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Potential impact of chemical stress on freshwater invertebrates: A sensitivity assessment on continental and national scale based on distribution patterns, biological traits, and relatedness.

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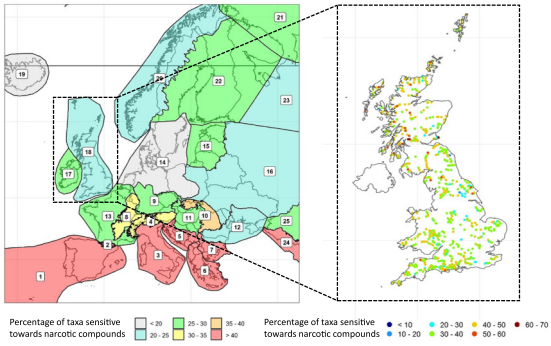
HIGHLIGHTS

- A chemical sensitivity assessment of freshwater invertebrate assemblages
- Increased mechanistic understanding by combining traits and taxonomy
- We identified geographical hotspots of species chemical sensitivity.
- Patterns of endemic biodiversity explain found sensitivity hotspots.
- A first next step towards a new predictive ecotoxicology framework

GRAPHICAL ABSTRACT



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ABSTRACT

Current chemical risk assessment approaches rely on a standard suite of test species to assess toxicity to environmental species. Assessment factors are used to extrapolate from single species to communities and ecosystem effects. This approach is pragmatic, but lacks resolution in biological and environmental parameters. Novel modelling approaches can help improve the biological resolution of assessments by using mechanistic information to identify priority species and priority regions that are potentially most impacted by chemical stressors. In this study we developed predictive sensitivity models by combining species-specific information on acute chemical sensitivity (LC50 and EC50), traits, and taxonomic relatedness. These models were applied at two spatial scales to reveal spatial differences in the sensitivity of species assemblages towards two chemical modes of action (MOA): narcosis and acetylcholinesterase (AChE) inhibition. We found that on a relative scale, 46% and 33% of European species were ranked as more sensitive towards narcosis and AChE inhibition, respectively. These more sensitive species were distributed with higher occurrences in the south and north-eastern regions, reflecting known continental patterns of endemic macroinvertebrate biodiversity. We found contradicting sensitivity patterns depending on the MOA for UK scenarios, with more species displaying relative sensitivity to narcotic MOA in north and north-western regions, and more species with relative sensitivity to AChE inhibition MOA

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in south and south-western regions. Overall, we identified hotspots of species sensitive to chemical stressors at two spatial scales, and discuss data gaps and crucial technological advances required for the successful application of the proposed methodology to invertebrate scenarios, which remain underrepresented in global conservation priorities.

1. Introduction

The scientific community is rapidly developing new ecological models to increase realism in environmental risk assessment (ERA, e.g. De Laender et al., 2015; Windsor et al., 2018). However, what so far has remained unclear is which organisms need to be modelled. Common standard test species are usually not representative of all species present in ecosystems with regards to their sensitivity to stressors (Nagai, 2016). Indeed, it has already been argued for over 30 years that there is not a single species or a specific group of species which is always the most sensitive (all the time, everywhere, and towards every compound). This has been coined the 'myth of the most sensitive species' (Cairns, 1986). However, since in reality both compound multiplicity as well as species diversity occur simultaneously, it is not feasible to acquire all possible sensitivity data with laboratory toxicity testing. Therefore, there is a need to develop models that can help identify priority species, which are species that are likely to be intrinsically most sensitive to chemical stressors.

Several studies have tried to determine which species are intrinsically most sensitive to chemical stressors by using species traits, and were able to explain up to 87% of the variation in species sensitivity using only four traits (Rico and Van den Brink, 2015; Rubach et al., 2012; Rubach et al., 2010; van den Berg et al., 2019). A large advantage of using traits-based approaches is that they add mechanistic understanding of the sensitivity process by describing characteristics that make a species more or less sensitive towards chemical stressors. This largely reduces the chances of overfitting models to the training data (Johnson and Omland, 2004). In addition to that, describing aquatic communities in terms of their biological traits increases the generality of such characterizations and their subsequent transferability between regions (Van den Brink et al., 2011). Also, correlations between species traits and species sensitivity might exist, potentially resulting in unexpected effects at the community level (Baert et al., 2017).

Other studies (Malaj et al., 2016) concerned with determining which species were most sensitive to chemical stressors, combined phylogenetic information with chemical properties. They were to a great extent (R^2 of ~0.8) capable of predicting species sensitivity to pesticides (Guénard et al., 2014) and heavy metals (Malaj et al., 2016). Furthermore, some studies have demonstrated that indeed traits and phylogeny (or other measures of relatedness between species) both explain a unique part of the sensitivity process (Pilière et al., 2016; Poteat et al., 2015). However, phylogenetic approaches do not unravel any concrete mechanisms of sensitivity, and are therefore more susceptible to overfitting on the training data. For this reason, we think that a combination of both traits and phylogenetic information has the most potential for identifying priority species at a large spatial scale.

We envision these priority species to, in the future, become part of environmental scenarios, a simplified (model) representation of exposed aquatic ecosystems which provides a sufficient amount of ecological realism, enabling us to conduct an appropriate ERA (Rico et al., 2016). There are clear benefits associated with the development of scenarios for use in risk assessment, the most important ones being reduction of animal tests, integration of exposure and effect assessments, and increased realism with respect to spatial-temporal dimensions and species biodiversity (Rohr et al., 2016). However, for obtaining more realism in respect to spatial-temporal dimensions and biodiversity, we require not only the identification of priority species, but also the spatial-temporal dimensions at which these species occur. Therefore, after identifying priority species, looking into the distribution patterns of these species can help to identify priority regions, that is, regions where these priority species are more abundant. These regions can assist in delivering realistic ranges of important landscape parameters (e.g. temperature, discharge, alkalinity) as input for environmental scenarios, enabling more realistic landscape level ERA (Franco et al., 2016; Rico et al., 2016). Additionally, these regions can become the focus of conservation and management efforts.

