class indicates a different level of predicted pesticide pressure (PP), whereas level PP1 reflects reference condition and level PP5 indicates samples with the highest pesticide pressure.

The number of samplings per site is 2.2 on average for the selected time period (from April to June) and for all of the years included in the analysis. The maximum number of samples taken at one site was 19. These repeated measurements are negligible because of the high total number of samples included in this analysis.

2.4 Calculation of invertebrate frequency

Along the gradient of predicted pesticide pressure, for each species we calculated the response related to its frequency in order to determine the respective vulnerability. We assessed the frequency for each species and level of predicted pesticide pressure as follows:

$$\mathrm{frequency}_{\mathrm{p,i}} = \frac{\mathrm{n}_{\mathrm{p,i}}}{n_p} \cdot 100 \qquad (3)$$

where i is the respective species, p is the level of pesticide pressure, $n_{p,i}$ is the number of samples for species i and pesticide pressure p, and n_p is the total number of samples for pesticide pressure p. If a species was not present at a certain level of pesticide pressure, the frequency was set to 0.

To reduce uncertainty within data analysis related to species with low data availability, only those species were taken into account that i) occurred at least in 100 samples, ii) showed a maximal frequency of > 5% at one of the 5 levels of pesticide pressure, iii) occurred in at least 10 samplings at each of 3 consecutive levels of pesticide pressure.

Applying these criteria, we reduced the species in the dataset from originally 1163 to 269 (step i), 156 (step ii) and 139 (step iii) species, for which frequency was finally determined. Step iii also lead to the exclusion of potentially extremely vulnerable species, which were only present at reference sites. This was done in order to ensure that we exclude species that are potentially dependent on landscape conditions typical of reference sites.

To enable frequency patterns to be compared, species' frequencies were converted to relative values by setting the highest value, irrespective of the level of pesticide pressure, to 100%. The relative frequency was calculated as:

$$\text{relative frequency}_{\mathrm{p,i}} = \frac{frequency_{p,i}}{max.\ frequency_i} \cdot 100 \quad (4)$$

where i is the respective species, p is the level of pesticide pressure, $\text{frequency}_{\mathrm{p,i}}$ is the frequency of species i at pesticide pressure p and max. $\text{frequency}_{\mathrm{i}}$ is the highest frequency value of species i, irrespective of the level of pesticide pressure.

To generalize the response pattern of the species, in a next step, the change of the relative frequency between the reference condition (PP1) and the highest level of pesticide pressure (PP5), referred to as Pesticide Associated Response (PARe), was calculated for each species as:

$$PARe_i = relative\ frequency\ (PP5)_i - relative\ frequency\ (PP1)_i \quad (5)$$

where i is the respective species, PP1 indicates reference conditions and PP5 is the highest level of pesticide pressure. With this calculation we intended to identify species with a clear negative or positive trend in frequency along the gradient of pesticide pressure.

To group species with similar response patterns, we defined the following three species groups according to PARe:

- Decreasing species group: species with PARe ≤ -70
- Nonspecific species group: species with PARe > -70 and < 70
- Increasing species group: species with PARe ≥ 70

2.5 Selection of ecological traits

The pesticide vulnerability of single species, expressed as pesticide associated response (PARe), was related to the laboratory based physiological sensitivity according to the index $SPEAR_{pesticides}$, derived from www.systemecology.eu/indicate (version 1.0.0, June 2019). The PARe-value of each species was additionally compared to the classification of species as SPEAR and NotSPEAR.

Finally, we investigated the influence of ecological traits not included in $SPEAR_{pesticides}$ on PARe. This analysis was conducted separately for species classified as SPEAR and NotSPEAR to avoid any potential confounding influence of the traits already included in $SPEAR_{pesticides}$. Ecological trait values for the 139 selected species were derived from the website www.freshwaterecology.info (version 7, November 2018). Available trait data originated from two types of sources: first from Tachet et al. (2010) and second from a compilation of different sources (Aspöck, 1995; Bauernfeind et al., 1995; Car et al., 1995; Eder et al., 1995; Graf et al., 1995a; Graf et al., 1995b; Hörner et al., 1995; Jäch et al., 1995; Janecek et al., 1995a; Janecek et al., 1995b; Moog, 1995; Nesemann and Moog, 1995; Nesemann and Reischütz, 1995; Zettel, 1995; Schmedtje and Colling, 1996; AQEM expert consortium, 2002; Graf et al., 2008; Buffagni et al., 2009; Graf et al., 2009; Tachet et al., 2010; Brabec et al., 2018; Buffagni et al., 2018; Graf et al., 2018a; Graf et al., 2018b).

Those traits were selected for which a link to pesticide vulnerability or sensitivity was to be expected or indicated by other studies (Rubach et al., 2010; Rico and Van den Brink, 2015; Wiberg-Larsen et al., 2016; Knillmann et al., 2018) and according to the data availability for the 139 species. To reduce the uncertainty of the trait analysis, only those traits were taken into account where a minimum of 30 data points were available for species at risk and not at risk respectively. The following traits were selected (n indicates the number of species for which trait information was available): (a) saprobic value (n=108), (b) substrate preference (n=75), (c) feeding type (n=131) and (d) respiration (n=82). Due to limited data availability, the ecological traits maximum potential size, resistance forms, life cycles and reproduction could not be included in the analysis.

Trait data for substrate preference, feeding type and respiration was available in a fuzzy coded way, whereas the height of the number determines the preference for the respective trait class. For each species and for each of the two sources, the trait class with the maximum number was extracted separately. When the maximum trait class of both sources was not identical, both trait classes were considered for the respective species in the analysis. In case the maximum number was assigned to several trait classes within one source, all of these trait classes were extracted for the respective species. Finally, a dataset was compiled which contained the trait class, or classes if applicable, indicated by the maximum number for each species. Trait data for the saprobic value for Germany was available in numbers (DEV, 2003). Due to the cumulative occurrence of single values and for a better comparability with other traits, we defined three classes for the saprobic value.

2.6 Statistical analysis

To test for differences between frequencies at the five different levels of pesticide pressure within the decreasing, unspecific and increasing species groups respectively, data was first tested for the homogeneity of variances (Levene’s Test) and normal distributions of the residuals (Shapiro-Wilk Normality Test). The frequency was log-transformed. Differences in the means of the average frequency were tested with an analysis of variance (one-way ANOVA) followed by a post-hoc test (Tukey's HSD Test). Data was tested at influential data points (Cook's Distance). A trend analysis according to Jonckheere (1954) was conducted for each species group to test whether frequency significantly follows a specific pattern (Jonckheere-Terpstra Test, package ‘DescTools’). This test is specifically designed to identify trends and their direction. It indicates whether an assumed trend (monotonic, increasing or decreasing) of class means is significant.

To identify whether the ecological traits of species (see section 2.5) have a significant impact on the Pesticide Associated Response (PARe) both for species at risk and species not at risk from pesticide pressure according to $SPEAR_{pesticides}$, data was tested for the homogeneity of variances (Levene's Test) and the normal distributions of the residuals (Shapiro-Wilk Normality Test). As the assumptions were not met, a non-parametric Kruskal-Wallis test was then applied to test the explained variance of PARe by the respective trait. If PARe was not significantly different between several categories of a respective trait, the categories were grouped to simplify the model. In case of more than two categories for one trait (as for the saprobic value), a non-parametric pairwise test for multiple comparisons of mean rank sums (Dunn's-Test) was conducted to test for significant differences of PARe between the categories.

