This is the preprint of the contribution published as:

Liebmann, L., Vormeier, P., Weisner, O., Liess, M. (2022): Balancing effort and benefit – How taxonomic and quantitative resolution influence the pesticide indicator system SPEAR_{pesticides} *Sci. Total Environ.* **848**, art. 157642

The publisher's version is available at:

http://dx.doi.org/10.1016/j.scitotenv.2022.157642

1	Balancing effort and benefit - how taxonomic and
2	quantitative resolution affect the ecological assessment of
3	pesticide effects
4	Author list
5	Liana Liebmann ^{1,2} , Philipp Vormeier ^{1,3,} Oliver Weisner ¹ , Matthias Liess ^{1,3}
6	Affiliations
7	¹ Helmholtz Centre for Environmental Research – UFZ, Permoserstr. 15, 04318 Leipzig,
8	Germany
9	² Department Evolutionary Ecology & Environmental Toxicology (E3T), Institute of Ecology,
10	Diversity and Evolution, Faculty of Biological Sciences, Goethe University Frankfurt, 60438
11	Frankfurt am Main, Germany
12	³ Institute for Environmental Research (Biology V), RWTH Aachen University, 52062 Aachen,
13	Germany
14	E-mail: Liana.Liebmann@ufz.de

24 Abstract

25 Biological indices aim to reflect the ecological quality of streams based on the community's 26 species or trait composition. Accordingly, the capability to predict the ecological quality 27 depends on (i) the knowledge on the association of taxa or traits with stressors and (ii) the 28 taxonomic and quantitative resolution of taxa. Generally speaking, a higher resolution is 29 associated with a better linkage between environmental condition and biological response but 30 also with higher efforts and costs. So far it is unknown how the taxonomic and quantitative 31 resolution affect the ecological quality assessment of streams related to pesticide effects when 32 applying the invertebrate-based indicator SPEAR_{pesticides}. We investigated the ecological quality 33 of 101 streams considering four taxonomic levels (species, genus, family, order) and three 34 quantitative resolutions (abundance, three abundance classes, and presence-absence). In a 35 multiple linear regression analysis between 13 investigated stressors and SPEAR_{pesticides}, the 36 full models' explained variance remained fairly constant with decreasing taxonomic and 37 quantitative resolution. As expected, the highest association between pesticide pressure and 38 SPEAR_{pesticides} was reached at a species/abundance resolution yielding an R² of 0.43. In 39 contrast, the lowest quantitative resolution of order level combined with presence-absence 40 information revealed an explained variance of 0.28 R². We suggest the family/abundance class 41 resolution ($R^2 = 0.38$) as the best trade-off between effort and accuracy for large-scale 42 monitoring. Due to a comparable linear regression at family/abundance class resolution, the 43 assigned ecological quality classes were largely congruent (69%) to species/abundance 44 resolution. We conclude that the ecological quality assessment with SPEAR_{pesticides} at 45 family/abundance class resolution can be used to link pesticide contamination and invertebrate 46 community structure with less taxonomic expertise and less quantification effort.

47 Introduction

The ecological status of streams can be determined by applying biological indices. These indices use the community's taxa or trait composition to assess the ecological quality of streams. Indices such as the %EPT (Lenat, 1988), Saprobic Index (Kolkwitz R. and Marsson M., 1909) and SPEAR_{pesticides} (Liess and Ohe, 2005) aim to reflect the general degradation,

52 oxygen deficiency, or pesticide contamination of streams. Species have specific ecological and 53 environmental requirements but also share ecological niches, increasingly so when comparing 54 taxonomic levels above the genus and family levels. Accordingly, with increasing taxonomic 55 resolution, the taxa composition can be linked more accurately to environmental conditions. 56 Similarly, increasing quantitative resolution of sampling, meaning the counting of individuals, 57 strengthens the association between community expression and environmental conditions. In 58 this trade-off between effort and accuracy, some countries use the taxonomic level of family 59 (e.g. Spain: Alba-Tercedor, J and Sánchez-Ortega, A (1988); USA: Barbour et al. (1999); 60 Australia: Smith et al. (1999); UK: Environment Agency (2008)). Instead, other countries apply 61 species level for determining aquatic indices (e.g. USA, Germany, the Netherlands, Slovakia, 62 and South Korea; Buss et al. (2015)). However, the strength of the relationship between 63 environmental factors and biological indicators also depends on the accuracy of the available 64 autecological information on the taxa investigated.

The Rhithron Feeding Type Index RETI (Schweder, 1992) and the SPEAR_{pesticides} index (Liess 65 66 and Ohe, 2005), for example, contain taxonomic information for species, genus, family, and 67 order level, whereas the Saprobic Index is mainly based on species level information with less 68 information at genus level and almost no information at family or order level. Accordingly, 69 Schmidt-Kloiber et al. (2004) revealed that the AQUEM Assessment software (ASS, 2000) is 70 not applicable at genus and family level, since some indices like the Saprobic Index do not 71 have sufficient information available at lower taxonomic levels. They found that when lowering 72 taxonomic resolution to genus level, already half of the sites investigated were assigned a 73 divergent ecological quality class. Regarding the trade-off between effort and accuracy for a 74 lower quantitative resolution, Buchner et al. (2019) analyzed how presence-absence resolution 75 affects the accuracy of ecological class assignment for biological indices. The Saprobic Index 76 showed with a mean Spearman's p of 0.93 a strong significant correlation between abundance 77 and presence-absence data. For the General Degradation Metrics (GDM from ASS 2002), 78 75% of the ecological quality classes were identical with abundance resolution (Buchner et al., 79 2019). Accordingly, a higher taxonomic and quantitative resolution of invertebrate monitoring

80 improves the ecological quality assessment with biological indices that involve both abundance81 and taxonomy.