The two main objectives of the present study therefore are i) to construct models predicting the sensitivity of aquatic macroinvertebrates based on mode of action (MOA), traits and relatedness, and ii) to reveal spatial differences in the sensitivity of species composition assemblages by applying the developed models at the continental and national scale. The community composition of European freshwater ecoregions (ERs, based on Illies, 1978) is used for the application of our models at the continental scale, while the reference database of the RIVPACS (River In-Vertebrate Prediction And Classification System) tool is used for river-type scale within the United Kingdom (Wright, 1994). We conduct the first trait-based chemical sensitivity assessment of freshwater macroinvertebrate assemblages, extensively test the influence of spatial scale on sensitivity patterns, and provide key recommendations for its robust application in data-poor taxa.

2. Methods

The whole methodology of this study has been developed in R, a free software environment (R Core Team, 2018). The R project, along with all scripts and data necessary to reproduce the models and figures performed in this study are available at Figshare (https://doi.org/10.6084/m9.figshare.11294450) (van den Berg, 2019).

2.1. Modelling approach

We extracted toxicological data from Van den Berg et al. (2019; original data from ECOTOX (USEPA, 2017)), which comprised Mode Specific Sensitivity (MSS) values for 36 and 32 macroinvertebrate genera towards baseline (narcosis) and AChE inhibiting toxicants respectively. Briefly, the MSS value represents the average relative sensitivity of each species to a group of chemicals with the same MOA (original MOA classification from Barron et al., 2015), where an MSS value below zero indicates that the species is more sensitive than average, and an MSS value above zero indicates that the species is less sensitive than average. The MOAs narcosis and AChE inhibition were selected for this study, because they were the most data rich (van den Berg et al., 2019). Narcosis, also called baseline toxicity, is found toxic at similar internal concentration across all organisms (Escher and Hermens, 2002; Wezel and Opperhuizen, 1995). Therefore, differences in sensitivity for this MOA are expected to be small, equally distributed across taxonomic groups, and mainly explained by traits related to toxicokinetics (i.e. uptake, biotransformation, and elimination). AChE inhibition is a more specific MOA, and therefore shows large differences in effect concentrations depending on taxonomic group (van den Berg et al., 2019). For this MOA we, therefore, expect a stronger phylogenetic signal. To justify a separate analysis for the two MOAs, we made a correlation plot of the measured MSS values of species that were tested on both MOAs (Fig. A.7). The lack of a significant relationship between species sensitivity towards the two MOAs indicates that sensitivity towards

them is independent. We therefore chose to perform a separate analysis for both MOAs in this study.

The dataset from Van den Berg et al. (2019) also contained data on genus name, unique identifier (UID from the NCBI database, Benson et al., 2009; Sayers et al., 2009), and traits (original data from Tachet et al., 2000; Usseglio-Polatera et al., 2000). In this study, we added relatedness to this dataset by constructing a taxonomic tree, since detailed phylogenetic data was still largely unavailable or incoherent for most freshwater macroinvertebrates (we looked, for instance, in Genbank, Benson et al., 2009), and Guénard et al. (2014) have provided sufficient proof that taxonomic relatedness explains around the same amount of variation in species sensitivity as phylogenetic data when a wide taxonomic range is taken into consideration. This taxonomic tree is subsequently converted to Phylogenetic Eigenvector Maps (PEMs), from which species scores are extracted which subsequently serve as predictors of relatedness in model construction (Griffith and Peres-Neto, 2006; Guénard et al., 2013).

2.1.1. Constructing the taxonomic tree

We constructed the taxonomic tree by extracting taxonomic data from the NCBI (National Centre for Biotechnology Information) database (Benson et al., 2009; Sayers et al., 2009), followed by applying the *class2tree* function from the **taxize** package in R (version 0.9.3, Chamberlain and Szöcs, 2013). Both the model species (for which we had sensitivity data available) and the target species (whose sensitivity we wanted to predict) were included in the tree. The simultaneous incorporation of both model and target species was necessary, because the PEM would change if the large number of target species would be added to the tree at a later point.

2.1.2. Phylogenetic eigenvector maps

As descriptors of the taxonomic tree, phylogenetic eigenvectors were obtained from the PEM (see Guénard et al., 2013 for details). PEMs work on a similar basis as principal component analysis (PCA; Legendre and Legendre, 2012). Briefly, the eigenvectors of a PEM are obtained from a decomposition of the among-species covariance's and represent a set of candidate patterns of taxonomic variation of the response variables (i.e. the sensitivities to different chemicals). As is the case for a traditional PCA, this decomposition results in n – 1 eigenvectors (Legendre and Legendre, 2012), where in our analysis n was the number of model species. The calculation of a PEM is obtained from both the structure of the taxonomic tree and from the dynamics of the (in our case) sensitivity evolution. The dynamics of the sensitivity evolution depends on the strength of a steepness parameter (parameter α; related to Pagels' parameter κ (Pagel, 1999), where $\alpha = 1 - \kappa$). This parameter represents the relative evolution rate of the sensitivity to the MOA, takes values between 0 (natural evolution) and 1 (strong natural selection), and was in our study estimated from the known sensitivity of the model species. We constructed the PEMs with the **MPSEM** package (version 0.3–4, Guénard, 2018; Guénard et al., 2013).

2.1.3. Model construction

For the narcosis dataset, two leverage points were discovered during the modelling process (Figs. A.1 and A.2). Since we doubted the validity of these points (they were exactly identical) and were unable to assess their validity (there was no data available on closely related species, and the reference was inaccessible), they were removed from the dataset, reducing the number of species for which toxicity data was available to 34. For the AChE inhibition dataset, only the 27 Arthropoda species present in the dataset were included in the analysis, because this MOA works in a more specific manner, making differences in MOA among different phyla more likely (Maltby et al., 2005). Eventually, 33 and 26 eigenvectors were included as taxonomic predictors for narcosis and AChE inhibition respectively (in the modelling process, taxonomic predictors were indicated with a 'V', see Figs. A.3 and A.4 for examples of such predictors), and were added to the sensitivity and trait data. To reduce the number of predictors going into the final model building process (required due to memory limitations of the algorithm), an exhaustive search was performed using the *regsubsets* function from the **leaps** package (version 3.0, Lumley and Miller, 2017). From this, traits or phylogenetic eigenvectors that were least frequently included in the best 1% of the models, ordered according to the Bayesian Information Criterion (BIC), were removed from the analysis. Next, an exhaustive regression was performed between the remaining predictors and the available MSS values, allowing a maximum of 4 predictors in the models. The best model was the model with the lowest AICc (Aikaike's Information Criterion with a correction for small sample size, Johnson and Omland, 2004). The modelling exercise was repeated using only traits-, and a combination of traits- and taxonomic- predictors. We did not consider taxonomy-only models, because we were primarily interested in obtaining more mechanistic understanding of the sensitivity process.

