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### Potential impact of chemical stress on freshwater invertebrates

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

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16 Predictive ecotoxicology, macroinvertebrate assemblage sensitivity, chemical stress, species  
17 traits, phylogenetic modelling, chemical mode of action

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19 Primary research article

20

21 **Abstract**

22 Current chemical risk assessment approaches rely on a standard suite of test species to assess  
23 toxicity to environmental species. Assessment factors are used to extrapolate from single  
24 species to communities and ecosystem effects. This approach is pragmatic, but lacks  
25 resolution in biological and environmental parameters. Novel modelling approaches can help  
26 improve the biological resolution of assessments by using mechanistic information to identify  
27 priority species and priority regions that are potentially most impacted by chemical stressors.  
28 In this study we developed predictive sensitivity models by combining species-specific  
29 information on acute chemical sensitivity (LC50 and EC50), traits, and taxonomic  
30 relatedness. These models were applied at two spatial scales to reveal spatial differences in  
31 the sensitivity of species assemblages towards two chemical modes of action (MOA): narcosis  
32 and acetylcholinesterase (AChE) inhibition. We found that on a relative scale, 46% and 33%  
33 of European species were ranked as more sensitive towards narcosis and AChE inhibition,  
34 respectively. These more sensitive species were distributed with higher occurrences in the  
35 south and north-eastern regions, reflecting known continental patterns of endemic  
36 macroinvertebrate biodiversity. We found contradicting sensitivity patterns depending on the  
37 MOA for UK scenarios, with more species displaying relative sensitivity to narcotic MOA in  
38 north and north-western regions, and more species with relative sensitivity to AChE inhibition  
39 MOA in south and south-western regions. Overall, we identified hotspots of species sensitive  
40 to chemical stressors at two spatial scales, and discuss data gaps and crucial technological  
41 advances required for the successful application of the proposed methodology to invertebrate  
42 scenarios, which remain underrepresented in global conservation priorities.

## 43 **1. Introduction**

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

57 Several studies have tried to determine which species are intrinsically most sensitive to  
58 chemical stressors by using species traits, and were able to explain up to 87 percent of the  
59 variation in species sensitivity using only four traits (Rico & Van den Brink, 2015; Rubach et  
60 al., 2012; Rubach, Baird, & Van den Brink, 2010; van den Berg et al., 2019). A large  
61 advantage of using traits-based approaches is that they add mechanistic understanding of the  
62 sensitivity process by describing characteristics that make a species more or less sensitive  
63 towards chemical stressors. This largely reduces the chances of overfitting models to the  
64 training data (Johnson & Omland, 2004). In addition to that, describing aquatic communities  
65 in terms of their biological traits increases the generality of such characterizations and their  
66 subsequent transferability between regions (Van den Brink et al., 2011). Also, correlations

67 between species traits and species sensitivity might exist, potentially resulting in unexpected  
68 effects at the community level (Baert, De Laender, & Janssen, 2017).

69 Other studies (Malaj, Guénard, Schäfer, & Van der Ohe, 2016) concerned with determining  
70 which species were most sensitive to chemical stressors, combined phylogenetic information  
71 with chemical properties. They were to a great extent ( $R^2$  of ~0.8) capable of predicting  
72 species sensitivity to pesticides (Guénard, von der Ohe, Walker, Lek, & Legendre, 2014) and  
73 heavy metals (Malaj et al., 2016). Furthermore, some studies have demonstrated that indeed  
74 traits and phylogeny (or other measures of relatedness between species) both explain an  
75 unique part of the sensitivity process (Pilière et al., 2016; Poteat, Jacobus, & Buchwalter,  
76 2015). However, phylogenetic approaches do not unravel any concrete mechanisms of  
77 sensitivity, and are therefore more susceptible to overfitting on the training data. For this  
78 reason, we think that a combination of both traits and phylogenetic information has the most  
79 potential for identifying priority species at a large spatial scale.

80 We envision these priority species to, in the future, become part of environmental scenarios, a  
81 simplified (model) representation of exposed aquatic ecosystems which provides a sufficient  
82 amount of ecological realism, enabling us to conduct an appropriate ERA (Rico, Van den  
83 Brink, Gylstra, Focks, & Brock, 2016). There are clear benefits associated with the  
84 development of scenarios for use in risk assessment, the most important ones being reduction  
85 of animal tests, integration of exposure and effect assessments, and increased realism with  
86 respect to spatial-temporal dimensions and species biodiversity (Rohr, Salice, & Nisbet,  
87 2016). However, for obtaining more realism in respect to spatial-temporal dimensions and  
88 biodiversity, we require not only the identification of priority species, but also the spatial-  
89 temporal dimensions at which these species occur. Therefore, after identifying priority  
90 species, looking into the distribution patterns of these species can help to identify priority  
91 regions, that is, regions where these priority species are more abundant. These regions can

92 assist in delivering realistic ranges of important landscape parameters (e.g. temperature,  
93 discharge, alkalinity) as input for environmental scenarios, enabling more realistic landscape  
94 level ERA (Franco et al., 2016; Rico et al., 2016). Additionally, these regions can become the  
95 focus of conservation and management efforts.

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

## 107 **2. Methods**

108 The whole methodology of this study has been developed in R, a free software environment  
109 (R Core Team, 2018). The R project, along with all scripts and data necessary to reproduce  
110 the models and figures performed in this study are available at Figshare  
111 ([10.6084/m9.figshare.11294450](https://www.figshare.com/projects/10.6084/m9.figshare.11294450)) (van den Berg, 2019).