82 Our aim was to identify the optimum trade-off between effort and accuracy when lowering 83 taxonomic and quantitative resolution with SPEAR_{pesticides}. For this analysis, we evaluated an 84 extensive Germany-wide data set comprising relevant environmental factors including high 85 resolution sampling of pesticides and high resolution sampling and determination of 86 invertebrates. In detail, we (i) assessed the specificity of SPEAR_{pesticides} to indicate pesticide 87 pressure among multiple stressors when reducing taxonomic and quantitative resolution, (ii) 88 evaluated the indication power under reduced resolution, (iii) investigated how ecological 89 quality classification is impacted, and (iv) derived implications for pesticide effect monitoring.

90 **2** Material and methods

91 **2.1 Sampling sites**

92 The data analyzed in this study was collected and presented by Liess et al. (2021). The 93 macroinvertebrates and pesticide concentrations were sampled in two field campaigns from 94 April to July in 2018 and 2019 in a Germany-wide monitoring study. This study provides 95 information on 101 sampling sites with a varying degree of agricultural use (0 - 100%) in the 96 hydrological catchment (n = 41 with less than 10 km²; n = 60 with 10 - 30 km²), covering 13 97 different stream types (EU Commision, 2000); see SI Liess et al. (2021)). These 101 sampling 98 sites contained 86 agricultural (more than 20% agricultural land use in the catchment area) 99 and 15 non-agricultural sites (less than 20% agricultural land use in the catchment area). For 100 the 11 sites monitored in both campaigns, the indicator values and environmental factors were 101 averaged.

102 **2.2 Pesticide monitoring and analyses**

Water samples (n = 320) were taken with event-driven automated samplers (MAXX TP5, Rangendingen, Germany) to capture the peak concentrations induced by significant rise of water level (5 cm, depending on stream bed, Liess et al. (1999)). After the device was activated, a mixed sample of 500 mL with 40 subsamples (every 5 minutes) was collected over

107 a total period of 3 hours and 20 minutes (Liess et al., 2021). Additionally, streams were 108 sampled regularly with 250 ml grab samples (n = 520) every three weeks according to WFD 109 standards (EU Commision, 2000). 75 pesticides and 33 pesticide metabolites were selected 110 by prioritization according to active substance-related sale quantities, under consideration of 111 the current environmental quality standards (EQS) and the regulatory acceptable 112 concentrations (RAC) (Wick et al., 2018) (see list of substances in SI Liess et al. (2021)). All 113 830 water samples were analyzed for these 108 substances via target analysis with high 114 pressure liquid chromatograph coupled with a high-resolution tandem mass spectrometer LC-115 HRMS/MS without enrichment by multiple-reaction-monitoring (MRM, (Halbach et al., 2021; 116 Reemtsma et al., 2013)).

117 2.3 Transferring measured concentrations into invertebrate toxicity

118 All pesticide concentrations from EDS and grab samples were converted into invertebrate 119 toxicity by calculating Toxic Units (TU; Sprague (1970)) with the substance-related acute LC₅₀ 120 value of Daphnia magna or Chironomus sp. (most sensitive organism selected per substance) 121 (Münze et al., 2017). LC₅₀ values were collected from the Pesticide Property Data Base (PPDB 122 and the US EPA ECOTOXicology knowledgebase in case of lacking Chironomus data) (Lewis 123 et al., 2016). Peak exposure in streams to pesticides toxic to invertebrates was determined by 124 the local maximum single substance insecticidal toxicity measured according to Liess et al. 125 (2021).

126 **2.4 Macroinvertebrate sampling**

Macroinvertebrates were sampled after the main application of pesticides in June in 2018 and 2019 at each site (Liess et al., 2021). The macroinvertebrate sampling followed the Water Framework Directive (WFD) guideline (Meier et al., 2006) commonly used by the German federal states. At each site, a 50-meter section downstream of the monitoring site was sampled. Regarding all microhabitat types, 20 subsamples were taken with a kicking net according to their share in the stream section (at least 5%). Subsequently, macroinvertebrates were filtered, sorted, and conserved in 90% ethanol until laboratory determination. Taxa were

identified to the highest possible taxonomic resolution using a binocular (Zeiss, Stereo
Discovery V.20, Carl Zeiss Microscopy GmbH; Jena, Germany).

136 **2.5 Calculation of the biological indicator - SPEAR**_{pesticides}

The effects of pesticides on macroinvertebrates were quantified using the bio-indicator SPEAR_{pesticides} by (Liess and Ohe, 2005). SPEAR_{pesticides} provided the relative abundance of vulnerable species within a community and was normalized to indicator values under pristine conditions according to Liess et al. (2021).