2.2. Predicting unknown taxa

The best model found for narcosis and the best model found for AChE inhibition were subsequently applied to the prediction of the sensitivity of species composition assemblages at two different spatial scales, continental and national. For the continental scale, the community composition of European freshwater ecoregions (ERs) was downloaded from https://www.freshwaterecology.info/ (Schmidt-Kloiber and Hering, 2015). Although we realize that these data do not exactly resemble species assemblage data, it was the only dataset currently available at this spatial scale. For the national scale, the reference database of the RIVPACS tool was downloaded from the website of the Centre for Ecology and Hydrology (https://www.ceh.ac.uk/services/rivpacs-reference-database). The RIVPAC database was selected, because it is the only easily accessible database that provides detailed community level data at this spatial scale. The database contains macroinvertebrate assemblages at 685 reference sites, and was originally used to assess the ecological quality of UK rivers under the Water Framework Directive. To assess the ecological quality, the 685 sites have in an earlier study been grouped into 43 end groups based on biological and environmental variables (Davy-Bowker et al., 2008). For descriptive summary purposes, these 43 end-groups were furthermore combined into 7 higher level super-groups (Davy-Bowker et al., 2008, Table 1), such that these super-groups can be considered river-types at a relatively broad scale. In this study, we will use the super-groups to assess differences in species sensitivity on a river-type scale (Table 1).

The Tachet database was used as a source of traits data (Tachet et al., 2000; Usseglio-Polatera et al., 2000). In order to make species-traits matching between the two community compositions (ERs and

Table 1
Division of the 685 reference sites into the 7 super-groups, along with a description of the dominant characteristics of the super-groups (taken from Davy-Bowker et al., 2008).

RIVPACS super-group	N sites	Dominant characteristics
1	64	All in Scotland, mostly islands
2	148	Upland streams, mainly in Scotland and Northern England
3	169	Intermediate rivers, South-East Scotland, Wales, North and South-West England
4	48	Small steeper streams, within 13 km of source
5	115	Intermediate size lowland streams, including chalk, South-East England
6	84	Small lowland streams, including chalk, South-East England
7	57	Larger, lowland streams, South-East England, larger, finer sediments

RIVPACS) and the Tachet database possible, the taxonomy of the three databases was aligned with the NCBI database using the **taxize** package (version 0.9.3, Chamberlain and Szöcs, 2013). Species from the ER and RIVPACS communities could then be matched with traits from the Tachet database using the UIDs from the NCBI database. This matching was done at genus level. Since the traits in the Tachet database are coded using a fuzzy coding approach (describing a species by its affinity to several trait modalities, see Chevenet et al., 1994 for more information), a transformation was required before this data could be used. Continuous traits were transformed using a weighted averaging of the different trait modalities, whilst for factorial traits the modality for which the species had the highest affinity was selected (as in van den Berg et al., 2019).

At this point, taxonomic and trait data of all the target species (species for which we want to predict sensitivity) were complete, and PEM scores had to be added. To do this, the locations of the target species were extracted from the taxonomic tree, and subsequently transformed into PEM scores using the **MPSEM** package (version 0.3–4, Guénard, 2018; Guénard et al., 2013). The PEM scores were then combined with the traits data, which allowed us to predict the sensitivity (MSS values) towards narcotic and AChE inhibiting chemicals using the two best models developed earlier.

The sensitivity of each ER or river type was determined by calculating the percentage of species with an MSS value below 0, comparable to (Hering et al., 2009). For RIVPACS, this was initially done both on abundance and presence-absence data, on the seasons spring, summer and autumn separately, and averaged over the three seasons. Eventually, we focused on presence-absence data averaged over the three seasons only, due to higher uncertainty (e.g. due to sampling error and seasonality) associated with the other data subsets. The results were projected on maps by colouring the ERs and river types according to the percentage of sensitivity species (MSS < 0) present. To construct the maps, we downloaded a map of the world from the Natural Earth website (https://www.naturalearthdata.com/downloads/10m-cultural-vectors/). The shape files for the ERs were obtained from the European Environment Agency (https://www.eea.europa.eu/data-and-maps/data/ecoregions-for-rivers-and-lakes), and their projection was transformed to match the projection of the world map using the *spTransform* function form the **sp** package (version 1.3–1, Pebesma and Bivand, 2005). Coordinates of all the RIVPACS sites were available in the RIVPACS database.

2.3. Statistics

A Kruskal-Wallis Rank Sum Test was done to check if there were any statistically significant differences in sensitivity between ERs or RIVPAS groups. If this was true, multiple comparisons of all the groups were done with Kruskal Wallis using the *kruskal* function from the **agricolae** R package (version 1.2–8, Mendiburu, 2017). Fisher's least significant difference criterion was used as a post-hoc test, and we used the Bonferroni correction as p-adjustment method.

3. Results

3.1. Sensitivity models

Incorporating taxonomic relatedness slightly improved the predictive capacity of models for invertebrate sensitivity towards narcotic and AChE inhibiting chemicals (higher adjusted R^2), compared to models without taxonomy (Table 2). Interestingly, the trait 'mode of respiration' was incorporated in the taxonomy & traits model of narcosis (Fig. A.3) and was also present in the traits-only model. For AChE inhibition, mode of respiration was included in the taxonomy & traits model (Fig. A.4), but not in the traits-only model. Considering the taxonomic predictors, V14, V2 and V4 were present in both the taxonomy-only and the taxonomy & traits model for narcosis. For AChE inhibition, the predictors V7 and V3 were present in both the taxonomy-only and the taxonomy & traits model.