### 112 2.1. Modelling approach

113 We extracted toxicological data from Van den Berg et al. (2019; original data from ECOTOX  
114 (USEPA, 2017)), which comprised Mode Specific Sensitivity (MSS) values for 36 and 32  
115 macroinvertebrate genera towards baseline (narcosis) and AChE inhibiting toxicants

116 respectively. Briefly, the MSS value represents the average relative sensitivity of each species  
117 to a group of chemicals with the same MOA (original MOA classification from Barron,  
118 Lilavois, & Martin, 2015), where an MSS value below zero indicates that the species is more  
119 sensitive than average, and an MSS value above zero indicates that the species is less  
120 sensitive than average. The MOAs narcosis and AChE inhibition were selected for this study,  
121 because they were the most data rich (van den Berg et al., 2019). Narcosis, also called  
122 baseline toxicity, is found toxic at similar internal concentration across all organisms (Escher  
123 & Hermens, 2002; Wezel & Opperhuizen, 1995). Therefore, differences in sensitivity for this  
124 MOA are expected to be small, equally distributed across taxonomic groups, and mainly  
125 explained by traits related to toxicokinetics (i.e. uptake, biotransformation, and elimination).  
126 AChE inhibition is a more specific MOA, and therefore shows large differences in effect  
127 concentrations depending on taxonomic group (van den Berg et al., 2019). For this MOA we,  
128 therefore, expect a stronger phylogenetic signal. To justify a separate analysis for the two  
129 MOAs, we made a correlation plot of the measured MSS values of species that were tested on  
130 both MOAs (Figure A.7). The lack of a significant relationship between species sensitivity  
131 towards the two MOAs indicates that sensitivity towards them is independent. We therefore  
132 chose to perform a separate analysis for both MOAs in this study.

133 The dataset from Van den Berg et al. (2019) also contained data on genus name, unique  
134 identifier (UID from the NCBI database, Benson, Karsch-Mizrachi, Lipman, Ostell, & Sayers,  
135 2009; Sayers et al., 2009), and traits (original data from Tachet, Richoux, Bournaud, &  
136 Usseglio-Polatera, 2000; Usseglio-Polatera, Bournaud, Richoux, & Tachet, 2000). In this  
137 study, we added relatedness to this dataset by constructing a taxonomic tree, since detailed  
138 phylogenetic data was still largely unavailable or incoherent for most freshwater  
139 macroinvertebrates (we looked, for instance, in Genbank, Benson et al., 2009), and Guénard  
140 and Von der Ohe et al. (2014) have provided sufficient proof that taxonomic relatedness

141 explains around the same amount of variation in species sensitivity as phylogenetic data when  
142 a wide taxonomic range is taken into consideration. This taxonomic tree is subsequently  
143 converted to Phylogenetic Eigenvector Maps (PEMs), from which species scores are extracted  
144 which subsequently serve as predictors of relatedness in model construction (Griffith & Peres-  
145 Neto, 2006; Guénard, Legendre, & Peres-Neto, 2013).

#### 146 2.1.1. Constructing the taxonomic tree.

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

#### 155 2.1.2. Phylogenetic eigenvector maps.

156 As descriptors of the taxonomic tree, phylogenetic eigenvectors were obtained from the PEM  
157 (see Guénard et al., 2013 for details). PEMs work on a similar basis as principal component  
158 analysis (PCA; Legendre & Legendre, 2012). Briefly, the eigenvectors of a PEM are obtained  
159 from a decomposition of the among-species covariance's and represent a set of candidate  
160 patterns of taxonomic variation of the response variables (i.e. the sensitivities to different  
161 chemicals). As is the case for a traditional PCA, this decomposition results in  $n - 1$   
162 eigenvectors (Legendre & Legendre, 2012), where in our analysis  $n$  was the number of model  
163 species. The calculation of a PEM is obtained from both the structure of the taxonomic tree  
164 and from the dynamics of the (in our case) sensitivity evolution. The dynamics of the



165 sensitivity evolution depends on the strength of a steepness parameter (parameter  $\alpha$ ; related to  
166 Pagels' parameter  $\kappa$  (Pagel, 1999), where  $\alpha = 1 - \kappa$ ). This parameter represents the relative  
167 evolution rate of the sensitivity to the MOA, takes values between 0 (natural evolution) and 1  
168 (strong natural selection), and was in our study estimated from the known sensitivity of the  
169 model species. We constructed the PEMs with the **MPSEM** package (version 0.3-4, Guénard,  
170 2018; Guénard et al., 2013).

### 171 2.1.3. Model construction.

172 For the narcosis dataset, two leverage points were discovered during the modelling process  
173 (Figure A.1 and A.2). Since we doubted the validity of these points (they were exactly  
174 identical) and were unable to assess their validity (there was no data available on closely  
175 related species, and the reference was inaccessible), they were removed from the dataset,  
176 reducing the number of species for which toxicity data was available to 34. For the AChE  
177 inhibition dataset, only the 27 Arthropoda species present in the dataset were included in the  
178 analysis, because this MOA works in a more specific manner, making differences in MOA  
179 among different phyla more likely (Maltby, Blake, Brock, & Van den Brink, 2005).