Different taxonomic resolutions were considered by aggregating the taxa to the respective taxonomic level and summing up the abundances before transforming the data. For quantitative resolutions, the abundances were adjusted to presence-absence and three different abundance classes (see 2.7.1). In conclusion, the same SPEAR_{pesticides} formula were applied to all taxonomic and quantitative resolutions.

146 SPEAR_{pesticides} is calculated by using the following equation (Knillmann et al., 2018):

147
$$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n} log 10(4x_i + 1) \cdot y_i}{\sum_{i=1}^{n} log 10(4x_i + 1)}$$

where *n* is the total number of taxa in a sample, x_i is the abundance of taxon i (given as individuals per m²), and *y* is set to 1 if taxon i is classified as "at risk" (Liess and Ohe, 2005) – i.e. vulnerable to pesticides under regular exposure events – and set to 0 otherwise.

Trait information on vulnerability is allotted to 1,581 species in the SPEAR_{pesticides} database, 1,325 of which are sensitive and 256 insensitive. At the genus level, 255 taxa are assigned sensitivity information, divided into 186 sensitive and 69 insensitive. Information for 53 sensitive and 111 insensitive families is available, while at the order level only four orders are classified as sensitive (Ephemeroptera, Trichoptera, Plecoptera, Megaloptera) and 11 as insensitive. According to the results of this study, the groups Diptera Gen. sp. and Crustacea Gen. sp. were classified as not vulnerable and the order Megaloptera Gen. sp. as vulnerable.

2.6 Biotic and abiotic parameters

To reveal the relationships between environmental factors and ecological status, we collected biotic and abiotic parameters as described in Liess et al. (2021). Oxygen content, water level, conductivity, and water pressure were measured continuously from April to June (Liess et al., 2021). Nutrients and metals were analyzed in EDS and grab samples. Flow velocity and water level were measured every three weeks. Hydromorphological parameters were identified once according to the guidelines of the WFD. For more in-depth information regarding each method and parameter, please refer to Liess et al. (2021).

166 **2.7 Data analysis**

167 **2.7.1 Taxonomic and quantitative resolutions**

In a first step, the taxa numbers corresponding to each taxonomic level were summed. For lower quantitative resolutions, the abundance was set to one or zero for presence-absence or grouped into three classes for abundance class resolution. The smallest class contained abundances from one to three, the middle class comprised abundances from four to 100, and the third class included abundances greater than 100. Ultimately, the same SPEAR_{pesticides} equation (see chapter 2.5) was applied to all taxonomic levels of abundance, presenceabsence, and abundance class resolution.

In a second step, we evaluated the differences between SPEAR_{pesticides} values resulting from four taxonomic (species, genus, family, and order) and three quantitative (abundance, abundance class, and presence-absence) resolutions visually using linear correlation (see SI figure 1) and with a paired Wilcoxon-test (see SI table 1). As for other German invertebrate metrics under the WFD, the four SPEAR_{pesticides} boundaries separating the five even quality classes equal Ecological Quality Ratio values of 0.8, 0.6, 0.4, and 0.2 (Environment Agency, 2008; EU Commission, 2008; Liess et al., 2021).

182 **2.7.2** Linear regressions, visualization, and ecological class assignment

183 The prediction quality of all resolutions was evaluated by a multiple linear regression analysis

184 with a two-way interaction of 13 environmental variables (for the environmental variables refer

to Liess et al. (2021)). The relative importance of each variable was calculated with the relaimp
 package (version 2.2-3) in R and given in R² (Grömping, 2006).

The effects of pesticide pressure on macroinvertebrate communities evaluated by SPEAR_{pesticides} were modelled by single linear regressions for the different taxonomic and quantitative resolutions. The indication power of different levels of taxonomic and quantitative resolutions were assessed by comparing linear model R² values. The regression slopes + yintercepts of SPEAR_{pesticides} were compared.

- In addition, the percentage of congruence of the assigned ecological quality classes for all
 lower taxonomic and quantitative resolutions compared to the species/abundance resolution
 was evaluated. Data was processed using the software R (version 4.0.2, R Core Team, 2020.
- All diagrams were generated with the "ggplot2" version 3.3.2 in R (Wickham, 2009).

2.7.3 Compensation factor at order level

At the order level, for each quantitative resolution, a compensation factor was empirically derived. The compensation factor approximates the order-level SPEAR_{pesticides} values to those at species/abundance resolution. The validity of the approximation is represented by a similar slope and y-intercept of the regression lines compared to species/abundance resolution as well as by a higher congruence in ecological class assignment (Fig. 2, B and C).