As for the physiological sensitivity according to $SPEAR_{pesticides}$, a Spearman's Rank correlation with PARe was performed. All statistical tests were performed with R Studio (version 1.1.463).

3 Results

3.1 Pesticide Associated Response (PARe) of single species

The available monitoring data from the field enabled the Pesticide Associated Response to be assessed (PARe, see section 2.4) for 139 macroinvertebrates (Table 2 and Fig. A.1). Based on this change in relative frequency, we were able to distinguish between (i) 71 'decreasing species' with a PARe of -70 or less, (ii) 13 'increasing species' with a PARe of +70 or more and (iii) 55 'nonspecific species' with a PARe between -70 and +70.

The dynamics of the mean frequencies of all investigated species are shown for each of the three species groups separately in Fig. 2. For the decreasing and increasing species groups, the change in frequency over the gradient of pesticide pressure was statistically significant (Jonckheere-Terpstra Test, $p = 0.001$), whereas nonspecific species showed no common monotone change in frequency (Jonckheere-Terpstra Test, $p > 0.05$). In the following, p-values in parentheses indicate significant differences from reference conditions (PP1). Decreasing species show a maximum frequency of 19.7% at PP1, from where the frequency decreases to 11.0%, 7.1%, 4.1% and 1.7% at the four levels of increasing PP (Tukey's HSD Test, $p < 0.001$ for all comparisons). The frequency of nonspecific species increases at moderate PP (Tukey's HSD Test, $p < 0.01$ at PP3 and $p < 0.05$ at PP4). However, no difference at the highest PP could be found compared to the reference conditions (Tukey's HSD Test, $p > 0.05$). Increasing species show an increase in frequency from 1.8% at PP1 to 4.7% (Tukey's HSD Test, $p < 0.05$), 7.6%, 9.0%, 11.4% (Tukey's HSD Test, $p < 0.001$) at the four levels of increasing PP.

Fig. 3a shows the relation of the Pesticide Associated Response (PARe) and the classification according to $SPEAR_{pesticides}$ as species at risk (SPEAR) and not at risk (NotSPEAR) towards pesticide pressure. A significant difference of PARe between SPEAR and NotSPEAR could be found (Kruskal-Wallis Test, $p < 0.001$). The classification system according to $SPEAR_{pesticides}$ was also compared with our classification of species as decreasing, nonspecific and increasing (see Fig. 3b). For SPEAR, both classification systems show similar results (35 decreasing species, 11 nonspecific species). However, NotSPEAR are distributed over all three species groups (36 decreasing, 44 nonspecific and 13 increasing species). This difference in classification indicates that other traits or stressors, which co-occur with pesticide pressure, might affect species occurrence (see also section 3.2).

Moreover, the distribution of decreasing, nonspecific and increasing species within different taxonomic groups was analyzed (Fig. 3c). Decreasing species are composed of species from eleven taxonomic groups (order or higher level of taxonomy), seven of them belonging to the class of insects. The taxonomic groups where decreasing species are predominant are Plecoptera (80%), Ephemeroptera (78%), Trichoptera (74%) and Coleoptera (71%). All other taxonomic groups are dominated by nonspecific species with the largest percentages identified for Bivalvia (100%), Heteroptera (100%) and Hirudinea (86%). The nonspecific species group contains species from all investigated 14 systematic orders and classes and therefore shows the highest diversity. Increasing species pertain to seven taxonomic groups with the highest shares of Oligochaeta (38%), Gastropoda (36%) and Diptera (29%).

3.2 Link between PARe and ecological traits

We identified a moderately negative but significant relationship of the Pesticide Associated Response (PARe) and the laboratory based physiological sensitivity according to $SPEAR_{pesticides}$ with a high variance (Spearman's Rank correlation, $r = -0.44$, $p < 0.001$, Fig. A.2). Hence, high values of physiological sensitivity indicate a relatively high vulnerability of the given species. Accordingly, we observed a decrease in the PARe-value with increasing physiological sensitivity. However, the high variance of the relationship indicates that PARe may also be influenced by other traits.

In a second step the relationship between PARe and different ecological traits namely (a) the saprobic value, (b) the substrate preference, (c) feeding type and (d) respiration were analyzed in order to investigate whether the traits have an impact on species' occurrence in the field in addition to the traits included in the index $SPEAR_{pesticides}$. Therefore, the analysis was conducted separately for SPEAR and NotSPEAR according to $SPEAR_{pesticides}$ (see also section 2.5). Values of the traits for each species as well as their classification according to $SPEAR_{pesticides}$ can be found in Table A.1.

For NotSPEAR a significant increase in PARe could be found with increasing saprobic value (Dunn's-Test, $p < 0.001$, see Fig. 4, 1b). Similarly, NotSPEAR preferring macrophytes or mud as a substrate were found to have a comparatively high PARe (Kruskal-Wallis Test, $p < 0.01$, see Fig. 4, 2b). Hence, the frequency of NotSPEAR with high saprobic values or preferring macrophytes/mud as a substrate is more likely to increase or remain about constant, while NotSPEAR with low saprobic values or preferring any other substrate will show a decrease in frequency.

For SPEAR, the variance of PARe cannot be explained by substrate preference (see Fig. 4, 2a). However, similar to NotSPEAR, a significant increase in PARe could be found with an increasing saprobic value from 1-1.9 to 2-2.4 (Dunn's-Test, $p < 0.05$, see Fig. 4, 1a). Hence, SPEAR with high saprobic values will show a less decreasing frequency.

No significant relation could be identified between PARe and the traits ‘feeding type’ and ‘respiration’ (Kruskal-Wallis Test, $p > 0.05$, see Fig. A.3). The ecological traits ‘maximum potential size’, ‘resistance forms’, ‘life cycles’ and ‘reproduction’ had to be excluded from the analysis due to limited data availability.

4 Discussion

We ranked the vulnerability of 139 aquatic macroinvertebrate species, expressed as Pesticide Associated Response (PARe) along a gradient of predicted pesticide pressure (PP) using large-scale monitoring data from 4147 stream sites. The predicted pesticide pressure was determined with the trait-based bioindicator $SPEAR_{pesticides}$ which is known for its general robustness against other environmental factors that often co-occur with pesticide pressure (Liess et al., 2008; Knillmann et al., 2018). Hence, we assume that the calculated $SPEAR_{pesticides}$ values largely result from pesticide pressure. Both variables, PARe and PP, are based on evaluations of the macroinvertebrate occurrence. By this, the general problem of circular reasoning arises. For example, sites with a low PP according to $SPEAR_{pesticides}$ will also have a high proportion of species at risk. We circumvent this issue by calculating the response to pesticide pressure (PARe) solely on the species level. Accordingly, the pesticide pressure - the independent variable - is determined by the whole community at a given site and the occurrence of single species only marginally influences the calculated pesticide pressure (PP). Therefore, the vulnerability ranking of each single species – the dependent variable - is largely independent from its contribution to the pesticide pressure.