Cross-validation of the model species resulted in the correct classification of 82% and 74% of the genera as sensitive or tolerant for respectively narcosis and AChE inhibiting chemicals (Fig. 1). For narcosis, the Diptera *Paratanytarsus* and *Mochlonyx*, the Odonata *Ophiogompus*, the Ephemeroptera *Siphlonurus*, the Gastropoda *Aplexa*, and the Annelida *Chaetogaster* were misclassified (predicted on the wrong side of the zero line). For AChE inhibition, incorrect predictions were made in only two taxonomic groups, the Diptera *Glyptotendipes*, *Paratanytarsus*, *Tanytarsus*, and the Odonata *Anax*, *Crocothemis*, *Ophiogompus* and *Orthetrum*.

3.2. European freshwater ecoregions

3.2.1. Data availability

For the ER communities, taxonomic data was available for 97% of the species, and covered four crustacean orders (Amphipoda, Anostraca, Decopoda, and Isopoda), and six insect orders (Coleoptera, Diptera, Ephemeroptera, Lepidoptera, Plecoptera and Trichoptera). Fig. A.5 shows the taxonomic composition of all ERs at the order level. For 19% of these species there was no or incomplete trait data available, leading to the exclusion of these species from our analysis. Of the remaining species, only around 5% had toxicity data available. We therefore had to predict the sensitivity of around 95% of the species for which no toxicity data was available using the taxonomy & traits models for narcosis and AChE inhibition.

3.2.2. Taxonomic pattern

On the continental scale, 46 and 33% of the species were found sensitive (MSS < 0) towards narcotic and AChE inhibiting chemicals, respectively. For narcotic chemicals, 18 families contained only genera predicted as sensitive. Among these 18 families were all families belonging to the order of Isopoda (1 family), as well as a part of the Amphipoda (1 family), Plecoptera (6), and Trichoptera (10) families included in our study (Table A.1). Five families contained both sensitive and tolerant genera. Four of these families belonged to the order of the Trichoptera, and one to the order of Lepidoptera. The remaining

Table 2
Predictive models constructed for narcotic and AChE inhibiting chemicals, in- and excluding taxonomy. Taxonomic predictors are indicated with a V. See Figs. A.3 and A.4 for a visualization of the predictors incorporated in the taxonomy & traits models.

MOA	Type of model	Model	Adj. R^2	p - value
Narcosis	Taxonomy & traits	MSS = −0.44 + 1.63 * V14–1.95 * V2 + 0.32 * respiration mode + 1.27 * V4	0.47	<0.001
	Taxonomy-only	MSS = 0.16 + 1.66 * V4 + 1.64 * V14 + 1.16 * V5–1.14 * V2	0.42	<0.001
	Traits-only	MSS = 0.04–0.25 * dispersal mode + 0.39 * respiration mode	0.20	0.011
AChE inhibition	Taxonomy & traits	MSS = 0.74 + 2.94 * V7–1.62 * V3–1.04 * V13–0.29 * respiration mode	0.62	<0.001
	Taxonomy-only	MSS = 0.19 + 2.61 *V7 + 0.9 * V10–0.88 * V1–0.86 * V3	0.61	<0.001
	Traits-only	MSS = 6.93–0.84 * life cycle duration – 1.13 * cycles per year– 0.17 * feeding mode – 0. 78 * temperature preferendum	0.4	0.004

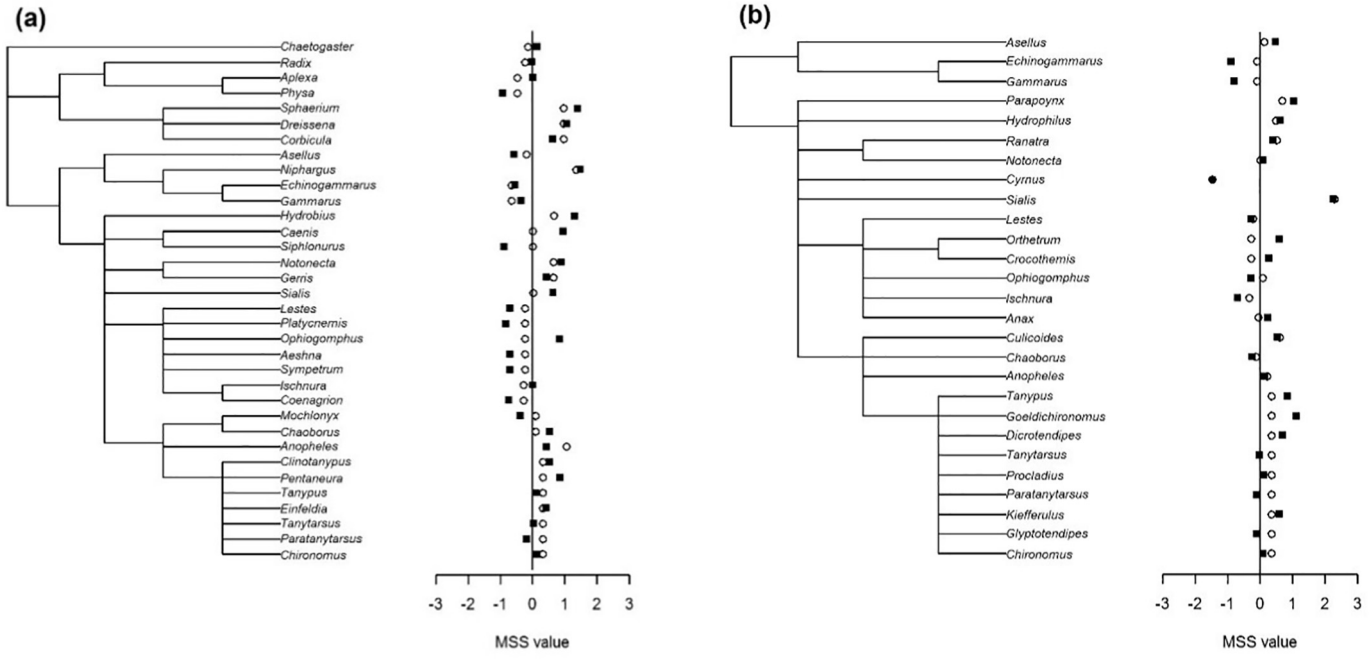

Fig. 1. Observed MSS values (filled squares) and values predicted (unfilled circles) using traits and taxonomy according to the best models for (a) narcotic (b) and AChE inhibiting chemicals. The dendrograms show the taxonomic relationship between species according to class, family, order, and genus.

