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Diagnosing the causes of river deterioration using stressorspecific metrics



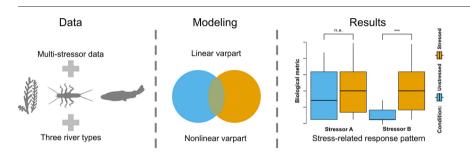
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HIGHLIGHTS

- We tested 437 biological metrics with respect to their stressor-specific indication
- Our data covers three different groups of stress and three broad river types.
- Stressor-specific indication depends on data-specific features attributable to the biogeographical context.
- Stressor-specific metrics provide evidence to guide appropriate mitigation measures by environmental managers.

GRAPHICAL ABSTRACT



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ABSTRACT

More often than not, rivers are impacted by multiple stressors simultaneously affecting water quality, ecological flow, habitat diversity and ultimately lotic biodiversity. Identifying individual stressors as specific causes of deterioration can help inform water managers about stressor hierarchy and appropriate management options. Here, we investigate whether biological metrics from bioassessment schemes hold diagnostic capabilities to distinguish between the impact of individual stressors. We hypothesise that stressor-specific responses occur, when individual stressors show independent 'modes of action' (i.e. the specific stress-induced changes of environmental factors that modify the ecological niches of the species constituting the biological community). The stress receptors comprised three aquatic organism groups (macrophytes, benthic invertebrates, fish) represented by 437 biological metrics relevant in aquatic bioassessment. The stressor groups under investigation were physicochemical, hydromorphological and hydrological stress. The data originated from official monitoring programmes with 769 sampling sites located at three broad river types in Western and Central Germany. Linear and non-linear variance partitioning was performed separately for each river type, with the non-linear analysis using a combination of boosted regression tree modeling and variance partitioning. We considered metrics to be potentially stressor-specific, if the corresponding models were explained predominantly by one stressor group. The linear analyses revealed 16 metrics that met our criteria. Subsequent non-linear modeling resulted in two genuinely stressor-specific metrics, both based on invertebrate data: The Index of Biocoenotic Region (specifically indicating hydromorphological stress) and the Relative abundance of alien invertebrate species (specifically indicating physico-chemical stress). We conclude that stressor-specific metrics can be empirically derived based on available monitoring data, and thus help support decision making in environmental management. However, their applicability is restricted to specific regions (e.g. river basin districts) reflecting the case-specific circumstance to which these metrics are conditioned.

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1. Introduction

Aquatic ecosystems are impacted by multiple human pressures (Hering et al., 2015) such as point source pollution from urban areas and diffuse pollution from agricultural land use. Together with severe hydrological and hydromorphological modifications, these pressures are widespread in Europe and continue to impact on aquatic biodiversity in lakes, rivers, estuaries, and coastal waters (EEA, 2018). These pressure impacts are exerted by numerous stressors, i.e. measurable environmental factors that exceed the range of natural variation and thus cause biological deterioration (Odum, 1985; Underwood, 1989; Birk, 2018). Eutrophication, and more specifically, the loads of rivers and lakes with nitrogen and phosphorus are quantified by the concentrations of respective nutrient compounds, for example, ammonia, nitrite, nitrate, or soluble reactive phosphorus. This kind of stressor data is usually surveyed in parallel with biological monitoring schemes.

At present, comprehensive monitoring and country-specific assessment data on biology and environmental stressors is available for about 120,000 water bodies in Europe covering various ecological types of surface waters (EEA, 2018). This data provides the basis for ecological status assessment and, subsequently, for the derivation of appropriate management and restoration options to improve ecological status according to the European Water Framework Directive 2000/ 60/EC (WFD). However, while biological assessment is relatively straightforward in Europe (Birk et al., 2012), the derivation of suitable management options is not. Often, ecological status assessment combines multiple stressor effects reflected by one or several biological metrics that can be used in a multi-metric index (Karr and Chu, 1997). In brief, current multi-metric bioassessment schemes usually integrate stressor effects (e.g. pollution, hydrological and morphological degradation) across different spatial scales (e.g. catchment, stream segment, reach, site). The commonly used relative abundance of Ephemeroptera, Plecoptera, and Trichoptera taxa (%EPT) is one example of such an integrative metric. Many taxa are not specifically sensitive to one stressor, which is why %EPT can be found in numerous studies addressing evenly numerous combinations of stressors at different spatial scales (e.g. Böhmer et al., 2004; Hering et al., 2006a; Collier, 2014).