202 **3 Results and discussion**

3.1 Indication power (performance) of SPEAR_{pesticides} using different taxonomic and quantitative resolutions

3.1.1 Indicating pesticide pressure among other stressors

SPEAR_{pesticides} reflects the effect of pesticide pressure on the macroinvertebrate community composition. To evaluate the specificity of the different resolutions of SPEAR_{pesticides}, we assessed the response of SPEAR_{pesticides} to 13 environmental and anthropogenic stressors considered relevant for the benthic invertebrate community. Here, we used a multiple linear regression analysis to identify the relative importance of each of the potential stressors: pesticide pressure; deficient hydromorphology; deficient bed habitat structure; O₂ deficiency; 212 nutrient pollution by NH₄; NO₂; total phosphorus; flow velocity; metal toxicity; temperature; pH; 213 and stream morphology stressors (Liess et al., 2021). Two of the 13 stressors included were 214 associated with SPEAR_{pesticides} at all taxonomic and quantitative resolutions investigated (Fig. 215 1). In the full models, the explained variance ranged from an R^2 of 0.55 to 0.61 for all taxonomic 216 and quantitative resolutions, while remaining relatively independent of the resolution of 217 SPEAR_{pesticides}. All full models reflected the specificity of SPEAR_{pesticides} through the identified 218 main driver pesticide pressure (TU_{max}) , which contributed an average explained variance of 219 0.26 R² up to the family level. Deficient hydromorphology added an average explained variance 220 of 0.16 R² to the full model for all resolutions. NH₄ was only identified as relevant by abundance 221 class and presence-absence resolution - but at all taxonomic levels - and added an average 222 explained variance of 0.08 R² to the full model. Deficient bed habitat structure contributed an 223 average R² of 0.07 and was found to be relevant mainly for abundance resolution but also for 224 the genus and family level at the two lower quantitative resolution. O_2 deficiency added an 225 average R² of 0.06 to the explained variance in the full models affecting only the order-level 226 full models' explained variance (Fig. 1). At the order level, the relevance of the main driver 227 pesticide pressure is underestimated. However, our results indicate that the specificity of 228 SPEAR_{pesticides} toward toxic pressure decreased at lower quantitative resolution while NH₄ and 229 hydromorphology explain more and toxicity less of the variance. The specificity of 230 SPEAR_{pesticides} is validated regarding the explanatory power of lower taxonomic resolutions up 231 to the family level in terms of multiple stressors.



232

Fig. 1 Overview of the multiple linear regression analysis. The relative importance of each of the five significant parameters for the different taxonomic and quantitative resolutions of SPEAR_{pesticides} is specified in explained variance by R² values. Black dots indicate the full models explained variance, whereas blue and red dots reflect the positive and negative influence of each stressor contributing to the full models' explained variance. Stars next to the R² indicate the level of significance (p < 0.05 = *, p < 0.01 = **, p < 0.001 = ***).

With the multiple linear regression analysis, we confirmed the specificity of SPEAR_{pesticides} toward pesticide pressure at all quantitative resolutions up to the family level. In the following, we will focus on the indication power of SPEAR_{pesticides} at lower taxonomic and quantitative resolution. In a single linear regression analysis between SPEAR_{pesticides} and pesticide pressure, resulting R² values for all combinations of taxonomic and quantitative resolution ranged from 0.32 (order/presence-absence; Fig. 2 A) to 0.43 (species/abundance; Fig. 2 A). In general, R² values increased with increasing taxonomic and quantitative resolution, except 246 at the genus level (Fig. 2 A). We found only a slightly decreasing explanatory power (R²) up to 247 the family level. With reducing the taxonomic resolution, the trait information basis changed. 248 At the genus level, genus sensitivity information was only available for 11% of genus taxa of 249 all sites, while 84% of genus taxa were linked at family level and 5% at order level. At species 250 level, only 17% of species taxa of all sites had sensitivity information. The majority of 80% had 251 sensitivity information at family level. Although the effect of pesticide pressure on the 252 invertebrate community could be linked up to the order level according to the R² values, in 253 some cases considerably deviating SPEAR_{pesticides} values indicated a higher prediction 254 uncertainty at order level.

255 Order level lead to an increase of most SPEAR_{pesticides} values. These higher SPEAR_{pesticides} 256 values at order level are not shifted in parallel (Fig. 2 A; SI). Streams with high ecological 257 quality deviated more than streams with lower ecological quality. Aggregating all higher 258 taxonomic trait information at order level is not feasible for every order, as many contradictory 259 higher trait information are combined. Some families classified as insensitive at the family level 260 (Hydropsychidae Gen. sp., Leptociridae Gen. sp., Sericostomidea Gen. sp., Ephemerellidae 261 Gen. sp., Leptophlebiidae Gen. sp., Nemouridae Gen. sp.) belong to the EPT group. The EPT 262 group is classified as sensitive at order level, thus increased the SPEAR_{pesticides} values. In the 263 SPEAR_{pesticides} database, 15 orders are implemented to which vulnerability information could 264 be assigned. Four orders are categorized as sensitive and 11 as insensitive towards 265 pesticides. To overcome the systematic shift of EPT rich streams at the order level, we derived 266 and applied a compensation factor of 1.2 for order/abundance and 1.3 for abundance class 267 and presence-absence resolution. This compensation factor approximated order level values 268 to that of species/abundance resolution to enable order level prediction (Fig. 2 B; SI). Despite 269 the compensation factor, many order-level SPEAR_{pesticides} values still diverged from species-270 level SPEAR_{nesticides} values which can be explained by reduced numbers of taxa at order level. 271 Linearly adjusting the order-level SPEAR_{pesticides} values only compensated the systematic shift 272 but did not allow to improve the prediction quality in terms of R².



273

Fig. 2 SPEAR_{pesticides} as a function of maximum pesticide toxicity expressed (TU_{max}) for 101 sampling sites. The three figures show the (A) explanatory power of the linear regression, (B) their respective slope, and (C) y-intercept. The points for each taxonomic level were connected as each level also contains some lower taxonomic resolutions.