180 Eventually, 33 and 26 eigenvectors were included as taxonomic predictors for narcosis and  
181 AChE inhibition respectively (in the modelling process, taxonomic predictors were indicated  
182 with a 'V', see Figures A.3 and A.4 for examples of such predictors), and were added to the  
183 sensitivity and trait data. To reduce the number of predictors going into the final model  
184 building process (required due to memory limitations of the algorithm), an exhaustive search  
185 was performed using the *regsubsets* function from the **leaps** package (version 3.0, Lumley &  
186 Miller, 2017). From this, traits or phylogenetic eigenvectors that were least frequently  
187 included in the best 1% of the models, ordered according to the Bayesian Information  
188 Criterion (BIC), were removed from the analysis. Next, an exhaustive regression was  
189 performed between the remaining predictors and the available MSS values, allowing a

190 maximum of 4 predictors in the models. The best model was the model with the lowest AICc  
191 (Aikaike's Information Criterion with a correction for small sample size, Johnson & Omland,  
192 2004). The modelling exercise was repeated using only traits-, and a combination of traits-  
193 and taxonomic- predictors. We did not consider taxonomy-only models, because we were  
194 primarily interested in obtaining more mechanistic understanding of the sensitivity process.

## 195 2.2. Predicting unknown taxa

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

215 The Tachet database was used as a source of traits data (Tachet et al., 2000; Usseglio-Polatera  
216 et al., 2000). In order to make species-traits matching between the two community  
217 compositions (ERs and RIVPACS) and the Tachet database possible, the taxonomy of the  
218 three databases was aligned with the NCBI database using the **taxize** package (version 0.9.3,  
219 Chamberlain & Szöcs, 2013). Species from the ER and RIVPACS communities could then be  
220 matched with traits from the Tachet database using the UIDs from the NCBI database. This  
221 matching was done at genus level. Since the traits in the Tachet database are coded using a  
222 fuzzy coding approach (describing a species by its affinity to several trait modalities, see  
223 Chevenet, Dolédec, & Chessel, 1994 for more information), a transformation was required  
224 before this data could be used. Continuous traits were transformed using a weighted averaging  
225 of the different trait modalities, whilst for factorial traits the modality for which the species  
226 had the highest affinity was selected (as in van den Berg et al., 2019).

227 At this point, taxonomic and trait data of all the target species (species for which we want to  
228 predict sensitivity) were complete, and PEM scores had to be added. To do this, the locations  
229 of the target species were extracted from the taxonomic tree, and subsequently transformed  
230 into PEM scores using the **MPSEM** package (version 0.3-4, Guénard, 2018; Guénard et al.,  
231 2013). The PEM scores were then combined with the traits data, which allowed us to predict  
232 the sensitivity (MSS values) towards narcotic and AChE inhibiting chemicals using the two  
233 best models developed earlier.

234 The sensitivity of each ER or river type was determined by calculating the percentage of  
235 species with an MSS value below 0, comparable to (Hering et al., 2009). For RIVPACS, this  
236 was initially done both on abundance and presence-absence data, on the seasons spring,  
237 summer and autumn separately, and averaged over the three seasons. Eventually, we focused  
238 on presence-absence data averaged over the three seasons only, due to higher uncertainty (e.g.  
239 due to sampling error and seasonality) associated with the other data subsets. The results were

240 projected on maps by colouring the ERs and river types according to the percentage of  
241 sensitivity species ( $MSS < 0$ ) present. To construct the maps, we downloaded a map of the  
242 world from the Natural Earth website ([https://www.naturalearthdata.com/downloads/10m-](https://www.naturalearthdata.com/downloads/10m-cultural-vectors/)  
243 [cultural-vectors/](https://www.naturalearthdata.com/downloads/10m-cultural-vectors/)). The shape files for the ERs were obtained from the European Environment  
244 Agency (<https://www.eea.europa.eu/data-and-maps/data/ecoregions-for-rivers-and-lakes>), and  
245 their projection was transformed to match the projection of the world map using the  
246 *spTransform* function from the **sp** package (version 1.3-1, Pebesma & Bivand, 2005).  
247 Coordinates of all the RIVPACS sites were available in the RIVPACS database.

### 248 2.3. Statistics

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

255

256 **Table 1.** Division of the 685 reference sites into the 7 super-groups, along with a description  
 257 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