From a purely ecological viewpoint, the holistic evaluation of ecosystem status neither expects nor desires stressor-specific biological response (Verdonschot, 2000). On the other hand, multimetric bioassessment often refers to the concept of stressor-specific bioindication (Hering et al., 2006b); yet concrete empirical evidence is largely pending. Furthermore, using integrative assessment schemes or integrative composite metrics, it is often difficult to distinguish individual stressors effects (Gieswein et al., 2017). This situation bears a serious challenge for river basin management, because to improve ecological status water managers need tools to distinguish the stressors' importance and their hierarchy to derive appropriate management options (Murphy et al., 2013; Townsend et al., 2008; Matthaei and Lange, 2016).

Our mechanistic understanding of stressor-specific responses is based on general concepts of multiple stressor effects in biological communities (Breitburg et al., 1998, Vinebrooke and Cottingham, 2004): Stressor effects are determined by their 'modes of action' (Escher and Hermens, 2002), understood here as the specific stress-induced changes of environmental factors that modify the ecological niches of the species constituting the biological community (Hutchinson, 1957). These changes in niche factors affect the species adapted to these niches, i.e. these species are sensitive to the stressor and will ultimately disappear from the stressed biological community. We hypothesise that in a multi-stressed environment stressor-specific biological responses can be observed if:

- The individual stressors show independent 'modes of action', and
- the 'modes of action' affect different features of the biological community (according to the concept of 'negatively correlated species cotolerances' sensu Vinebrooke and Cottingham, 2004).

River impoundment for hydropower, for instance, lowers the oxygen concentration at the river bottom (=mode of action), altering the environmental conditions crucial for organisms sensitive to oxygen availability. River salinization from road de-icers, for instance, increases the osmotic stress for aquatic organisms (=mode of action), affecting organisms incapable of regulating this stress.

Inspired by the few studies that demonstrated successful applications of the concept of stressor-specific biological response (e.g. Clews and Ormerod, 2009; Statzner and Bêche, 2010; Baattrup-Pedersen et al., 2016), we here analyse an extensive dataset to systematically scrutinise the biological response patterns of different stressor groups. The different community features mentioned above are represented by altogether 437 bioassessment metrics computed from the sampling data of three aquatic organism groups, which are mandatory for the status assessment of rivers according to the WFD (macrophytes, benthic invertebrates, fish). These metrics quantify the presence and abundance of taxa, and the proportion of individuals within the biological community that share the same ecological and biological traits (e.g. taxonomic affiliations; life-history, physiological, or morphological traits; habitat preferences). Against this plethora of possible community response gradients, we investigate stressor effects across up to three co-acting groups of stressors: physico-chemistry, hydromorphology and hydrology. Based on the analyses of general stressor groups, we additionally refer to a visualization of response patterns of stressor-specific metrics. This represents a methodology to identify stressor-specific bioassessment metrics to support the diagnosis of stressor effects in river basins.

2. Material and methods

2.1. Data

We obtained WFD data from three German Federal States (North Rhine-Westphalia, Saxony-Anhalt, Hesse) covering 769 sites at three European broad river types (BT; ETC/ICM, 2015) (Fig. 1): Medium to large lowland rivers of calcareous or mixed catchment geology (BT4, n=92 sites; corresponding to German River Types 15, 15g, 17 according to Pottgiesser and Sommerhäuser, 2008), small lowland rivers of calcareous or mixed catchment geology (BT5, n=392 sites; corresponding to German River Types 14, 16, 18), and small mid-altitude rivers of siliceous catchment geology (BT9, n=285 sites; corresponding to German River Types 5 and 5.1). Biological data covered three organism groups (macrophytes, benthic invertebrates, fish) and non-biological data covered physico-chemistry, hydromorphology, hydrology (only for BT4), catchment size and altitude (Table 1).

2.2. Biological data

Macrophyte data was available for 505 sites and from 846 surveys conducted between 2005 and 2010. Species composition and abundance of aquatic plants (macroalgae, bryophytes, angiosperms) were recorded by visually inspecting representative river stretches of 100 m length during the growing season (May to September; Schaumburg et al., 2004).

Data on taxonomic composition and abundance of benthic invertebrates was available for 615 sites and 874 samples taken from 2004 to 2013. Benthic invertebrates were collected during spring and summer following a multi-habitat sampling protocol (Hering et al., 2004): For each sample, 20 representative sampling units were taken that cover all important microhabitat types (at least 5% of the sample reach) using a kick-net with $25 \times 25 \, \mathrm{cm}^2$ frame and a mesh size of 500 µm. Benthic invertebrates were identified to species level where possible.