25 families were predicted to only contain tolerant genera (MSS > 0), and included all of the families belonging to the order of Anostraca (1 family), Decapoda (5), Diptera (1), and Ephemeroptera (12), as well as the remaining Amphipoda (2 families), Plecoptera (1), and Trichoptera (3) families included in this study (Table A.2).

For AChE inhibiting chemicals, there was little variation in sensitivity of the genera belonging to the same family, and the whole family was either predicted to contain only sensitive (MSS < 0) or only tolerant (MSS > 0) genera. All genera belonging to the order of the Trichoptera and all genera belonging to the family of the Gammaridae were predicted as sensitive (Table A.3), while all other families included in this study were predicted to contain only tolerant genera (Table A.4).

3.2.3. Geographical pattern

For both MOAs, we noticed that the South of Europe (e.g. ER 1) has the highest proportion of sensitive species (MSS < 0), whilst Iceland (ER 19) is the ecoregion containing the lowest proportion of sensitive species (Fig. 2). Central Europe (e.g. ER 14) contains the lowest percentages of sensitive species. ER 6 contains the largest percentage (57%) of species sensitive to narcotic chemicals, whilst ER 24 contains the largest percentage (45%) of species sensitive to AChE inhibiting chemicals.

When comparing the assigned sensitivity class of each ER for the two MOAs, we find that 8 of the 25 ERs were grouped into the same class for both MOAs (ER 1, 3, 5, 11, 18, 19, 21, 24, Fig. A.5). ER 2, 4, and 6–10 were classified one or two classes lower for sensitivity towards AChE

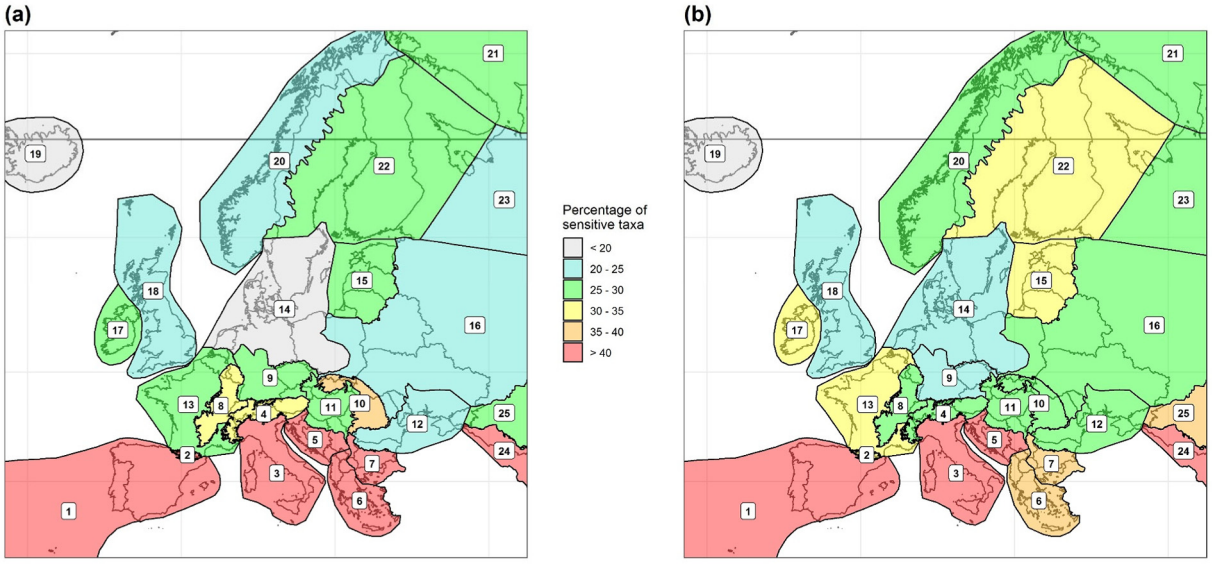

Fig. 2. Percentage of sensitive taxa (MSS < 0) to narcotic (a) and AChE inhibiting (b) chemicals in European freshwater ecoregions. The numbers refer to the ecoregion number (ER 1 through ER 25).

inhibiting chemicals compared to sensitivity towards narcotic chemicals, whilst the opposite was true for ER 12–17, 20, 22, 23, and 25 (Fig. A.6).

3.3. RIVPACS river types

3.3.1. Data availability

For the RIVPACS end-group communities, taxonomic data was available for 98% of the species. To ensure that model predictions did not trespass the taxonomic range on which the model was calibrated, any phylum that was not represented by one of the model species was removed from the analysis. Consequently, sensitivity towards narcotic chemicals was predicted for genera belonging to the phyla Annelida, Mollusca, and Arthropoda, whilst sensitivity towards AChE inhibiting chemicals was predicted only for Arthropoda. Coincidentally, in case of both datasets (Annelida, Mollusca, and Arthropoda, versus Arthropoda only), 34% of the species had no or incomplete traits data available, leading to the exclusions of these species from the analysis. Of the remaining species, <10% had toxicity data available. We therefore had to predict the sensitivity of 90% of the species for which no toxicity data was available using the taxonomy & traits models for narcosis and AChE inhibition.

3.3.2. Taxonomic pattern

Within the UK, 38, and 25% of the species were found sensitive (MSS < 0) to narcotic and AChE inhibiting chemicals respectively. For narcotic chemicals, 37 families contained only genera predicted as sensitive, with an MSS value below zero. Among these 37 families were all families belonging to the order of Annelida (9 families), Isopoda (1), and Odonata (7), as well as a part of the Amphipoda (1), Plecoptera (6), Trichoptera (8), and Gastropoda (5) families included in our study (Table A.5). Four families contained both sensitive and tolerant genera, all of them belonging to the order of Trichoptera. The 49 remaining families were predicted to only contain tolerant genera, with an MSS value above zero. Among them were all families belonging to the order of Arguloida (1 family), Coleoptera (7), Decapoda (1), Diptera (5), Ephemeroptera (9), Hemiptera (7), Lepidoptera (1), Megaloptera (1), Neuroptera (2), and Bivalvia (4), as well as the remaining Amphipoda (3), Plecoptera (1), Trichoptera (3), and Gastropoda (4) families (Table A.6).