The field response of the investigated 139 species related to pesticide pressure ranged from a complete disappearance in frequency with a PARe of -100 to a maximal increase with a

PARe of +100, enabling us to group species into roughly 50% 'decreasing species', 10% 'increasing species' and 40% 'nonspecific species'. We compared the PARe-value of investigated species with their given classification according to $SPEAR_{pesticides}$. Species, which are classified as 'at risk' from pesticide pressure (referred to as SPEAR) mainly belonged to the 'decreasing species' with a mean PARe of -80 (SD = 69.8). For example, Plecoptera, which is the order with the highest number of 'decreasing species', was identified in laboratory based sensitivity rankings as containing very sensitive or even the most sensitive species towards pesticides (Von der Ohe and Liess, 2004; Rubach et al., 2010; Rico and Van den Brink, 2015). By contrast, those species which are classified as 'not at risk' from pesticide pressure (referred to as NotSPEAR) showed no clear agreement with their PARe and only partially belonged to the group of 'increasing species'. The mean PARe of all NotSPEAR is -12.5 (SD = 33.4) with more than one third even belonging to the group of 'decreasing species' with a PARe of < -70. The decreasing NotSPEAR group mainly comprised Coleoptera, *Hydropsyche* (Trichoptera) and 'refuge taxa' as described by Knillmann et al. (2018). Out of 21 investigated refuge species, 15 are classified as decreasing in our analysis with a mean PARe of -92.4 (SD=9.5). Hence, our analysis demonstrated their typically high physiological sensitivity towards pesticides. These species are nevertheless classified as pesticide-invulnerable according to $SPEAR_{pesticides}$ because they are able to quickly recolonize from forested or grassland upstream sections (Knillmann et al., 2018). The monitoring data investigated in this study does not contain information on the presence of refuge areas close to the sampling sites. However, the observed decline in refuge species might be due to the fact that also the nearby presence of refuge areas is declining with the increase of pesticide pressure or agricultural intensity.

The decline of other NotSPEAR apart from refuge species may be attributed to the occurrence of multiple stressors in streams with an agricultural influence. Species responses in the field may depend on several co-occurring environmental factors (Sundermann et al., 2015). Rasmussen et al. (2012) showed that an increase of pesticide pressure in the field often co-occurs with the reduction of oxygen and the increasing presence of soft substrates.

Hence, species' requirements towards parameters as the habitat or their sensitivity towards other stressors such as oxygen deficiency could be associated with PARe. To identify the potential additional effect of other stressors on the species' occurrence, we performed a trait analysis. We showed that many Coleoptera, which represent a large share of decreasing NotSPEAR, are characterized by a comparatively low saprobic value (www.freshwaterecology.info, November 2018) and hence by an expected high oxygen demand of species. The latter has also been recorded in several studies: In a review of the family Elmidae, Elliott (2008) stated that especially adult species require water with high amount of dissolved oxygen, as they rely upon plastron respiration. The relation to high oxygen supply was also supported by Jacobsen and Marín (2008) who found the density of Elmidae populations to be closely related to minimum oxygen saturation based on a field investigation in a Bolivian stream. Kolar and Rahel (1993) tested the effect of hypoxia of *Hydaticus modestus* from the family Dytiscidiae in experimental chambers and showed the larvae to be intolerant towards low oxygen. Hence, a high oxygen demand could be one factor associated with the observed decrease in frequency, as shown for Coleoptera in this study.

Furthermore, the trait analysis of our study revealed a significant relationship between the PARe-value of NotSPEAR and the trait 'substrate preference'. Species, such as from the genus *Hydropsyche* and the majority of refuge species, preferring organic substrates, sand, gravel, stones or root substrates are characterized by a significantly lower PARe than those species preferring mud or macrophytes. However, the impact of the structural quality on the derivation of $SPEAR_{pesticides}$ has been considered in several field studies and only explained a minor share of community response (Bunzel et al., 2014; Knillmann et al., 2018). Hence, we assume the habitat quality to be intercorrelated with the pesticide pressure but not primarily cause the field response of the mentioned species.

Moreover, the decline of some NotSPEAR species may also be a result of indirect effects following the impact of pesticide pressure, such as changed ecological interactions with species directly affected by pesticides. Indirect effects can lead to negative or positive responses of species and have been shown to play an important role for toxicant effects in communities in previous studies such as Peterson (2001), Preston (2002), Fleeger et al. (2003), Knillmann et al. (2012), and Sundermann et al. (2015).

Finally, it should also be noted that the trait values themselves are subject to uncertainties, which could have led to a misclassification of species as NotSPEAR. Except for refuge species, most decreasing NotSPEAR are classified as NotSPEAR because of their low physiological sensitivity. This trait value is based on only a limited number of laboratory toxicity tests that will in several cases not reflect pesticide vulnerability under field conditions. Also, for a majority of species sensitivity information needed to be aggregated at a higher taxonomic level as species specific values are often not available. One example is the genus *Hydropsyche,* where low physiological sensitivity is assigned to all respective decreasing NotSPEAR species. However, studies show that even species of the same genus can differ in their physiological sensitivity. While Wiberg-Larsen et al. (2016) confirmed a comparatively high insensitivity for *H. angustipennis* towards a pyrethroid within a laboratory study, Rico and Van den Brink (2015) identified that several species of the genus *Hydropsyche* were sensitive towards organophosphates, pyrethroids and carbamates. This shows the urgent need for more detailed physiological sensitivity values at species level from standard laboratory toxicity tests. For a more sound information basis, we further recommend a detailed comparison of laboratory based sensitivity values with species occurrence related to pesticide pressure in the field, as has been done for salinity tolerance by Kefford et al. (2004). The uncertainty caused by the current lack of species specific sensitivity data is one reason for the binary classification of species within $SPEAR_{pesticides}$. As $SPEAR_{pesticides}$ indicates effects based on several species, the indicator is relatively robust towards mis-classification of single species.

Overall, we conclude that our ranking of species regarding their field response can in most cases be traced back to pesticide effects. This applies especially for sensitive species according to $SPEAR_{pesticides}$. In comparison, the decrease of frequency of species classified as insensitive according to $SPEAR_{pesticides}$ may be explained by confounding factors and insufficient information on their vulnerability.

In the present study we revealed detailed knowledge about single species' responses to pesticides in the field. This ranking of species' vulnerability may be useful to determine particularly threatened species. Although all of the species analyzed in our study belong to macroinvertebrates with a comparatively high occurrence throughout Germany, we identified 42 (mostly insect) species, which were not present at any sampling site assigned to high or highest level of pesticide pressure. This leads to the conclusion that these species are particularly at risk due to current agricultural practices with high pesticide usage. A comparison of the above mentioned 42 species with the red list of threatened species in Germany (BfN, 2018) shows surprisingly little consistency. Only the Limnephilidae *Drusus annulatus* is categorized as 'near threatened', and another 23 species are not even listed in the data base. Therefore, we suggest complementing the classification of endangered species with a calculation-based approach using macroecological monitoring investigations as presented in this study.

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Appendix

Fig. A.1

Fig. A.2

Fig. A.3

Table A.1

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Figure captions

Fig. 1: Sampling sites, provided for the analysis by the German federal state authorities, sampled during the period 2004-2013 and selected according to our criteria for site selection.