258

### 259 **3. Results**

#### 260 3.1. Sensitivity models

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

270

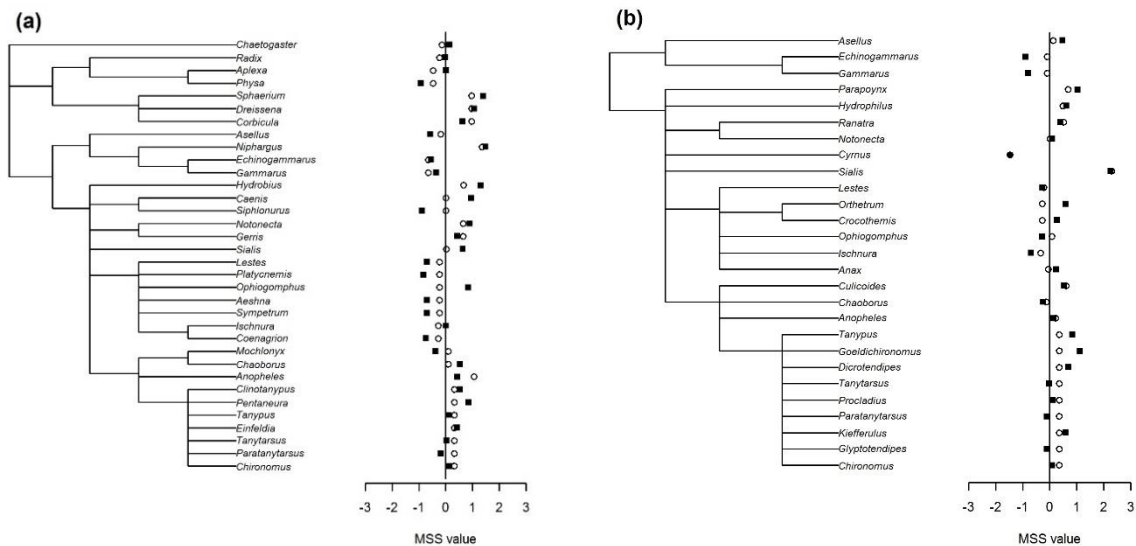
271 **Table 2.** Predictive models constructed for narcotic and AChE inhibiting chemicals, in- and  
 272 excluding taxonomy. Taxonomic predictors are indicated with a V. See Figures A.3 and A.4  
 273 for a visualization of the predictors incorporated in the taxonomy & traits models.

MOA	Type of model	Model	Adj. R <sup>2</sup>	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

274

275 Cross-validation of the model species resulted in the correct classification of 82% and 74% of  
 276 the genera as sensitive or tolerant for respectively narcosis and AChE inhibiting chemicals  
 277 (Figure 1). For narcosis, the Diptera *Paratanytarsus* and *Mochlonyx*, the Odonata  
 278 *Ophiogomphus*, the Ephemeroptera *Siphonurus*, the Gastropoda *Aplexa*, and the Annelida  
 279 *Chaetogaster* were misclassified (predicted on the wrong side of the zero line). For AChE

280 inhibition, incorrect predictions were made in only two taxonomic groups, the Diptera  
 281 *Glyptotendipes*, *Paratanytarsus*, *Tanytarsus*, and the Odonata *Anax*, *Crocothemis*,  
 282 *Ophiogomphus* and *Orthetrum*.



283  
 284 **Figure 1.** Observed MSS values (filled squares) and values predicted (unfilled circles) using  
 285 traits and taxonomy according to the best models for (a) narcotic (b) and AChE inhibiting  
 286 chemicals.

## 287 3.2. European freshwater ecoregions

### 288 3.2.1. Data availability.

289 For the ER communities, taxonomic data was available for 97% of the species, and covered  
 290 four crustacean orders (Amphipoda, Anostraca, Decapoda, and Isopoda), and six insect orders  
 291 (Coleoptera, Diptera, Ephemeroptera, Lepidoptera, Plecoptera and Trichoptera). Figure A.5  
 292 shows the taxonomic composition of all ERs at the order level. For 19% of these species there  
 293 was no or incomplete trait data available, leading to the exclusion of these species from our  
 294 analysis. Of the remaining species, only around 5% had toxicity data available. We therefore

295 had to predict the sensitivity of around 95% of the species for which no toxicity data was  
296 available using the taxonomy & traits models for narcosis and AChE inhibition.

### 297 3.2.2. Taxonomic pattern.

298 On the continental scale, 46 and 33% of the species were found sensitive ( $MSS < 0$ ) towards  
299 narcotic and AChE inhibiting chemicals, respectively. For narcotic chemicals, 18 families  
300 contained only genera predicted as sensitive. Among these 18 families were all families  
301 belonging to the order of Isopoda (1 family), as well as a part of the Amphipoda (1 family),  
302 Plecoptera (6), and Trichoptera (10) families included in our study (Table A.1). Five families  
303 contained both sensitive and tolerant genera. Four of these families belonged to the order of  
304 the Trichoptera, and one to the order of Lepidoptera. The remaining 25 families were  
305 predicted to only contain tolerant genera ( $MSS > 0$ ), and included all of the families  
306 belonging to the order of Anostraca (1 family), Decapoda (5), Diptera (1), and Ephemeroptera  
307 (12), as well as the remaining Amphipoda (2 families), Plecoptera (1), and Trichoptera (3)  
308 families included in this study (Table A.2).