For AChE inhibiting chemicals, there was little variation in sensitivity of the genera belonging to the same family, and, as for the ER assemblages, the whole family was either predicted to only contain sensitive (MSS < 0) or tolerant (MSS > 0) genera. In total, 25 families contained genera that were all predicted as sensitive. This encompassed all families belonging to the order of Trichoptera (15 families), as well as a part of the Amphipoda (1), Diptera (2), Neuroptera (1), and Odonata (6) families (Table A.7). The remaining 43 Arthropod families were predicted to only contain tolerant species, and included all Arguloida (1 family), Coleoptera (7), Decapoda (1), Ephemeroptera (9), Hemiptera (7), Isopoda (1), Lepidoptera (1), Megaloptera (1), and Plectopera (7), as well as the rest of the Amphipoda (3), Diptera (3), Neuroptera (1), and Odonata (1) families (Table A.8).

3.3.3. Geographical pattern

Considering the RIVPACS sites, geographical patterns show opposite results for the two MOAs (Fig. 3). Regions containing more species sensitive towards narcotic chemicals were observed in the west and north of the UK, while regions containing more species sensitive towards AChE inhibiting chemicals were found in the south, south-west of the UK (Fig. 3). RIVPACS sites located in small to intermediate lowland streams contained more sensitive species towards AChE inhibiting

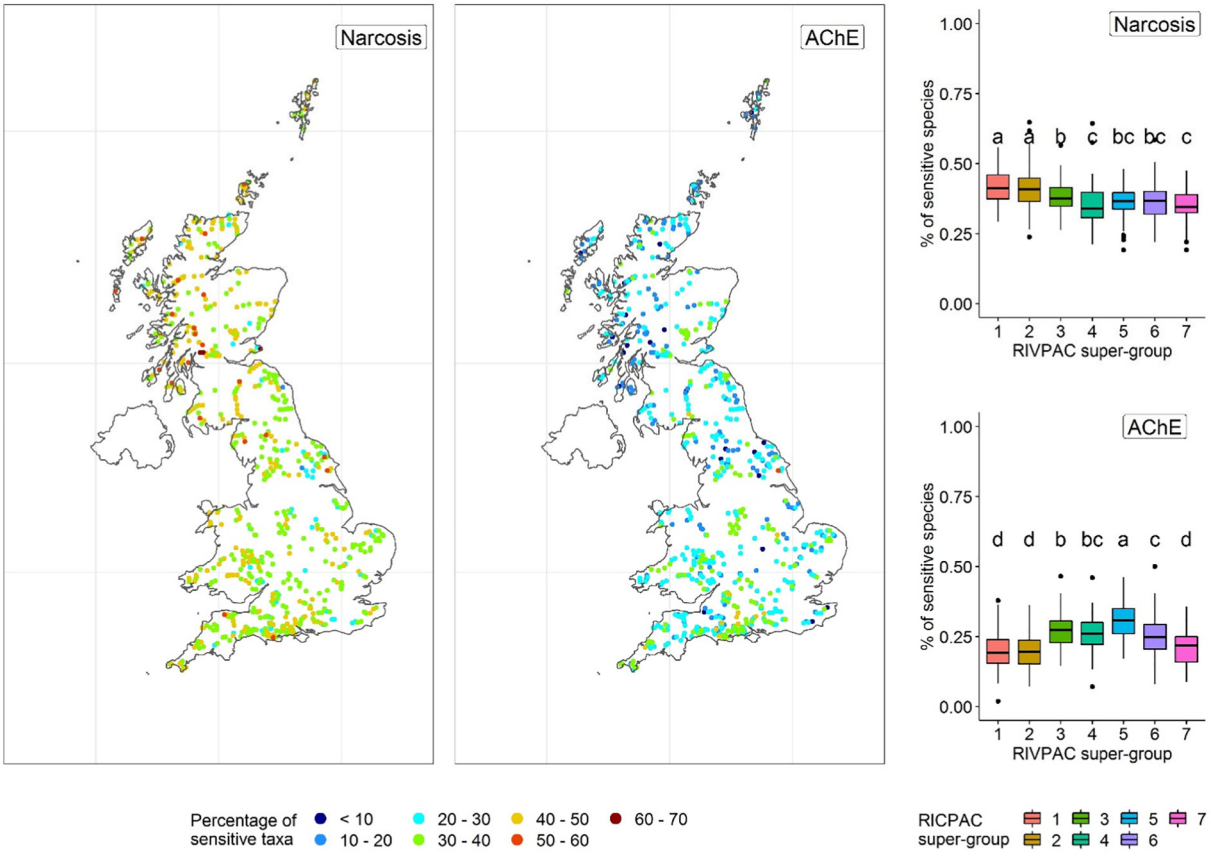

Fig. 3. Map of the UK showing the percentage of sensitive taxa (MSS < 0) present at all RIVPACS sites, and boxplots of the percentage of sensitive species (MSS < 0) present in each RIVPACS super-group to narcotic and AChE inhibiting chemicals. Letters in boxplots indicate significant differences ($p < .05$).

chemicals (super-groups 3, 4 and primarily 5, boxplots Fig. 3), whilst for narcotic chemicals most sensitive species were found at sites located in upland rivers, mainly located in Scotland and Northern England (super-groups 1 and 2, boxplots Fig. 3). For both MOAs, larger, lowland streams located in South-East England (super-group 7), contained the smallest percentage of sensitive species.

4. Discussion

4.1. Traits and taxonomic predictor selection, and how this can be improved

For both MOAs, mode of respiration was selected as an important trait for explaining species sensitivity (Table 2). Several studies have investigated the relationship between respiration and AChE inhibiting chemicals before (Buchwalter et al., 2002; Rico and Van den Brink, 2015; Rubach et al., 2012; Rubach et al., 2010; van den Berg et al., 2019), and have frequently found respiration important for determining species sensitivity, primarily due to an influence of respiration mode on uptake rates. The relationship between narcosis and respiration has been studied less, and there is to our knowledge only one study available that performed an analysis with narcotic chemicals (van den Berg et al., 2019). The result of that study closely aligns with ours, undoubtedly due to the large overlap in the data included in both studies.