Fig. 2: Absolute frequency over the time period April to June of (a) decreasing species, (b) nonspecific species and (c) increasing species along a predicted gradient of pesticide pressure (PP). The points indicate the mean of all species belonging to the respective species group. The error bars indicate the 25% and 75% quantiles. Asterisks indicate significant differences from PP1 (Tukey's HSD Test, significance code: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Fig. 3: Relation of the Pesticide Associated Response (PARe) and the classification as species 'at risk' and 'not at risk' defined by the index $SPEAR_{pesticides}$ (a; Kruskal-Wallis Test, $p < 0.001$). Distribution of decreasing, nonspecific and increasing species within the two classes of pesticide vulnerability according to $SPEAR_{pesticides}$ (b) as well as within different taxonomic orders and classes (respective highest taxonomic level according to Asterics (2011)), sorted by the number of decreasing species, whereas taxa marked in bold belong to the class of insects (c). The x-axis in (b) and (c) indicates a Pesticide Associated Response (PARe) of 0. PARe is < 0 below the x-axis and > 0 above the x-axis.

Fig. 4: Relationship between Pesticide Associated Response (PARe) and the traits saprobic value (1a, 1b) as well as substrate preference (2a, 2b), analyzed separately for species classified as 'at risk' (first row) and 'not at risk' (second row) according to $SPEAR_{pesticides}$. The number between the category name and the box indicates the number of species belonging to the category. The number [%] in the right upper corner displays the share of species for which data could be included in the respective analysis in relation to all 139 species. The horizontal line displays the median PARe of all species belonging to the respective category. The end of the box indicates the 25th and 75th percentile respectively. The whiskers represent the most extreme data point which is at maximum 1.5 times the length of the box away from the box. Asterisks indicate significant differences between the different categories

or groups of categories (1a and 1b Dunn's-Test, 2a and 2b Kruskal-Wallis Test, Significance code: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

Fig. A.1: Absolute frequency (y-axis) over the time period April to June of all 139 species along a predicted gradient of pesticide pressure (PP, x-axis). PARe indicates the Pesticide Associated Response, defined as the change of relative frequency from PP1 (reference conditions) to PP5 during the main period of pesticide application (see section 2.4). The number at the top of each plot window indicates the number of the row in Table 2 and Table A.1 for the respective species.

Fig. A.2: Spearman's rank correlation of the Pesticide Associated Response (PARe) and physiological sensitivity according to Von der Ohe and Liess (2004). Grey dots show NotSPEAR and black dots SPEAR.

Fig. A.3: Relationship between the Pesticide Associated Response (PARe) and the traits feeding type (1a, 1b) and respiration (2a, 2b), analyzed separately for species classified as 'at risk' (first row) and 'not at risk' (second row) according to $SPEAR_{pesticides}$. The number between the category name and the box indicates the number of species belonging to that category. The number [%] in the upper right corner shows the share of species for which data could be included in the respective analysis in relation to all 139 species. The horizontal line shows the median PARe of all species in that category. The end of the box indicates the 25th and 75th percentile respectively. The whiskers represent the most extreme data point which is a maximum of 1.5 times the length of the box away from the box.

1

Author contributions

Lena Reiber: Conceptualization, Data collection, Methodology, Formal analysis, Software, Writing - Original Draft

Saskia Knillmann: Conceptualization, Methodology, Software, Validation, Writing - Review & Editing

Kaarina Foit: Conceptualization, Methodology, Software, Validation, Writing - Review & Editing

Matthias Liess: Conceptualization, Writing - Review & Editing, Supervision

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Table 1: Overview on five levels of predicted pesticide pressure with boundaries (in TU) and number of samplings in the respective class

Level of predicted pesticide pressure (PP)	**Toxic Unit (TU)**	**number of samplings**
PP1 (reference)	≤ −4	2347
PP2	> −4 to ≤ −3	1256
PP3	> −3 to ≤ −2	1413
PP4	> −2 to ≤ −1	1123
PP5	> −1	803

Table 2: Species and their Pesticide Associated Response (PARe), divided into increasing, nonspecific and decreasing species groups. No indicates the number listed in the plot displaying the trend of absolute frequency along pesticide pressure of the respective species in Fig. A.1. In column Gr, the taxonomic order or class is listed (Biv: Bivalvia, Col: Coleoptera, Cru: Crustacea, Dip: Diptera, Eph: Ephemeroptera, Gas: Gastropoda, Het: Heteroptera, Hir: Hirudinea, Meg: Megaloptera, Odo: Odonata, Oli: Oligochaeta, Ple: Plecoptera, Tri: tricjoptera, Tur: Turbellaria). PP1 and PP5 indicate the absolute frequency at pesticide pressure level 1 (PP1, reference conditions) and the highest pesticide pressure (PP5) during the main period of pesticide application.

No	Decreasing Species	Gr	PARe	PP1	PP5
1	*Rhithrogena semicolorata*	Eph	-100	54.3	0
2	*Polycentropus flavomaculatus ssp.*	Tri	-100	37.4	0
3	*Habroleptoides confusa*	Eph	-100	36.5	0
4	*Anomalopterygella chauviniana*	Tri	-100	31.7	0
5	*Ecdyonurus venosus*	Eph	-100	28.8	0
6	*Baetis muticus*	Eph	-100	28.5	0
7	*Lepidostoma hirtum*	Tri	-100	25.4	0
8	*Habrophlebia lauta*	Eph	-100	24.8	0
9	*Torleya major*	Eph	-100	23.4	0
10	*Rhyacophila nubila*	Tri	-100	23.2	0
11	*Lepidostoma basale*	Tri	-100	23.1	0
39	*Leuctra nigra*	Ple	-100	6.3	0
40	*Sericostoma flavicorne*	Tri	-100	5.8	0
41	*Athripsodes albifrons*	Tri	-100	5.7	0
42	*Hydropsyche incognita*	Tri	-100	5.7	0
43	*Hydraena gracilis*	Col	-96.1	36.2	1.4
44	*Potamophylax latipennis*	Tri	-92.5	4.9	0
45	*Silo pallipes*	Tri	-91.2	18.1	1.6
46	*Plectrocnemia conspersa ssp.*	Tri	-90.9	16.5	1.5
47	*Serratella ignita*	Eph	-89.8	28.4	2.9
48	*Limnius volckmari*	Col	-89.7	37.8	3.9