278 3.1.2 Congruence and divergence between species and family SPEAR_{pesticides}

279 For most stream monitoring sites, species and family SPEAR_{pesticides} values were similar. With 280 an explained variance of $R^2 = 0.41$, family/abundance resolution associate pesticide pressure 281 and SPEAR_{pesticides} second accurately. Additionally, an identical slope and y-intercept of the 282 linear regression at family level indicated a high congruence to species level regression 283 SPEAR_{pesticides} values (Fig. 2 B, C). This congruence can be explained by three possible 284 reasons. Firstly, trait information in the SPEAR_{pesticides} indicator at the species level is only 285 available for one-third of our determined species, while most of the identified species are linked 286 to family level trait information. The frequency of each taxa was not considered here. Secondly, 287 if clear identification characteristics were absent due to damaging of individuals during the 288 sampling process or too small to determine the taxa at species level, a lower taxonomic 289 determination level was chosen to ensure the correct determination of the taxa. Thirdly, some 290 trait information in the SPEAR_{pesticides} database was extrapolated from higher taxonomic levels 291 to the species level due to missing information at the species level.

These reasons are expected to explain the insignificant differences of the SPEAR_{pesticides} values between "species" and "family" level at the same quantitative resolution (p = 0.44, Wilcoxon test, Tab. 1; SI). 295 Still, some family SPEAR_{pesticides} values deviated from species-level SPEAR_{pesticides} values. For 296 instance, family values increased due to some insensitive taxa classified as sensitive at the 297 family level (Rhyacophilidae Gen. sp., Phryganeidae Gen. sp., Goeridae Gen. sp., 298 Limnephelidae Gen. sp., Hydroptilidae Gen. sp.). Decreasing family SPEAR_{nesticides} values can 299 be explained by sensitive taxa aggregated into insensitive families (Hydropsychidae Gen. sp.) 300 at lower taxonomic resolution. Additionally, if there are many species of the same family in one 301 stream, the SPEAR_{pesticides} values at the family level decreased because the number of taxa 302 before log-transformation decreased.

303 In fact, family-level or even lower taxonomic levels are known to work with some 304 macroinvertebrate indices: the BMWP (Moolna et al., 2019), ASPT (Moolna et al., 2019), RETI 305 (Schmidt-Kloiber, A. & Nijboer, R. C., 2004), or Anglers' Riverfly Monitoring Initiative Index 306 (ARMI (Di Fiore and Fitch, 2016)). Taxonomic sufficiency (Ellis, 1985) is the taxonomic 307 resolution required for a specific endpoint to predict and judge impairment or change of an 308 ecosystem reliably. For macroinvertebrate indices, the level of adequate taxonomic resolution 309 depends on the determination depth of invertebrates but mainly on the available taxonomic 310 information in the index. Subsequently, depending on the invertebrate index and accordingly 311 the integrated taxonomic information, family level is sufficient to link multiple environmental 312 stressor to the macroinvertebrate community composition (SIGNAL, (Chessman, 1995)) or 313 show weak to no associations between environmental conditions and community composition 314 compared to the species level (SWAMPS, (Crowns et al., 1992)). Although species level has 315 the highest precision in reflecting the ecological status of streams (Norris and Hawkins, 2000; 316 Resh et al., 1995; Schmidt-Kloiber, A. & Nijboer, R. C., 2004) and is, thus, often treated as the 317 gold standard, similar to previous studies on other indicators, our analysis for SPEAR_{nesticides} 318 showed that family/abundance resolution can be used as a surrogate for species/abundance 319 resolution in terms of specificity and indication power.

320 **3.1.3 Effects of lower quantitative resolution on prediction quality**

The two lower quantitative resolutions analyzed – abundance class and presence-absence –
 showed slightly less powerful associations between pesticide pressure and SPEAR_{pesticides} of

323 0.33 to 0.38 R² (Fig. 2 A) compared to abundance resolution. At lower quantitative resolutions, the association between toxic pressure and SPEAR_{pesticides} was observed to be relatively 324 325 independent from the taxonomic resolution. Within the same lower quantitative resolutions, the 326 explanatory power is similar for all taxonomic levels (Fig. 2 A). By converting abundances into 327 abundance classes or presence-absence data the relevance of the number of taxa increased 328 while the relevance of high abundances declined with a lower quantitative resolution. The 329 minor role of the abundance is reflected by the still high association (R²) of SPEAR_{pesticides} and 330 pesticide pressure at abundance class and presence-absence resolution, even at order level 331 (Fig. 2 A). Our correlation results at lower quantitative resolution showed for the first time that 332 abundance class and presence-absence resolution can also provide a powerful link between 333 pesticide exposure and SPEAR_{pesticides.}

334 3.2 Indicating the ecological quality

335 We analyzed how taxonomic and quantitative resolutions affect the assigned ecological quality 336 in terms of SPEAR_{pesticides} classes. Lower taxonomic resolution only slightly affected the 337 ecological class assignments up to the family level. Family/abundance resolution yielded 88% 338 congruence in ecological class assignment compared to species/abundance resolution (Fig. 3 339 A). At the order level, SPEAR_{pesticides} provided a distinctly reduced congruence of 56% in 340 ecological class assignment (Fig. 3; SI). In contrast, the AQUEM Assessment Software (ASS), 341 mainly focused on species taxonomic resolution, which only reached 50% congruence at the 342 genus level and 40% congruence at the family level in ecological classification (Schmidt-343 Kloiber, A. & Nijboer, R. C., 2004).