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

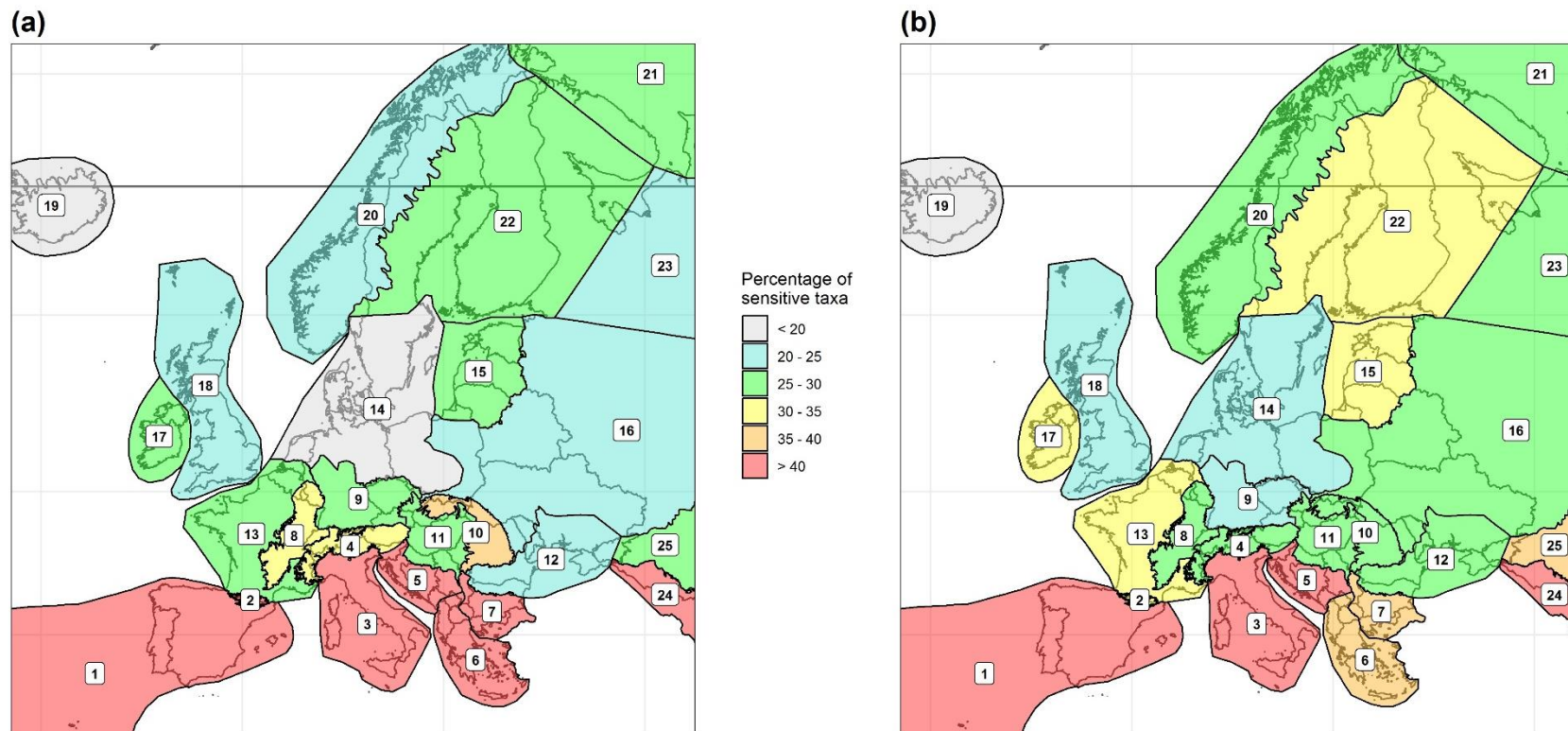
### 315 3.2.3. Geographical pattern.

316 For both MOAs, we noticed that the South of Europe (e.g. ER 1) has the highest proportion of  
317 sensitive species ( $MSS < 0$ ), whilst Iceland (ER 19) is the ecoregion containing the lowest  
318 proportion of sensitive species (Figure 2). Central Europe (e.g. ER 14) contains the lowest



319 percentages of sensitive species. ER 6 contains the largest percentage (57%) of species  
320 sensitive to narcotic chemicals, whilst ER 24 contains the largest percentage (45%) of species  
321 sensitive to AChE inhibiting chemicals.

322 When comparing the assigned sensitivity class of each ER for the two MOAs, we find that 8  
323 of the 25 ERs were grouped into the same class for both MOAs (ER 1, 3, 5, 11, 18, 19, 21, 24,  
324 Figure A.5). ER 2, 4, and 6 -10 were classified one or two classes lower for sensitivity  
325 towards AChE inhibiting chemicals compared to sensitivity towards narcotic chemicals,  
326 whilst the opposite was true for ER 12 -17, 20, 22, 23, and 25 (Figure A.6).