12	*Odontocerum albicorne*	Tri	-100	23.1	0
13	*Atherix ibis*	Dip	-100	21.3	0
14	*Limnius perrisi*	Col	-100	20.5	0
15	*Oreodytes sanmarkii*	Col	-100	19.7	0
16	*Ephemerella mucronata*	Eph	-100	19.2	0
17	*Centroptilum luteolum*	Eph	-100	18.9	0
18	*Ecdyonurus torrentis*	Eph	-100	18.8	0
19	*Sialis fuliginosa*	Meg	-100	18.3	0
20	*Brachyptera risi*	Ple	-100	18.3	0
21	*Baetis scambus*	Eph	-100	17.7	0
22	*Baetis lutheri*	Eph	-100	12.7	0
23	*Potamophylax cingulatus/latipennis/ luctuosus*	Tri	-100	12.4	0
24	*Heptagenia sulphurea*	Eph	-100	11.6	0
25	*Potamophylax cingulatus ssp.*	Tri	-100	11	0
26	*Drusus annulatus*	Tri	-100	10.4	0
27	*Calopteryx virgo*	Odo	-100	10.3	0
28	*Leuctra geniculata*	Ple	-100	9.9	0
29	*Esolus parallelepipedus*	Col	-100	9.6	0
30	*Haplotaxis gordioides*	Oli	-100	9.3	0
49	*Elmis maugetii*	Col	-89.2	36.2	3.9
50	*Caenis luctuosa*	Eph	-89.1	4.9	0
51	*Hydropsyche instabilis*	Tri	-88.1	25.2	3
52	*Sericostoma personatum*	Tri	-88.1	10.1	1.2
53	*Paraleptophlebia submarginata*	Eph	-85.1	18.1	2.7
54	*Hydropsyche siltalai*	Tri	-83.4	55.9	9.3
55	*Orectochilus villosus*	Col	-82.8	29.6	5.1
56	*Oulimnius tuberculatus*	Col	-82.6	25.8	4.5
57	*Ancylus fluviatilis*	Gas	-81.9	43	7.8
58	*Athripsodes bilineatus ssp.*	Tri	-81.3	7.5	1.4
59	*Rhyacophila fasciata ssp.*	Tri	-80.6	6.2	1.2
60	*Dugesia gonocephala*	Tur	-79.9	41.3	8.3
61	*Hydropsyche pellucidula*	Tri	-79.5	22.9	4.7
62	*Elmis aenea/maugetii/ rietscheli/rioloides*	Col	-77.3	11	2.5
63	*Stylodrilus heringianus*	Oli	-77.2	26.8	6.1
64	*Silo nigricornis*	Tri	-76.4	7.2	1.7

31	*Silo piceus*	Tri	-100	9.2	0
32	*Baetis fuscatus*	Eph	-100	8.4	0
33	*Psychomyia pusilla*	Tri	-100	7.7	0
34	*Polycentropus irroratus*	Tri	-100	7.3	0
35	*Isoperla grammatica*	Ple	-100	6.6	0
36	*Oecetis testacea*	Tri	-100	6.6	0
37	*Agapetus fuscipes*	Tri	-100	6.6	0
38	*Caenis rivulorum*	Eph	-100	6.3	0

65	*Halesus digitatus ssp.*	Tri	-74.3	11.3	2.9
66	*Lype reducta*	Tri	-74.1	6.3	0
67	*Ephemera danica*	Eph	-74	51.5	13.4
68	*Hydropsyche saxonica*	Tri	-73.5	11.7	3.1
69	*Gammarus fossarum*	Cru	-73	37.4	10.1
70	*Elmis aenea*	Col	-72.9	35.1	9.5
71	*Chaetopteryx villosa ssp.*	Tri	-70.5	21	5

No	Nonspecific Species	Gr	PARe	PP1	PP5
72	*Aphelocheirus aestivalis*	Het	-67.3	3.5	0
73	*Goera pilosa*	Tri	-62.5	7.2	1.2
74	*Baetis rhodani*	Eph	-58.7	79.9	33
75	*Halesus radiatus*	Tri	-57.5	26.2	8.8
76	*Mystacides azurea*	Tri	-51	9.4	4.5
77	*Eiseniella tetraedra*	Oli	-45.8	32.5	17.6
78	*Calopteryx splendens*	Odo	-37.7	12	3.9
79	*Caenis horaria*	Eph	-36.9	4.5	1.4
80	*Athripsodes cinereus*	Tri	-33.1	7.6	3.9
81	*Molanna angustata*	Tri	-30.5	1.8	0

No	Nonspecific Species	Gr	PARe	PP1	PP5
102	*Lumbriculus variegatus*	Oli	29.7	14.9	21.2
103	*Pisidium casertanum ssp.*	Biv	32.7	2.5	4.2
104	*Erpobdella octoculata*	Hir	33.8	34.5	55.3
105	*Radix balthica*	Gas	34.5	19.9	30.4
106	*Prodiamesa olivacea*	Dip	35.8	24.4	40.3
107	*Erpobdella vilnensis*	Hir	36.7	9.2	15.1
108	*Sphaerium corneum*	Biv	39	12.1	23.4

82	*Baetis vernus*	Eph	-30.1	25.5	12.7
83	*Potamophylax rotundipennis*	Tri	-29	3.6	1.4
84	*Platycnemis pennipes*	Odo	-24.6	3.3	1.9
85	*Simulium vernum*	Dip	-24	4.9	3.6
86	*Sialis lutaria*	Meg	-20.7	13.2	8.8
87	*Platambus maculatus*	Col	-20	6.9	4.4
88	*Simulium ornatum*	Dip	-18.2	9.5	7
89	*Pisidium henslowanum*	Biv	-15.5	3	2.1
90	*Elodes minuta*	Col	-14	5.3	4
91	*Anabolia nervosa*	Tri	-8.6	25	21.5
92	*Habrophlebia fusca*	Eph	-4.9	2.5	2.2
93	*Cloeon dipterum*	Eph	-2.6	3.1	2.9
94	*Pisidium amnicum*	Biv	0	5.2	5.2
95	*Limnephilus rhombicus ssp.*	Tri	0	2.9	2.9
96	*Glossiphonia nebulosa*	Hir	4.9	4.8	5.2
97	*Apsectrotanypus trifascipennis*	Dip	14.8	3.4	4.2
98	*Gammarus roeselii*	Cru	18.3	11.2	16.4
99	*Gammarus pulex*	Cru	19.8	53.2	68.5
100	*Limnephilus lunatus*	Tri	20.2	11.8	18.7
101	*Erpobdella nigricollis*	Hir	28	3.1	5.2

109	*Glossiphonia complanata*	Hir	41.1	17.6	35.9
110	*Psammoryctides barbatus*	Oli	42.2	4.7	9.3
111	*Potamopyrgus antipodarum*	Gas	43.1	12.5	22.9
112	*Viviparus contectus*	Gas	45.1	0.8	3.1
113	*Pisidium subtruncatum*	Biv	46	3.7	8.3
114	*Laccophilus hyalinus*	Col	46	1.2	3.5
115	*Hydropsyche angustipennis ssp.*	Tri	50.4	4.9	16.2
116	*Gerris lacustris*	Het	53.1	3.8	8.1
117	*Haemopis sanguisuga*	Hir	55.2	0.9	4.1
118	*Asellus aquaticus*	Cru	55.8	24.6	57.4
119	*Dendrocoelum lacteum*	Tur	57.5	1.8	6
120	*Bithynia tentaculata*	Gas	58.3	6.8	18.8
121	*Gyraulus albus*	Gas	59.7	2.7	6.7
122	*Polycelis nigra/tenuis*	Tur	60	2.7	8.7
123	*Physa fontinalis*	Gas	60.6	3.1	8.8
124	*Nemoura cinerea ssp.*	Ple	63.7	3.6	11.5
125	*Nepa cinerea*	Het	65.3	1.7	6.4

126	*Dugesia lugubris/polychroa*	Tur	65.7	2.3	6.7

No	Increasing Species	Gr	PARe	PP1	PP5
127	*Lymnaea stagnalis*	Gas	70.3	1.9	7.8
128	*Proasellus coxalis*	Cru	72.1	5.5	19.7
129	*Helobdella stagnalis*	Hir	74.4	5.2	20.3
130	*Anisus vortex*	Gas	81.4	1.8	10.1
131	*Limnodrilus hoffmeisteri*	Oli	82.6	3.6	20.7
132	*Planorbis planorbis*	Gas	88.5	1	8.7
133	*Limnodrilus claparedeanus*	Oli	89.1	0.6	5.5