Reducing the quantitative resolution affected ecological class assignment stronger compared to a lower taxonomic resolution up to the family level. Species/abundance class resolution lead to 74% congruence compared to species/abundance resolution. Species/presence-absence resolution resulted in 65% congruence, the lowest accuracy at the species level in class assignment with SPEAR_{pesticides}. Hence, at lower taxonomic and quantitative resolution, a class shift of individual streams occurred. We found that a shift into a better ecological class was more likely than into a worse class (see EXCEL workbook; SI). As indicated by the Aquem

Assessment Software applied at lower taxonomic resolution (ASS: Schmidt-Kloiber, A. & Nijboer, R. C. (2004)) and its General Degradation Metrics applied with presence-absence resolution (GDM: Buchner et al. (2019)), we also observed a class shift of up to two classes but in our case only at the order level (Fig. 3; SI). We observed that class shifts mainly occurred when the species/abundance resolution SPEAR_{pesticides} value is close to an ecological class boundary. Therefore, we suggest that at lower taxonomic and quantitative resolution the assigned ecological class – but also the SPEAR_{pesticides} value itself – should be considered for

of



individual

streams.



358

evaluation

361 Fig. 3 Comparison of ecological classification in terms of SPEAR_{pesticides} under A) 362 species/abundance and family/abundance resolution (88% congruence), B) species/abundance 363 and family/abundance class resolution (69% congruence), and C) species abundance and 364 family/presence-absence resolution (62% congruence). The ecological classes are displayed 365 from "High" (blue) to "Bad" (red) and symbolize the ecological quality of invertebrate 366 communities for each of the 101 streams (y-axis). The light grey horizontal bars show that the 367 majority of streams are classified equally. The dark grey horizontal bars show streams shifting 368 one class higher or lower due to lowering the taxonomic and quantitative resolution.

369 **3.3 Implications for pesticide effect monitoring**

Large-scale monitoring implies working with multiple field personnel with different macroinvertebrate sampling skills, resulting in a higher likelihood of sampling errors that can occur when estimating macroinvertebrate abundances in the field (Metzeling et al., 2013). To avoid those sampling errors according to Giehl et al. (2014), many monitoring studies are based on presence-absence resolution, rather than abundance resolution. As an example,

375 Australian state-wide biomonitoring has adopted the use of family/presence-absence 376 resolution rather than species/abundance resolution (Marshall et al., 2002). Our study for 377 SPEAR_{pesticides} also revealed that abundance class resolution is a simplified approach for 378 differently skilled field personnel and less prone to sampling errors. This lower quantitative 379 resolution provides less accurate but still adequate indication of pesticide pressure and the 380 related ecological status. Whereas the trade-off for presence-absence resolution is higher as 381 the derived ecological status deviated in 35% of cases from the maximum resolution 382 assessment and should be restricted to monitoring where the scope is to only get an 383 impression of the analyzed invertebrate community composition. Reduced quantitative 384 resolution requires less material for the conservation of taxa and consequently the monetary 385 expenditure is lower (Bush et al., 2019; Marshall et al., 2006). Depending on the availability of 386 personnel, time, and material, the respective quantitative resolution should be chosen. 387 Accordingly, we suggest applying SPEAR_{pesticides} at family/abundance class resolution as a 388 good trade-off for large-scale monitoring. The deviating ecological classifications are minor 389 restrictions over the gained time efficiency and ensured determination validity in stream 390 monitoring. Classifying the sampled macroinvertebrates into abundance classes at the family 391 level in the field also leads to lower lethal effects in the community, as the proportion of 392 preserved sample for laboratory determination is reduced. Hence, already stressed but also 393 intact macroinvertebrate communities are protected, benefiting from lower taxonomic and 394 quantitative resolution. Taxonomically and quantitatively sufficient for SPEAR_{pesticides} is the 395 sampling and determination of macroinvertebrate data at family/abundance class resolution.

396 Conclusion

Family/abundance class resolution provides the best trade-off for time-efficient large
 scale pesticide effect monitoring and ecological stream assessment with
 SPEAR_{pesticides}. It represents a simplified but reliable approach which is also applicable
 for Citizen Science programs.

The multiple stressor analysis has shown that at reduced quantitative resolution, the
 SPEAR_{pesticides} specificity decreased slightly while NH4 also began to codetermine
 SPEAR_{pesticides}.

- The derived compensation factors at order level enabled an approximation of the order
 SPEAR_{pesticides} values to the species level SPEAR_{pesticides} values. Still, though, the
 indication power is reduced at order/abundance resolution.
- 407 Also order/presence-absence resolution can associate toxic pressure to the
 408 invertebrate community composition, but reflecting the assigned ecological status only
 409 half as accurate as species/abundance resolution.