327

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

### 330 3.3. RIVPACS river types

#### 331 3.3.1. Data availability.

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

#### 344 3.3.2. Taxonomic pattern.

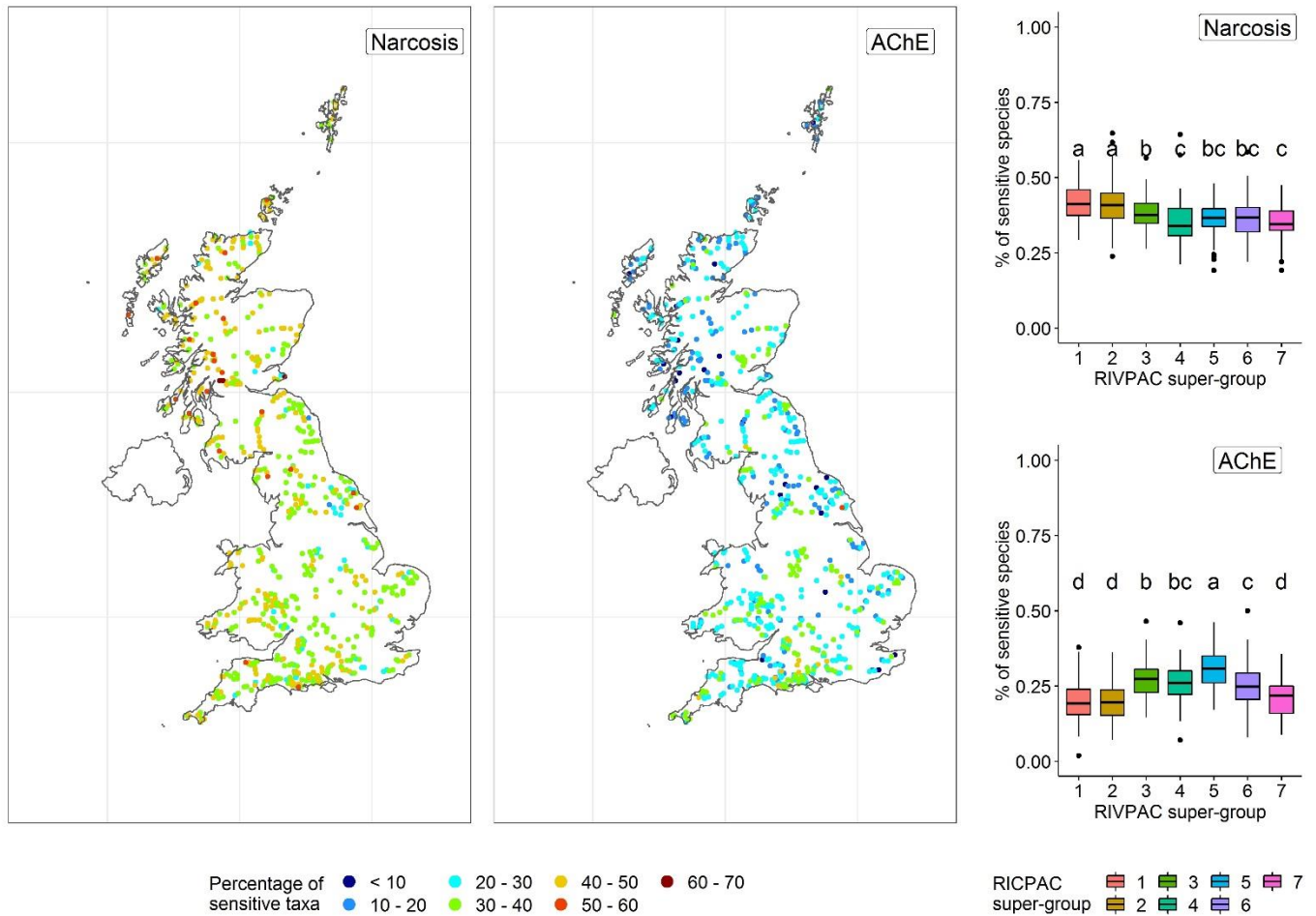
345 Within the UK, 38, and 25% of the species were found sensitive ( $MSS < 0$ ) to narcotic and  
346 AChE inhibiting chemicals respectively. For narcotic chemicals, 37 families contained only  
347 genera predicted as sensitive, with an MSS value below zero. Among these 37 families were  
348 all families belonging to the order of Annelida (9 families), Isopoda (1), and Odonata (7), as  
349 well as a part of the Amphipoda (1), Plecoptera (6), Trichoptera (8), and Gastropoda (5)  
350 families included in our study (Table A.5). Four families contained both sensitive and tolerant  
351 genera, all of them belonging to the order of Trichoptera. The 49 remaining families were  
352 predicted to only contain tolerant genera, with an MSS value above zero. Among them were  
353 all families belonging to the order of Arguloidea (1 family), Coleoptera (7), Decapoda (1),

354 Diptera (5), Ephemeroptera (9), Hemiptera (7), Lepidoptera (1), Megaloptera (1), Neuroptera  
355 (2), and Bivalvia (4), as well as the remaining Amphipoda (3), Plecoptera (1), Trichoptera (3),  
356 and Gastropoda (4) families (Table A.6).

357 For AChE inhibiting chemicals, there was little variation in sensitivity of the genera  
358 belonging to the same family, and, as for the ER assemblages, the whole family was either  
359 predicted to only contain sensitive ( $MSS < 0$ ) or tolerant ( $MSS > 0$ ) genera. In total, 25  
360 families contained genera that were all predicted as sensitive. This encompassed all families  
361 belonging to the order of Trichoptera (15 families), as well as a part of the Amphipoda (1),  
362 Diptera (2), Neuroptera (1), and Odonata (6) families (Table A.7). The remaining 43  
363 Arthropod families were predicted to only contain tolerant species, and included all Arguloidea  
364 (1 family), Coleoptera (7), Decapoda (1), Ephemeroptera (9), Hemiptera (7), Isopoda (1),  
365 Lepidoptera (1), Megaloptera (1), and Plectoptera (7), as well as the rest of the Amphipoda (3),  
366 Diptera (3), Neuroptera (1), and Odonata (1) families (Table A.8).

### 367 3.3.3. Geographical pattern.

368 Considering the RIVPACS sites, geographical patterns show opposite results for the two  
369 MOAs (Figure 3). Regions containing more species sensitive towards narcotic chemicals were  
370 observed in the west and north of the UK, while regions containing more species sensitive  
371 towards AChE inhibiting chemicals were found in the south, south-west of the UK (Figure 3).  
372 RIVPACS sites located in small to intermediate lowland streams contained more sensitive  
373 species towards AChE inhibiting chemicals (super-groups 3, 4 and primarily 5, boxplots  
374 Figure 3), whilst for narcotic chemicals most sensitive species were found at sites located in  
375 upland rivers, mainly located in Scotland and Northern England (super-groups 1 and 2,  
376 boxplots Figure 3). For both MOAs, larger, lowland streams located in South-East England  
377 (super-group 7), contained the smallest percentage of sensitive species.