No	Increasing Species	Gr	PARe	PP1	PP5
134	*Chironomus plumosus*	Dip	89.6	0.7	6.7
135	*Tubifex tubifex*	Oli	90.1	1.3	13.1
136	*Planorbarius corneus*	Gas	92	0.9	11.3
137	*Chironomus riparius*	Dip	92.7	0.6	8.2
138	*Ironoquia dubia*	Tri	93.7	0.6	9.5
139	*Haliplus lineatocollis*	Col	100	0	6.6

Table A.1: Overview of the ecological traits of each species. No. indicates the number listed in the plot showing the trend of absolute frequency along pesticide pressure of the respective species in Fig. A.1. In column Gr, the taxonomic order or class is listed (Biv: Bivalvia, Col: Coleoptera, Cru: Crustacea, Dip: Diptera, Eph: Ephemeroptera, Gas: Gastropoda, Het: Heteroptera, Hir: Hirudinea, Meg: Megaloptera, Odo: Odonata, Oli: Oligochaeta, Ple: Plecoptera, Tri: Trichoptera, Tur: Turbellaria). Column S indicates the classification according to $SPEAR_{pesticides}$ as species at risk (1) or species not at risk (0). In columns SV (saprobic value for Germany), substrate preference, feeding type and respiration, the categories of the respective traits are shown as included in Fig. 4 and Fig. A.3 (NA: no data available).

No	Decreasing species	Gr	S	SV	Substrate preference	Feeding type	Respiration
1	*Rhithrogena semicolorata*	Eph	1	1.6	gravel	scraper	gill, tegument
2	*Polycentropus flavomaculatus ssp.*	Tri	1	NA	NA	NA	tegument
3	*Habroleptoides confusa*	Eph	0	1.5	gravel	deposit feeder	gill, tegument
4	*Anomalopterygella chauviniana*	Tri	1	1.5	stones, gravel	scraper	gill, tegument
5	*Ecdyonurus venosus*	Eph	1	1.5	gravel	deposit feeder, scraper	gill, tegument
6	*Baetis muticus*	Eph	1	1.4	macrophytes	deposit feeder, scraper	gill, tegument
7	*Lepidostoma hirtum*	Tri	1	1.8	macrophytes, roots	scraper, shredder	gill, tegument
8	*Habrophlebia lauta*	Eph	0	1.7	organic	deposit feeder	gill, tegument
9	*Torleya major*	Eph	0	1.8	stones, macrophytes, organic, mud	deposit feeder, scraper	gill, tegument
10	*Rhyacophila nubila*	Tri	1	2	stones, gravel	predator	gill, tegument
11	*Lepidostoma basale*	Tri	1	1.8	sand, roots	scraper, shredder	gill, tegument
12	*Odontocerum albicorne*	Tri	1	1.4	gravel, stones, macrophytes, organic, sand, silt, roots	scraper, predator, shredder	gill, tegument
13	*Atherix ibis*	Dip	1	2	NA	predator	NA

14	*Limnius perrisi*	Col	0	1.4	NA	scraper	NA
15	*Oreodytes sanmarkii*	Col	0	1.6	NA	predator	NA
16	*Ephemerella mucronata*	Eph	0	1.4	stones, gravel	deposit feeder, scraper	gill, tegument
17	*Centroptilum luteolum*	Eph	1	2	macrophytes	deposit feeder, scraper	gill, tegument
18	*Ecdyonurus torrentis*	Eph	1	2	gravel	deposit feeder, scraper	gill, tegument
19	*Sialis fuliginosa*	Meg	1	2	NA	predator	NA
20	*Brachyptera risi*	Ple	1	1.2	gravel	scraper	tegument
21	*Baetis scambus*	Eph	1	2	gravel, macrophytes	deposit feeder, scraper	gill, tegument
22	*Baetis lutheri*	Eph	1	1.5	stones, gravel	deposit feeder, scraper	gill, tegument
23	*Potamophylax cingulatus/latipennis/luctuosus*	Tri	1	NA	organic	shredder	gill, tegument
24	*Heptagenia sulphurea*	Eph	1	2	gravel	deposit feeder, scraper	gill, tegument
25	*Potamophylax cingulatus ssp.*	Tri	1	1.5	NA	shredder	gill, tegument
26	*Drusus annulatus*	Tri	1	1	stones	scraper	gill, tegument
27	*Calopteryx virgo*	Odo	1	1.8	NA	predator	NA
28	*Leuctra geniculata*	Ple	1	1.6	gravel, stones, roots	deposit feeder, shredder	tegument
29	*Esolus parallelepipedus*	Col	0	1.6	NA	scraper	NA
30	*Haplotaxis gordioides*	Oli	0	1.5	gravel, mud	deposit feeder	tegument
31	*Silo piceus*	Tri	0	1.1	stones, gravel	scraper	gill, tegument
32	*Baetis fuscatus*	Eph	1	2.1	gravel, macrophytes, sand	deposit feeder, scraper	gill, tegument
33	*Psychomyia pusilla*	Tri	1	2.1	stones, gravel	scraper	tegument
34	*Polycentropus irroratus*	Tri	1	1.5	stones, gravel, macrophytes, organic, roots	predator	tegument
35	*Isoperla grammatica*	Ple	1	1.6	gravel	predator	tegument
36	*Oecetis testacea*	Tri	0	NA	stones, gravel	predator	gill, tegument
37	*Agapetus fuscipes*	Tri	1	1	stones, gravel	scraper	tegument
38	*Caenis rivulorum*	Eph	1	2	organic	deposit feeder	gill, tegument
39	*Leuctra nigra*	Ple	1	1.4	organic	deposit feeder	tegument
40	*Sericostoma flavicorne*	Tri	0	1.5	gravel, organic,	shredder	gill, tegument