Data on species composition and abundance of fishes was available for 275 sites and 352 samples taken from 2004 to 2009. The sampling procedure followed CEN (2003) using electrofishing along river

Legend

- Broad Type 4: Medium to large lowland rivers of calcareous or mixed catchment geology
- Broad Type 5: Small lowland rivers of calcareous or mixed catchment geology
- Broad Type 9: Small mid-altitude rivers of siliceous catchment geology

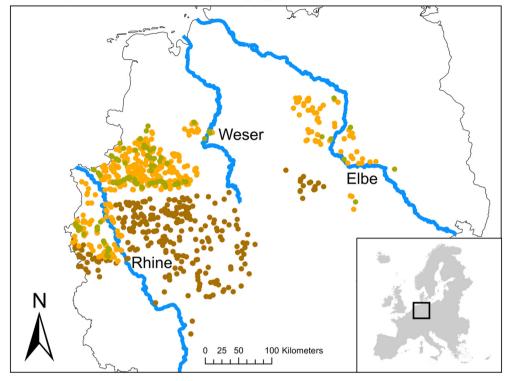


Fig. 1. Location of the 769 sampling sites used in this study covering macrophyte, benthic invertebrate and fish data at three broad river types in Western and Central Germany.

 Table 1

 Overview of explained variances (given as adjusted R^2) per Broad Type (BT) and organism group gained from linear Varpart. The joint fraction is the sum of explained variances shared by more than one stressor group. n = number of samples/number of metrics, 90th = 90th percentile.

Organism group	BT	n	Statistical descriptors	Full model	Individual fractions	Joint fraction			
					Physico-chemistry	Hydromorphology	Hydrology	Natural variables	
Macrophytes	4	314/148	Median	0.103	0.010	0.010	0.013	0.008	0.024
			90th	0.266	0.067	0.095	0.055	0.043	0.116
			Max	0.396	0.175	0.199	0.086	0.099	0.272
Benthic invertebrates	4	391/137	Median	0.167	0.002	0.016	0.026	0.024	0.052
			90th	0.355	0.026	0.051	0.085	0.137	0.179
			Max	0.556	0.107	0.126	0.136	0.402	0.368
Fish	4	280/113	Median	0.017	0.036	0.033	0.062	0.000	0.171
			90th	0.126	0.146	0.102	0.213	0.062	0.350
			Max	0.169	0.259	0.177	0.463	0.209	0.514
Macrophytes	5	322/145	Median	0.045	0.013	0.003	_	0.008	0.006
			90th	0.154	0.065	0.018	_	0.069	0.029
			Max	0.341	0.128	0.048	_	0.214	0.074
Benthic invertebrates	5	389/140	Median	0.098	0.015	0.023	_	0.027	0.015
			90th	0.264	0.061	0.075	_	0.108	0.064
			Max	0.356	0.124	0.135	_	0.194	0.124
Fish	5	280/110	Median	0.127	0.015	0.012	_	0.075	0.000
			90th	0.283	0.065	0.052	_	0.271	0.037
			Max	0.380	0.136	0.128	_	0.313	0.087
Macrophytes	9	203/152	Median	0.041	0.004	0.002	_	0.010	0.004
			90th	0.130	0.033	0.002	_	0.109	0.029
			Max	0.182	0.109	0.057	_	0.141	0.047
Benthic invertebrates	9	392/143	Median	0.155	0.048	0.010	_	0.012	0.069
			90th	0.380	0.125	0.036	_	0.043	0.210
			Max	0.544	0.187	0.083	-	0.124	0.323
Fish	9	187/105	Median	0.217	0.022	0.016	_	0.078	0.049
			90th	0.321	0.085	0.073	_	0.229	0.098
			Max	0.397	0.153	0.166	_	0.250	0.140

stretches of several 100 m in length. All sampled specimens were identified to species level, counted, measured in length and released after sampling (Dahm et al., 2013).

Based on the biological data, we calculated a total of 437 bioassessment metrics for the three organism groups (see Table S1 in the supplementary material). The metrics covered a wide range of categories, for example, measures of richness, abundance, diversity and sensitivity, biological and functional traits (Karr, 1981; Birk et al., 2012). 165 macrophyte metrics were computed referring to ecological classification methods (Birk and Willby, 2010), growth form types (Wiegleb, 1991), ecological attribute groups (Willby et al., 2000) and additional trait information (Baattrup-Pedersen et al., 2016). 159 benthic invertebrate metrics were calculated with ASTERICS Version 4.0.4 (Meier et al., 2006). Data on functional invertebrate traits were acquired from Schmidt-Kloiber and Hering (2015) including information according to Tachet et al. (2010). We computed invertebrate community average trait values weighted by taxa abundances according to Dolédec et al. (2011), 113 fish metrics were calculated using the autecological information of Holzer (2008) collated in the European research projects EFI + (EFI+ Consortium, 2009) and FAME (Kestemont and Goffaux, 2002).