378

379 **Figure 3.** Map of the UK showing the percentage of sensitive taxa (MSS < 0) present at all  
 380 RIVPACS sites, and boxplots of the percentage of sensitive species (MSS < 0) present in each  
 381 RIVPACS super-group to narcotic and AChE inhibiting chemicals. Letters in boxplots  
 382 indicate significant differences (p < 0.05).

### 383 4. Discussion

#### 384 4.1. Traits and taxonomic predictor selection, and how this can be improved

385 For both MOAs, mode of respiration was selected as an important trait for explaining species  
 386 sensitivity (Table 2). Several studies have investigated the relationship between respiration  
 387 and AChE inhibiting chemicals before (Buchwalter, Jenkins, & Curtis, 2002; Rico & Van den  
 388 Brink, 2015; Rubach et al., 2012; Rubach et al., 2010; van den Berg et al., 2019), and have  
 389 frequently found respiration important for determining species sensitivity, primarily due to an

390 influence of respiration mode on uptake rates. The relationship between narcosis and  
391 respiration has been studied less, and there is to our knowledge only one study available that  
392 performed an analysis with narcotic chemicals (van den Berg et al., 2019). The result of that  
393 study closely aligns with ours, undoubtedly due to the large overlap in the data included in  
394 both studies.

395 We find that combining traits with taxonomic information results in models with increased  
396 predictive power, although only marginal (Table 2). Previous studies likewise emphasize the  
397 importance of complementing traits approaches with taxonomic approaches (Pilière et al.,  
398 2016; Poff et al., 2006; Poteat et al., 2015). For example, Pilière and colleagues (2016) used  
399 boosted regression tree modelling to assess the environmental responses of single traits,  
400 orders and trait profile groups. They found that taxa belonging to the same trait profile group  
401 but to different orders showed different environmental responses. Similarly, they found that  
402 taxa belonging to the same order but to different trait profile groups showed different  
403 environmental responses (Pilière et al., 2016). This indicates that unique information related  
404 to the evolutionary history was captured by the order of a taxon, whilst another part was  
405 captured by the trait set of a taxon. We find a similar result in our study, where the taxonomy-  
406 only model explaining sensitivity towards narcotic chemicals has an explanatory power of  
407 0.42. This explanatory power increases to 0.46 when traits are included (Table 2). For AChE  
408 inhibition we see a similar result, although there the increase is only from 0.61 to 0.62 (Table  
409 2). Although the increase of predictive power is only slight, the increase in mechanistic  
410 explanation is large, since the traits reveal mechanistic information regarding species  
411 sensitivity, and the taxonomic predictors point out taxa which show a different response to the  
412 chemical. The taxonomic predictors can thereby focus future research on finding the actual  
413 mechanisms that are different between these taxa. For this reason, both traits and taxonomy  
414 should be taken into consideration simultaneously for maximum benefit to risk assessment.

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

#### 430 4.2. Sensitivity patterns at European scale

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

440 arthropod species are the most sensitive group towards AChE inhibiting chemicals, including  
441 non-arthropod species will result in relatively more sensitive arthropod species.

442 Considering both MOAs, our predictions show that river basins in central Europe contain  
443 fewer sensitive species than those situated in the south (Figure 2). We reason that this results  
444 from, on the one hand, chemical exposure patterns before and during the period that Illies  
445 recorded the community composition of the ERs (Illies, 1978), and on the other hand, from  
446 more ancient phylogeographical and ecological processes. Indeed, the pattern we find  
447 coincides with the emission pattern of multiple persistent organic contaminants commonly  
448 used in the 1960s, around the time when Illies was constructing his species database (Illies,  
449 1978). Chemicals like DDT (Dichloro-diphenyl-trichloroethane, Stemmler & Lammel, 2009),  
450 lindane (Prevedouros, MacLeod, Jones, & Sweetman, 2004), mercury (Pacyna, Pacyna,  
451 Steenhuisen, & Wilson, 2003), and PCDFs (polychlorinated dibenzofurans, Pacyna, Breivik,  
452 Münch, & Fudala, 2003) were more extensively used in central Europe, potentially reducing  
453 the occurrence of more sensitive species in those regions. However, we think that chemical  
454 exposure was not the main determinant for species composition, primarily because Moog and  
455 colleagues demonstrated that different ERs could always be differentiated from each other  
456 based on their community composition, even when heavily impacted by chemical stress  
457 (Moog, Schmidt-Kloiber, Ofenböck, & Gerritsen, 2004). Therefore, we argue that the main  
458 cause for the geographical pattern we see lies in the phylogeography of Europe, in which  
459 extreme climatic events wipe out more sensitive species, and mountainous regions  
460 consecutively serve as refugia and biodiversity hotspots (Rahbek, Borregaard, Antonelli, et  
461 al., 2019; Rahbek, Borregaard, Colwell, et al., 2019). During the last ice age, glaciers covered  
462 the majority of northern Europe, forcing most species towards refugia present in southern  
463 Europe or to ice free parts of high mountain areas (e.g. Schmitt & Varga, 2012). Indeed, there  
464 is a large overlap in biodiversity hotspots (Médail & Quézel, 1999; Mittermeier, Myers,



465 Thomsen, Da Fonseca, & Olivieri, 1998; Rahbek, Borregaard, Colwell, et al., 2019) or so-  
466 called regions of large endemism (Deharveng et al., 2000), with regions containing the  
467 highest percentage of sensitive species (Figure 2). Then after the last ice age, species  
468 recolonized northern Europe from these southern refugia, which is confirmed by the fact that  
469 almost all species occurring in northern European are also present in central and/or southern  
470 Europe (Hering et al., 2009). The relatively higher sensitivity of ER 22 and 15 (especially  
471 towards AChE inhibiting chemicals, Figure 2) can be explained due to migration of more  
472 sensitive species from Siberian refugia, e.g. located in the Ural mountains (Bernard, Heiser,  
473 Hochkirch, & Schmitt, 2011; Schmitt & Varga, 2012).