					sand		
41	*Athripsodes albifrons*	Tri	0	2.1	gravel	shredder	gill, tegument
42	*Hydropsyche incognita*	Tri	0	2	stones, gravel	filter feeder	gill, tegument
43	*Hydraena gracilis*	Col	0	1.5	NA	scraper	NA
44	*Potamophylax latipennis*	Tri	1	1.5	organic	shredder	gill, tegument
45	*Silo pallipes*	Tri	0	1.5	stones, gravel	scraper	gill, tegument
46	*Plectrocnemia conspersa ssp.*	Tri	1	NA	NA	NA	tegument
47	*Serratella ignita*	Eph	0	2	macrophytes	deposit feeder, scraper	gill, tegument
48	*Limnius volckmari*	Col	0	1.6	NA	scraper	NA
49	*Elmis maugetii*	Col	0	1.5	NA	scraper	NA
50	*Caenis luctuosa*	Eph	1	2	macrophytes, sand	deposit feeder	gill, tegument
51	*Hydropsyche instabilis*	Tri	0	1.5	stones, gravel	filter feeder	gill, tegument
52	*Sericostoma personatum*	Tri	0	1.5	gravel, organic, sand	shredder	gill, tegument
53	*Paraleptophlebia submarginata*	Eph	0	1.8	macrophytes	deposit feeder	gill, tegument
54	*Hydropsyche siltalai*	Tri	0	1.8	stones, gravel	filter feeder	gill, tegument
55	*Orectochilus villosus*	Col	0	2	NA	predator	NA
56	*Oulimnius tuberculatus*	Col	0	1.9	NA	scraper	NA
57	*Ancylus fluviatilis*	Gas	0	1.9	stones	scraper	tegument
58	*Athripsodes bilineatus ssp.*	Tri	0	NA	NA	NA	gill, tegument
59	*Rhyacophila fasciata ssp.*	Tri	0	NA	stones, gravel	NA	gill, tegument
60	*Dugesia gonocephala*	Tur	0	1.5	NA	predator	NA
61	*Hydropsyche pellucidula*	Tri	0	2	stones, gravel	filter feeder	gill, tegument
62	*Elmis aenea/ maugetii/rietscheli/rioloides*	Col	0	NA	NA	NA	NA
63	*Stylodrilus heringianus*	Oli	0	NA	gravel	deposit feeder	tegument
64	*Silo nigricornis*	Tri	0	1.5	stones, gravel	scraper	gill, tegument
65	*Halesus digitatus ssp.*	Tri	1	NA	NA	NA	NA
66	*Lype reducta*	Tri	1	NA	roots	scraper	tegument
67	*Ephemera danica*	Eph	0	1.8	sand	filter feeder	gill, tegument
68	*Hydropsyche saxonica*	Tri	0	1.5	stones, gravel	filter feeder	gill, tegument
69	*Gammarus fossarum*	Cru	0	1.5	NA	shredder	NA
70	*Elmis aenea*	Col	0	1.5	NA	scraper	NA
No	**Nonspecific species**	**Gr**	**S**	**SV**	**Substrate preference**	**Feeding type**	**Respiration**

72	*Aphelocheirus aestivalis*	Het	0	2	stones	piercer, predator	plastron, spiracle, tegument
73	*Goera pilosa*	Tri	1	2	stones, gravel	scraper	gill, tegument
74	*Baetis rhodani*	Eph	0	2.1	stones, gravel	deposit feeder, scraper	gill, tegument
75	*Halesus radiatus*	Tri	1	1.9	organic, roots	shredder	gill, tegument
76	*Mystacides azurea*	Tri	0	2.1	macrophytes	deposit feeder	gill, tegument
77	*Eiseniella tetraedra*	Oli	0	NA	macrophytes	deposit feeder	tegument
78	*Calopteryx splendens*	Odo	1	2.2	NA	predator	NA
79	*Caenis horaria*	Eph	1	2	mud	deposit feeder	gill, tegument
80	*Athripsodes cinereus*	Tri	0	2	gravel	predator	gill, tegument
81	*Molanna angustata*	Tri	1	NA	mud, sand	predator	gill, tegument
82	*Baetis vernus*	Eph	1	2.1	gravel	deposit feeder, scraper	gill, tegument
83	*Potamophylax rotundipennis*	Tri	1	2	organic	shredder	gill, tegument
84	*Platycnemis pennipes*	Odo	0	2.1	NA	predator	NA
85	*Simulium vernum*	Dip	0	1.5	NA	filter feeder	NA
86	*Sialis lutaria*	Meg	1	2.5	NA	predator	NA
87	*Platambus maculatus*	Col	0	2.2	NA	predator	NA
88	*Simulium ornatum*	Dip	0	2.5	NA	filter feeder	NA
89	*Pisidium henslowanum*	Biv	0	NA	NA	filter feeder	NA
90	*Elodes minuta*	Col	0	NA	NA	shredder	NA
91	*Anabolia nervosa*	Tri	0	2	macrophytes	shredder	gill, tegument
92	*Habrophlebia fusca*	Eph	0	1.7	gravel, macrophytes, mud, organic	deposit feeder	gill, tegument
93	*Cloeon dipterum*	Eph	1	2.3	macrophytes	deposit feeder, scraper	gill, tegument
94	*Pisidium amnicum*	Biv	0	2	NA	filter feeder	NA
95	*Limnephilus rhombicus ssp.*	Tri	1	NA	NA	shredder	gill, tegument
96	*Glossiphonia nebulosa*	Hir	0	2	NA	predator	NA
97	*Apsectrotanypus trifascipennis*	Dip	0	NA	NA	predator	NA
98	*Gammarus roeselii*	Cru	0	2.2	NA	shredder	NA
99	*Gammarus pulex*	Cru	0	2	NA	shredder	NA
100	*Limnephilus lunatus*	Tri	1	2	macrophytes	shredder	gill, tegument
101	*Erpobdella nigricollis*	Hir	0	2.5	NA	predator	NA
102	*Lumbriculus variegatus*	Oli	0	3	NA	deposit feeder	NA

103	*Pisidium casertanum ssp.*	Biv	0	NA	NA	filter feeder	NA
104	*Erpobdella octoculata*	Hir	0	2.8	NA	predator	NA
105	*Radix balthica*	Gas	0	2.3	NA	deposit feeder, scraper	NA
106	*Prodiamesa olivacea*	Dip	0	NA	NA	deposit feeder	NA
107	*Erpobdella vilnensis*	Hir	0	2.2	NA	predator	NA
108	*Sphaerium corneum*	Biv	0	2.4	NA	filter feeder	NA
109	*Glossiphonia complanata*	Hir	0	2.3	NA	predator	NA
110	*Psammoryctides barbatus*	Oli	0	NA	NA	deposit feeder	NA
111	*Potamopyrgus antipodarum*	Gas	0	2.3	mud	deposit feeder, shredder	gill
112	*Viviparus contectus*	Gas	0	2	NA	scraper	NA
113	*Pisidium subtruncatum*	Biv	0	NA	NA	filter feeder	NA
114	*Laccophilus hyalinus*	Col	0	NA	NA	predator	NA
115	*Hydropsyche angustipennis ssp.*	Tri	0	NA	stones, gravel	NA	gill, tegument
116	*Gerris lacustris*	Het	0	NA	NA	predator	NA
117	*Haemopis sanguisuga*	Hir	0	NA	stones	piercer, predator	tegument
118	*Asellus aquaticus*	Cru	0	2.8	macrophytes	deposit feeder, scraper, shredder	gill
119	*Dendrocoelum lacteum*	Tur	0	2.4	stones	predator	tegument
120	*Bithynia tentaculata*	Gas	0	2.3	NA	filter feeder	NA
121	*Gyraulus albus*	Gas	0	2	NA	scraper	NA
122	*Polycelis nigra/tenuis*	Tur	0	2	NA	predator	NA
123	*Physa fontinalis*	Gas	0	2	macrophytes	scraper	tegument
124	*Nemoura cinerea ssp.*	Ple	0	NA	NA	shredder	tegument
125	*Nepa cinerea*	Het	0	NA	NA	predator	NA
126	*Dugesia lugubris/polychroa*	Tur	0	2.1	NA	predator	NA

No	**Increasing species**	**Gr**	**S**	**SV**	**Substrate preference**	**Feeding type**	**Respiration**
128	*Proasellus coxalis*	Cru	0	2.8	NA	shredder	NA
129	*Helobdella stagnalis*	Hir	0	2.6	macrophytes	piercer, predator	tegument
130	*Anisus vortex*	Gas	0	2	NA	scraper	NA
131	*Limnodrilus hoffmeisteri*	Oli	0	3.3	NA	deposit feeder	NA
132	*Planorbis planorbis*	Gas	0	2.4	NA	scraper	NA