We find that combining traits with taxonomic information results in models with increased predictive power, although only marginal (Table 2). Previous studies likewise emphasize the importance of complementing traits approaches with taxonomic approaches (Pilière et al., 2016; Poff et al., 2006; Poteat et al., 2015). For example, Pilière et al. (2016) used boosted regression tree modelling to assess the environmental responses of single traits, orders and trait profile groups. They found that taxa belonging to the same trait profile group but to different orders showed different environmental responses. Similarly, they found that taxa belonging to the same order but to different trait profile groups showed different environmental responses (Pilière et al., 2016). This indicates that unique information related to the evolutionary history was captured by the order of a taxon, whilst another part was captured by the trait set of a taxon. We find a similar result in our study, where the taxonomy-only model explaining sensitivity towards narcotic chemicals has an explanatory power of 0.42. This explanatory power increases to 0.46 when traits are included (Table 2). For AChE inhibition we see a similar result, although there the increase is only from 0.61 to 0.62 (Table 2). Although the increase of predictive power is only slight, the increase in mechanistic explanation is large, since the traits reveal mechanistic information regarding species sensitivity, and the taxonomic predictors point out taxa which show a different response to the chemical. The taxonomic predictors can thereby focus future research on finding the actual mechanisms that are different between these taxa. For this reason, both traits and taxonomy should be taken into consideration simultaneously for maximum benefit to risk assessment.

Although our models already show a good fit on the available data (Table 2), we anticipate that technological advances both in molecular and computational technologies will lead to an improvement of our models over time. Applying sophisticated molecular approaches can help with resolving the taxonomy of currently still problematic organism groups, for instance, by increasingly basing taxonomy on DNA markers, ideally replacing taxonomy completely by phylogenetics in due time (Hebert et al., 2003). Additionally, basing phylogenetic trees on key target genes associated with Adverse Outcome Pathways (AOPs) might substantially improve phylogenetic predictive models for application in ecotoxicology (e.g. LaLone et al., 2013). Furthermore, our models could improve with increased computing power. Due to memory limitations and the structure of currently existing model selection algorithms, we had to restrict the number of predictors going into the model selection process. However, since we maintain strict rules to avoid overfitting (e.g. the use of AICc as a model selection criterion and the use of a multivariate approach for the taxonomic predictors), it would be possible to add more predictors to the model without increasing the chance of overfitting.

4.2. Sensitivity patterns at European scale

At the continental scale, we predict that around half of the species are sensitive (MSS < 0) towards narcotic chemicals. This matches our expectations, since MSS is a relative value, and there is not any taxonomic group known that is particularly sensitive towards narcotic compounds (Escher and Hermens, 2002). For AChE inhibiting chemicals we predict around one third of the arthropod species to be sensitive (MSS < 0). This is less than found in the sensitivity ranking of Rico and Van den Brink (2015), where on average 70% of the Arthropoda were found sensitive towards AChE inhibiting chemicals (organophosphates and carbamates). However, this difference likely originates from the fact that Rico and Van den Brink (2015) also included non-arthropod species. Since MSS is a relative value, and arthropod species are the most sensitive group towards AChE inhibiting chemicals, including non-arthropod species will result in relatively more sensitive arthropod species.

Considering both MOAs, our predictions show that river basins in central Europe contain fewer sensitive species than those situated in the south (Fig. 2). We reason that this results from, on the one hand, chemical exposure patterns before and during the period that Illies recorded the community composition of the ERs (Illies, 1978), and on the other hand, from more ancient phylogeographical and ecological processes. Indeed, the pattern we find coincides with the emission pattern of multiple persistent organic contaminants commonly used in the 1960s, around the time when Illies was constructing his species database (Illies, 1978). Chemicals like DDT (Dichloro-diphenyl-trichloroethane, Stemmler and Lammel, 2009), lindane (Prevedouros et al., 2004), mercury (Pacyna et al., 2003b), and PCDFs (polychlorinated dibenzofurans, Pacyna et al., 2003a) were more extensively used in central Europe, potentially reducing the occurrence of more sensitive species in those regions. However, we think that chemical exposure was not the main determinant for species composition, primarily because Moog and colleagues demonstrated that different ERs could always be differentiated from each other based on their community composition, even when heavily impacted by chemical stress (Moog et al., 2004). Therefore, we argue that the main cause for the geographical pattern we see lies in the phylogeography of Europe, in which extreme climatic events wipe out more sensitive species, and mountainous regions consecutively serve as refugia and biodiversity hotspots (Rahbek et al., 2019a; Rahbek et al., 2019b). During the last ice age, glaciers covered the majority of northern Europe, forcing most species towards refugia present in southern Europe or to ice free parts of high mountain areas (e.g. Schmitt and Varga, 2012). Indeed, there is a large overlap in biodiversity hotspots (Médail and Quézel, 1999; Mittermeier et al., 1998; Rahbek et al., 2019b) or so-called regions of large endemism (Deharveng et al., 2000), with regions containing the highest percentage of sensitive species (Fig. 2). Then after the last ice age, species recolonized northern Europe from these southern refugia, which is confirmed by the fact that almost all species occurring in northern European are also present in central and/or southern Europe (Hering et al., 2009). The relatively higher sensitivity of ER 22 and 15 (especially towards AChE inhibiting chemicals, Fig. 2) can be explained due to migration of more sensitive species from Siberian refugia, e.g. located in the Ural mountains (Bernard et al., 2011; Schmitt and Varga, 2012).

4.3. Sensitivity patterns at UK scale

We see that certain biases in the underlying data are revealed in the sensitivity patterns we find for the UK. For instance, at a national scale, fewer species were considered sensitive compared to the continental scale, both towards narcotic and AChE inhibiting chemicals. We think

this is caused by the interaction of two things. First, our models are biased in predicting entire families as sensitive or tolerant, in some cases resulting in entire phyla being predicted as sensitive or tolerant. Second, the RIVPACS communities are taxonomically uneven at genus level, the level we used to predict species sensitivity. Indeed, dipterans make up around 40% of all genera present which all are predicted to be tolerant towards the two MOAs. In this case, the taxonomic unevenness *at genus level* specifically, has a large influence on the percentage of species sensitive at the national scale. When we compare the ER and RIVPACS results at the family level, results between the two datasets are more consistent. For instance, for the ER dataset we predict that 33, 59, and 86% of respectively Amphipoda, Trichoptera, and Plecoptera families were sensitive towards narcotic compounds. This was 25, 53, and 86% of the families in the same orders in the RIVPACS dataset.