410 Acknowledgment

411 The authors thank the German Helmholtz long-range strategic research funding and the 412 "Pilotstudie zur Ermittlung der Belastung von Kleingewässern in der Agrarlandschaft mit Pflanzenschutzmittel-Rückständen"funded by the German Federal Ministry for the 413 414 Environment, Nature Conservation and Nuclear Safety (FKZ 3717 63 403 0). MOSES 415 (Modular Observation Solutions for Earth Systems) and TERENO (Terrestrial Environmental 416 Observations) projects provided additional funding. We would also like to thank all contributing 417 partners from the regional agencies which helped in the realization of the monitoring: Baden-418 Württemberg (Landesanstalt für Umwelt Baden-Württemberg – LUBW), Bavaria (Bayerisches 419 Landesamt für Umwelt – LfU), Brandenburg (Landesamt für Umwelt Brandenburg – LfU), 420 Hesse (Hessisches Landesamt für Naturschutz, Umwelt und Geologie – HLNUG), Lower 421 Saxony (Nieders. Landesbetrieb für Wasser- wirtschaft, Küsten- und Naturschutz – NLWKN), 422 Mecklenburg-Western Pomerania (Landesamt für Umwelt, Naturschutz und Geologie 423 Mecklenburg-Vorpommern – LUNG), North Rhine-Westphalia (Landesamt für Natur, Umwelt 424 und Verbraucherschutz Nordrhein-Westfalen – LANUV), Rhineland-Palatinate (Landesamt für 425 Umwelt Rheinland-Pfalz – LfU), Saxony (Sächsisches Landesamt für Umwelt, Landwirtschaft 426 und Geologie - LfULG), Saxony-Anhalt (Landesamt für Hochwasserschutz und 427 Wasserwirtschaft Sachsen-Anhalt – LHW), Schleswig-Holstein (Landesamt für Landwirtschaft,

428 Umwelt und ländliche Räume des Landes Schleswig-Holstein – LLUR) and Thuringia
429 (Thüringer Landesanstalt für Umwelt und Geologie – TLUBN.

430 **References**

- 431 Alba-Tercedor, J and Sánchez-Ortega, A. Un método rápido y simple para evaluar la
- 432 calidad biológica de las aguas corrientes basado en el de Hellawell. Limnética
 433 1988;4:50–6.
- 434 Barbour MT, Gerritsen J, Snyder BD, Stribling JB. Rapid bioassessment protocols for

435 use in streams and wadeable rivers: Periphyton, Benthic Macroinvertebrates and

436 Fish. EPA 841-B-99-002 1999;2:1–339.

- 437 Buchner D, Beermann AJ, Laini A, Rolauffs P, Vitecek S, Hering D et al. Analysis of
- 438 13,312 benthic invertebrate samples from German streams reveals minor
- deviations in ecological status class between abundance and presence/absence
 data. PloS one 2019;14(12):1-18.
- 441 Bush A, Compson ZG, Monk WA, Porter TM, Steeves R, Emilson E et al. Studying
- 442 Ecosystems With DNA Metabarcoding: Lessons From Biomonitoring of Aquatic
- 443 Macroinvertebrates. Front. Ecol. Evol. 2019;7:6247.
- 444 Buss DF, Carlisle DM, Chon T-S, Culp J, Harding JS, Keizer-Vlek HE et al. Stream
- biomonitoring using macroinvertebrates around the globe: a comparison of large-

scale programs. Environ. Monit. Assess. 2015;187(4132):1–21.

- 447 Chessman BC. Rapid assessment of rivers using macroinvertebrates: A procedure
- based on habitat specific sampling, family level identification and a biotic index.
- 449 Austral Ecol. 1995;20:122–9.
- 450 Crowns JE, Davis JA, Cheal F, Schmidt LG, Rosich RS, Bradley SJ. Multivariate
- 451 pattern analysis of wetland invertebrate communities and environmental variables
- 452 in Western Australia. Austral Ecol. 1992;17:275–88.

453	Di Fiore D, Fitch B. Riverfly monitoring initiative: structured community data gathering
454	informing statutory response. Environmental SCIENTIST 2016:37–41.
455	Ellis D. Taxonomic sufficiency in pollution assessment. Mar. Pollut. Bull.
456	1985;16(12):459.
457	Environment Agency. Freshwater biological indicators of pesticide contamination –
458	an adaptation of the SPEAR approach for the UK. Science Report
459	2008(SC030189/SR4):1–30.
460	EU Commision. Directive 2000/60/EC of the European Parliament and of the Council.
461	Establishing a framework for Community action in the field of water policy. Off. J.
462	EUr. Union 2000;327:1–72.
463	EU Commission. Establishing, pursuant to Directive 2000/60/EC of the European
464	Parliament and of the Council, the values of the Member State monitoring system
465	classifications as a result of the intercalibration exercise. Off. J. EUr. Union
466	2008;332(25).
467	Giehl NFdS, Dias-Silva K, Juen L, Batista JD, Cabette HSR. Taxonomic and
468	numerical resolutions of nepomorpha (insecta: heteroptera) in cerrado streams.
469	PloS one 2014;9(8):e103623.
470	Grömping U. Relative Importance for Linear Regression in R: The Package relaimpo.
471	J. Stat. Softw. 2006;17(1):1–27.
472	Halbach K, Moeder M, Schrader S, Liebmann L, Schaefer RB, Schneeweiss A et al.
473	Small streams – large concentrations? Pesticide monitoring in small agricultural
474	streams in Germany during dry weather and rainfall. Water Res. 2021;203(27):1–
475	10.
476	Knillmann S, Orlinskiy P, Kaske O, Foit K, Liess M. Indication of pesticide effects and
477	recolonization in streams.(630), 2018:1619–27.