133	*Limnodrilus claparedeanus*	Oli	0	3.3	NA	deposit feeder	NA
134	*Chironomus plumosus*	Dip	0	NA	NA	deposit feeder	NA
135	*Tubifex tubifex*	Oli	0	3.6	NA	deposit feeder	NA
136	*Planorbarius corneus*	Gas	0	2.2	macrophytes	scraper, shredder	tegument
137	*Chironomus riparius*	Dip	0	NA	NA	deposit feeder	NA
138	*Ironoquia dubia*	Tri	0	2	macrophytes, organic	shredder	gill, tegument
139	*Haliplus lineatocollis*	Col	0	NA	NA	scraper	NA

Highlights

- Single species' pesticide vulnerability derived from governmental monitoring data
- 71 species are threatened by pesticide pressure, 13 are profiting
- Species threatened by pesticide pressure are mostly insects

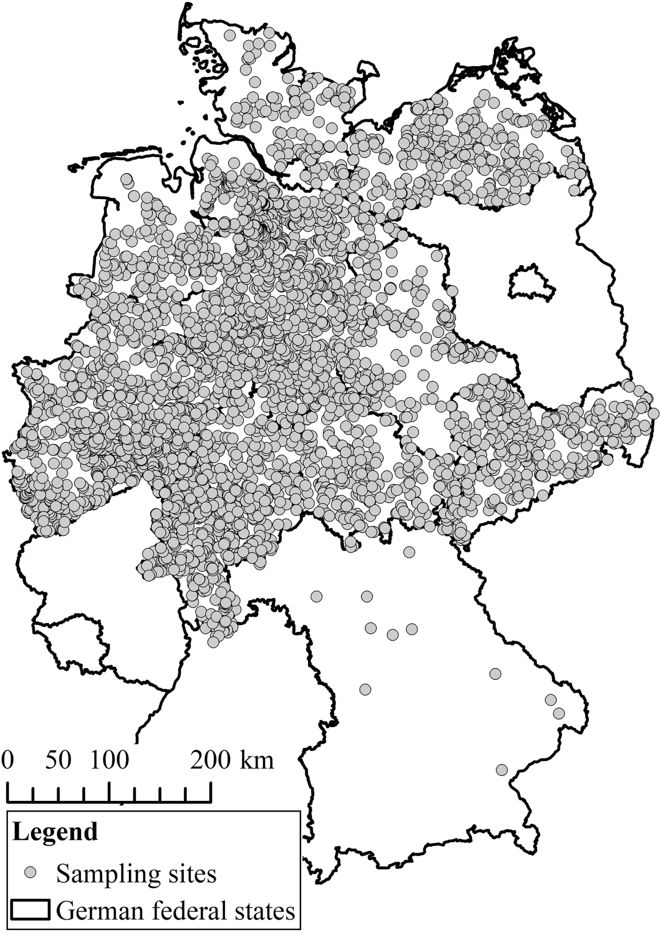

Figure 1

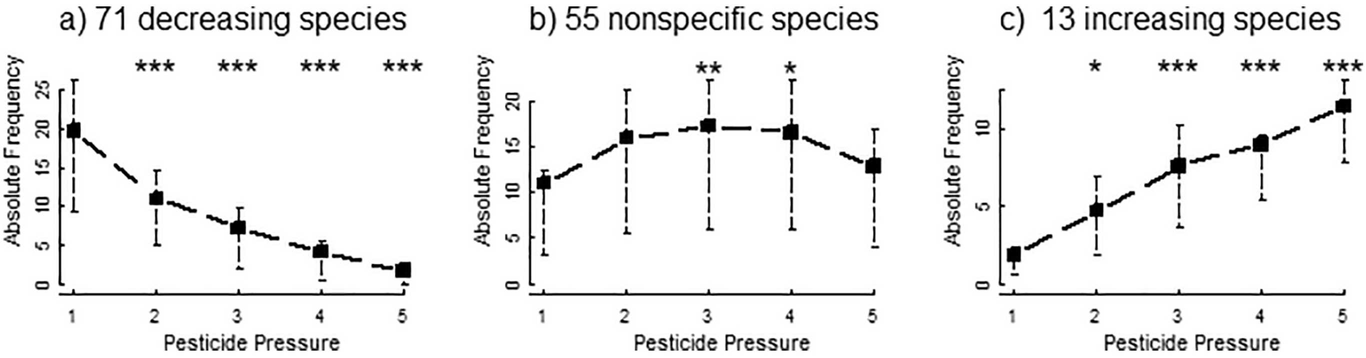
a) 71 decreasing species
b) 55 nonspecific species
c) 13 increasing species
***
**
*
Absolute Frequency
Pesticide Pressure
0
5
10
15
20
25
1
2
3
4
5

Figure 2

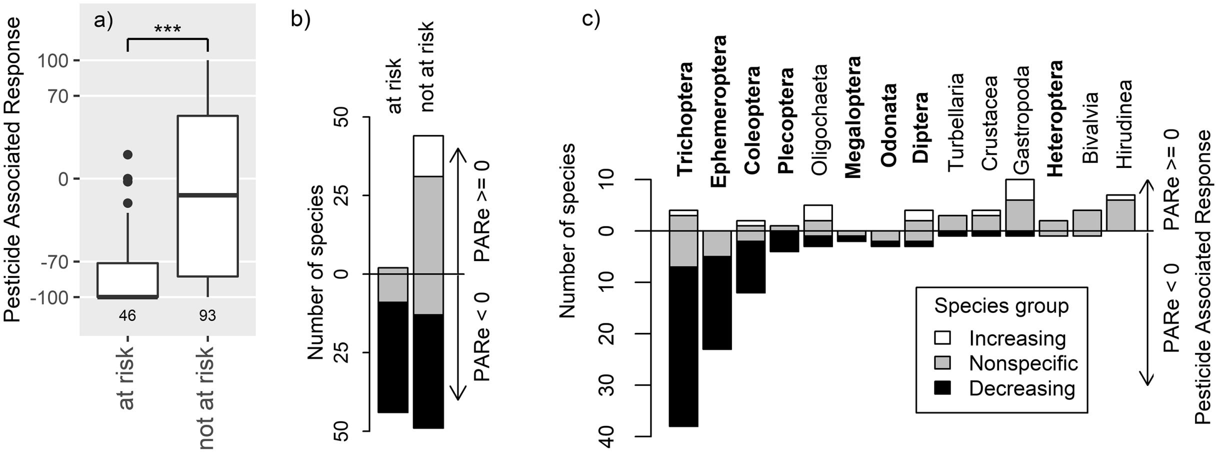
a)
***
Pesticide Associated Response
100
70
0
-70
-100
46
93
at risk
not at risk
b)
at risk
not at risk
Number of species
50
25
0
25
50
PARe >= 0
PARe < 0
c)
Trichoptera
Ephemeroptera
Coleoptera
Plecoptera
Oligochaeta
Megaloptera
Odonata
Diptera
Turbellaria
Crustacea
Gastropoda
Heteroptera
Bivalvia
Hirudinea
Number of species
10
0
10
20
30
40
Species group
Increasing
Nonspecific
Decreasing
PARe >= 0
PARe < 0
Pesticide Associated Response

Figure 3