#### 474 4.3. Sensitivity patterns at UK scale

475 We see that certain biases in the underlying data are revealed in the sensitivity patterns we  
476 find for the UK. For instance, at a national scale, fewer species were considered sensitive  
477 compared to the continental scale, both towards narcotic and AChE inhibiting chemicals. We  
478 think this is caused by the interaction of two things. First, our models are biased in predicting  
479 entire families as sensitive or tolerant, in some cases resulting in entire phyla being predicted  
480 as sensitive or tolerant. Second, the RIVPACS communities are taxonomically uneven at  
481 genus level, the level we used to predict species sensitivity. Indeed, dipterans make up around  
482 40% of all genera present which all are predicted to be tolerant towards the two MOAs. In this  
483 case, the taxonomic unevenness *at genus level* specifically, has a large influence on the  
484 percentage of species sensitive at the national scale. When we compare the ER and RIVPACS  
485 results at the family level, results between the two datasets are more consistent. For instance,  
486 for the ER dataset we predict that 33, 59, and 86% of respectively Amphipoda, Trichoptera,  
487 and Plecoptera families were sensitive towards narcotic compounds. This was 25, 53, and  
488 86% of the families in the same orders in the RIVPACS dataset.

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

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

508 As an explanation for the geographical pattern for AChE inhibiting compounds, the larger  
509 differences between the sensitivity of super-groups towards AChE inhibiting chemicals  
510 demonstrates that species sensitive towards AChE inhibition were more differentiated  
511 according to river type (i.e. the abiotic preferences of the species) than according to the  
512 availability of conservation areas. Additionally, the finding that the North to South pattern  
513 that we found at a European level was not noticeably present at the UK level is probably due

514 to smaller differences in environmental factors (e.g. temperature, precipitation,  
515 phylogeographic history) when considering the UK only, compared to when the whole of  
516 Europe is considered.

#### 517 4.4. Implications and outlook

518 Our analysis indicates that not only the taxonomic resolution of available trait databases is  
519 crucial, also the resolution of the model is important. Additionally, we are confident that our  
520 models will improve in the near future, for instance by the replacement of the taxonomic tree  
521 with a phylogenetic tree based on validated biomarkers (for instance, as in Simões et al.,  
522 2019). In that case, the successful application of our suggested approach is mainly limited by  
523 access to raw biological data (e.g. species abundance), which is currently still problematic  
524 because governmental agencies provide ecological status information based on general  
525 indices rather than species counts. Providing access to raw data, along with clear metrics on  
526 the quality of that data (e.g. meeting the criteria defined in Moermond, Kase, Korkaric, &  
527 Ågerstrand, 2016), would foster our understanding of the links between anthropogenic  
528 stressors and populations or communities. Subsequently combining this effect data with  
529 chemical concentration data would be the next logical step, and would require chemical  
530 concentration data on all chemicals that are being monitored, not only priority substances, to  
531 be made widely available by governmental agencies.

532 The current analysis provides an important new chapter in the development of environmental  
533 scenarios that can be used for the environmental risk assessment of chemicals at larger  
534 geographical scales (Franco et al., 2016; Rico et al., 2016). Our work is the first attempt to  
535 apply sensitivity models on community assemblage data previously grouped according to both  
536 biotic and abiotic parameters (e.g. invertebrate community composition, water depth,  
537 alkalinity and temperature, Davy-Bowker et al., 2008). This combination of both biological

538 and spatial data is required to successfully characterize exposure, effects and recovery of  
539 aquatic non-target species under realistic worst-case conditions. Currently, mismatches exist  
540 between parameter values and spatial-temporal scales of ecological models used to predict  
541 potential effects of chemicals (Rico et al., 2016). Our approach contributes to solving this  
542 mismatch by simultaneously incorporating biological and environmental factors.

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

561 Finally, our study demonstrates that sensitivity towards chemical stressors is spatially  
562 variable, and that although entire regions can be considered relatively tolerant, there might

563 still be certain river reaches with a large percentage of sensitive species. Applied at relevant  
564 geographic scales, the methodology described in this study has demonstrated the potential to  
565 identify hotspots of sensitive species for given chemical classes. When applied to current risk  
566 assessment approaches, this will both increase the biological realism of assessments, and  
567 reduce the need for overly conservative assessment factors.

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## 571 **Appendix A. Supplementary material**

572 Supplementary data to this article can be found online at *insert DOI*. The R project, along  
573 with all scripts and data necessary to reproduce the models and figures performed in this study  
574 are available at Figshare ([10.6084/m9.figshare.11294450](https://doi.org/10.6084/m9.figshare.11294450)) (van den Berg, 2019).

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