Kolkwitz R. and Marsson M. Ökologie der tierischen Saprobien. Beiträge zur Lehre
von der biologischen Gewässerbeurteilung. Internat. Rev. Hydrobiol.

480 1909(2):126–52.

- 481 Lenat DR. Water Quality Assessment of Streams Using a Qualitative Collection
- 482 Method for Benthic Macroinvertebrates. J. North Am. Benthol. Soc. (Journal of the
- 483 North American Benthological Society) 1988;7(3):222–33.
- Lewis KA, Tzilivakis J, Warner DJ, Green A. An international database for pesticide
- 485 risk assessments and management. Hum. Ecol. Risk Assess. (Human and
- 486 Ecological Risk Assessment: An International Journal) 2016;22(4):1050–64.
- 487 Liess M, Liebmann L, Vormeier P, Weisner O, Altenburger R, Borchardt D et al.
- 488 Pesticides are the dominant stressors for vulnerable insects in lowland streams.
- 489 Water Res. 2021;201:117262.
- 490 Liess M, Ohe PC von der. Analyzing effects of pesticides on invertebrate
- 491 communities in streams.(4), 2005:954–65.
- 492 Liess M, Schulz R, Liess MH-D, Rother B, Kreuzig R. Determination of insecticide
- 493 contamination in agricultural headwater streams. Water Res. 1999;33(1):239–47.
- 494 Marshall J, Hoang H, Choy S, Recknagel F. Relationships between habitat properties
- 495 and the occurrence of macroinvertebrates in Queensland streams (Australia)
- discovered by a sensitivity analysis with artificial neural networks. SIL
- 497 Proceedings, 1922-2010 2002;28(3):1415–9.
- 498 Marshall JC, Steward AL, Harch BD. Taxonomic Resolution and Quantification of
- 499 Freshwater Macroinvertebrate Samples from an Australian Dryland River: The
- 500 Benefits and Costs of Using Species Abundance Data. Hydrobiologia
- 501 2006;572(1):171–94.
- 502 Meier C, Haase P, Rolauffs P, Schindehütte K, Schöll F, Sundermann A et al.
- 503 Methodisches Handbuch Fließgewässerbewertung. Handbuch zur Untersuchung

- ⁵⁰⁴ und Bewertung von Fließgewässern auf der Basis des Makrozoobenthos vor dem
- 505 Hintergrund der EG-Wasserrahmenrichtlinie. 2006.
- 506 Metzeling L, Chessman BC, Hardwick R, Wong V. Rapid assessment of rivers using
- 507 macroinvertebrates: the role of experience, and comparisons with quantitative
- 508 methods. Hydrobiologia 2013;510:39–52.
- 509 Moolna A, Duddy M, Fitch B, White K. Citizen science and aquatic
- 510 macroinvertebrates: public engagement for catchment-scale pollution vigilance.
- 511 Écoscience 2019;27(4):303–17.
- 512 Münze R, Hannemann C, Orlinskiy P, Gunold R, Paschke A, Foit K et al. Pesticides
- 513 from wastewater treatment plant effluents affect invertebrate communities. Sci.
- 514 Total Environ. 2017;599-600:387–99.
- 515 Norris RH, Hawkins CP. Monitoring river health. Hydrobiologia 2000;435(1/3):5–17.
- 516 Reemtsma T, Alder L, Banasiak U. Emerging pesticide metabolites in groundwater
- and surface water as determined by the application of a multimethod for 150
- 518 pesticide metabolites. Water Res. 2013;47(15):5535–45.
- 519 Resh VH, Norris RH, Barbour MT. Design and implementation of rapid assessment
- 520 approaches for water resource monitoring using benthic macroinvertebrates.
- 521 Austral Ecol. 1995;20:108–21.
- 522 Schmidt-Kloiber, A. & Nijboer, R. C. The effect of taxonomic resolution on the
- 523 assessment of ecological water quality classes. Hydrobiologia 2004(516):269–83.
- 524 Schweder H. Neue Indizes für die Bewertung des ökologischen Zustandes von
- 525 Fließgewässern, abgeleitet aus der Makroinvertebraten-Ernährungstypologie.
- 526 Limnologie 1992(3):353–77.
- 527 Smith MJ, Kay WR, Edward D. H. D., Papas PJ, St. Richardson KJ, Simpsons JC et
- al. AusRivAS: using macroinvertebrates to assess ecological condition of rivers in
- 529 Western Australia. Freshwater Biology 1999(41):269–82.

- 530 Sprague JB. Measurement of pollutant toxicity to fish. II. Utilizing and applying
- 531 bioassay results. Water Res. 1970;4:3–32.
- 532 Wick A, Bänsch-Baltruschat B, Keller M, Scharmüller A, Schäfer R, Foit K et al.
- 533 Umsetzung des Nationalen Aktionsplans zur nachhaltigen Anwendung von
- 534 Pestiziden Teil 2 Konzeption eines repräsentativen Monitorings zur Belastung
- 535 von Kleingewässern in der Agrarlandschaft.: Teilvorhaben II.
- 536 Umweltforschungsplan des Bundesministeriums für Umwelt, Naturschutz, Bau
- 537 und Reaktorsicherheit 2018:1–154.
- 538 Wickham H. ggplot2. New York, NY: Springer New York; 2009.