The geographical distribution of sensitive species throughout the United Kingdom is less pronounced than at a European level, although the opposing results of the RIPVAC super-groups towards the two MOAs studied is striking. This contradictory result corresponds with the study of Van den Berg et al. (2019), where an inclusive database approach reveals large differences in species sensitivity depending on MOA. Their study shows that AChE and narcosis are on opposing ends of a dendrogram clustered on a matrix of species sensitivity towards six diverse MOAs, indicating that AChE and narcosis show the largest differences in species sensitivity among all MOAs tested. Additionally, we found alternative explanations that could explain the contradicting geographical patterns we found for the two MOAs.

As an explanation for the geographical pattern for narcotic compounds, we find a large overlap between hotspots of sensitivity towards narcotic toxicants and conservation areas in the UK (e.g. with Special Areas of Conservation, Special Protection Areas, Sites of Special Scientific Interest, (Gaston et al., 2006)). It is known that protected areas serve as establishment centres, enabling the colonization of new regions by species that are shifting their geographical ranges (Hiley et al., 2013; Thomas et al., 2012). Although all RIVPACS sites are considered reference sites and have been selected because of low anthropogenic influence, our results show that whether or not these sites are included or in close proximity to a conservational area leads to a higher support of sensitive species, likely due to an increased landscape and habitat heterogeneity.

As an explanation for the geographical pattern for AChE inhibiting compounds, the larger differences between the sensitivity of supergroups towards AChE inhibiting chemicals demonstrates that species sensitive towards AChE inhibition were more differentiated according to river type (i.e. the abiotic preferences of the species) than according to the availability of conservation areas. Additionally, the finding that the North to South pattern that we found at a European level was not noticeably present at the UK level is probably due to smaller differences in environmental factors (e.g. temperature, precipitation, phylogeographic history) when considering the UK only, compared to when the whole of Europe is considered.

4.4. Implications and outlook

Our analysis indicates that not only the taxonomic resolution of available trait databases is crucial, also the resolution of the model is important. Additionally, we are confident that our models will improve in the near future, for instance by the replacement of the taxonomic tree with a phylogenetic tree based on validated biomarkers (for instance, as in Simões et al., 2019). In that case, the successful application of our suggested approach is mainly limited by access to raw biological data (e.g. species abundance), which is currently still problematic because governmental agencies provide ecological status information based on general indices rather than species counts. Providing access to raw data, along with clear metrics on the quality of that data (e.g. meeting the criteria defined in Moermond et al., 2016), would foster our understanding of the links between anthropogenic stressors and populations or communities. Subsequently combining this effect data with chemical concentration data would be the next logical step, and would require chemical concentration data on all chemicals that are being monitored, not only priority substances, to be made widely available by governmental agencies.

The current analysis provides an important new chapter in the development of environmental scenarios that can be used for the environmental risk assessment of chemicals at larger geographical scales (Franco et al., 2016; Rico et al., 2016). Our work is the first attempt to apply sensitivity models on community assemblage data previously grouped according to both biotic and abiotic parameters (e.g. invertebrate community composition, water depth, alkalinity and temperature, Davy-Bowker et al., 2008). This combination of both biological and spatial data is required to successfully characterize exposure, effects and recovery of aquatic non-target species under realistic worst-case conditions. Currently, mismatches exist between parameter values and spatial-temporal scales of ecological models used to predict potential effects of chemicals (Rico et al., 2016). Our approach contributes to solving this mismatch by simultaneously incorporating biological and environmental factors.

In addition to this, the inclusion of traits in our models leads to an increased mechanistic understanding of cause-effect relationships, and allows for the application across wide biogeographical regions. This extrapolation enables, for instance, the comparison of ecological status across countries or regions that have so far remained unmonitored due to practical reasons (e.g. remote regions), for instance, by using species assemblages predicted by means of species distribution models (e.g. as in He et al., 2015). Also, patterns across wide geographical scales can easily be compared with other studies by means of geographical information systems (GIS) and simple additive models to reveal regions where multiple stressors might be causing an effect simultaneously (e.g. as in Fig. A.6, and see Vaj et al., 2011 for an example study). Take, for instance, the potential impact of climate change on aquatic insects. Hering et al. (2009) show that southern European regions contain the highest fraction of species sensitive towards climate change. Since this largely overlaps with the regions we found to be most sensitive towards chemical stressors (Fig. 2), there might be an increased overall effect on aquatic communities due to an unexpected interaction between climate change and chemical stress. In the north-east of Europe, a similar amplification effect may occur due to an overlap in regions with a relatively high chemical sensitivity (Fig. 2), and predicted increased potential of harmful arthropod pest invasions (Bacon et al., 2014).

Finally, our study demonstrates that sensitivity towards chemical stressors is spatially variable, and that although entire regions can be considered relatively tolerant, there might still be certain river reaches with a large percentage of sensitive species. Applied at relevant geographic scales, the methodology described in this study has demonstrated the potential to identify hotspots of sensitive species for given chemical classes. When applied to current risk assessment approaches, this will both increase the biological realism of assessments, and reduce the need for overly conservative assessment factors.

CRediT authorship contribution statement

Sanne J.P. Van den Berg: Conceptualization, Methodology, Software, Investigation, Visualization, Writing - original draft. **Cecilie Rendal:** Data curation, Methodology, Software, Writing - original draft, Project administration. **Andreas Focks:** Writing - review & editing, Conceptualization. **Emma Butler:** Writing - review & editing, Supervision, Project administration. **Edwin T.H.M. Peeters:** Writing - review & editing, Methodology. **Frederik De Laender:** Funding acquisition, Supervision, Writing - review & editing. **Paul J. Van den Brink:** Funding acquisition, Project administration, Conceptualization, Methodology, Writing - review & editing.

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Declaration of competing interest

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.139150. The R project, along with all scripts and data necessary to reproduce the models and figures performed in this study are available at Figshare (https://doi.org/10.6084/m9.figshare.11294450) (van den Berg, 2019).

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