2.3. Non-biological data

Non-biological data was available for three groups of stressors, i.e. physico-chemistry, hydromorphology and hydrology (Table S2). Physico-chemical parameters were acquired from routine water quality monitoring programs of the German Federal States (UBA, 2014) and were spatio-temporally matched to the biological sample sites using GIS (ArcGIS 10.4, ESRI Inc.). For the macrophyte samples, we computed annual average values of the parameters water temperature, oxygen concentration, chloride, total nitrogen and total phosphorus. The same parameters and time frame were selected for the benthic invertebrate samples, except total nitrogen was replaced by nitrate due to lacking data. For the fish samples, we collated the average annual values of water temperature, oxygen concentration, conductivity, chloride and total phosphorus.

Hydromorphological data on selected physical habitat quality features was available from standardised field surveys of 100 m river stretches (LAWA, 2000; LUA, 2001; Gellert et al., 2014), which we aggregated for 500 m reaches upstream of a biological sampling site. Ten different instream-, riparian- and floodplain-quality features were evaluated by scores ranging from 1 (=near-natural) to 7 (=totally impaired) (see Dahm et al., 2013;, Table S2). For BT4, data on hydrological alteration was acquired from the hydrological model PCR-GLOBWB adequately covering European rivers with catchment sizes > 1000 km² (van Beek and Bierkens, 2008). Score ratios of 81 indicators of hydrological alteration (IHA; Richter et al., 1996) derived from two model scenarios (i. altered hydrology including water abstraction and ii. nearnatural hydrology) were calculated on the level of the Functional Elementary Catchments (FEC) of the European catchments and rivers network system (EEA, 2012; Globevnik et al., 2017; Panagopoulos et al., 2017). We assigned the FEC-specific IHA score ratios to each biological sampling site located in the respective FEC. In addition, we used the altitude and catchment size of each site to account for natural variability in the data.

2.4. Data preparation

Histograms of all non-biological variables were visually inspected for normal distribution and, when necessary, transformed using Box-Cox transformation (Box and Cox, 1964). For each of the nine datasets (i.e. three biological organism groups times three broad river types), we minimized collinearity between the non-biological predictor variables by a stepwise reduction of variables with a variance inflation factor > 10 (Hair Jr. et al., 2010). The remaining collinearity was checked by Spearman rank correlation. All non-biological variables were then

centered and scaled. This reduced set of predictor variables was used for both further analyses to ensure the same input in the linear and non-linear models. To balance the number of explanatory variables in the linear *Varpart*, the parameters within each stressor group were reduced to the first three axes of the principal components analysis (PCA, Table S3).

We additionally compiled a subset of benthic invertebrate and respective non-biological data that covered 161 samples at 107 sites of the German River Type 5 (small coarse substrate-dominated siliceous highland rivers) sampled during spring. With this geographically and methodologically more homogeneous data subset within BT9, we intended to study the effects of data quality on the analytical outcomes.

2.5. Data analysis

We used variance partitioning analysis (*Varpart*) to investigate stressor-specific biological responses (Borcard et al., 1992; Peres-Neto et al., 2006). *Varpart* uses regression analysis of groups of predictor variables against a response variable to quantify the variance fractions (given as adjusted R² values) explained by each predictor group alone (individual fraction) or in combination (joint fraction, see Fig. 2). The groups of stressors (physico-chemical, hydromorphological, hydrological) and natural variables (altitude, catchment size) were each treated as groups of predictor variables. The bioassessment metrics were used as response variables.

To calculate all single fractions [a], [b], [c] and [d] as shown in Fig. 2, single and combined regression models of the predictor groups against the biological response variables were calculated. We performed linear *Varpart* in R using the *vegan* package (Oksanen et al., 2016) for all 437 bioassessment metrics. To compare linear and non-linear patterns of stressor-specific responses, we calculated *Varpart* fractions based on Boosted Regression Tree analysis in R (BRT; Elith and Leathwick, 2017) using the *dismo* (Hijmans et al., 2016) and *gbm* (Ridgeway, 2013) packages. This analysis was done only for a limited number of metrics due to the considerable time expenditure involved in this modeling procedure. The metrics for the BRT analyses were selected based on the results of the linear *Varpart*, promising results documented in the scientific literature and our own judgement. In both *Varpart* analyses, negative variance fractions were replaced by zero values according to Borcard et al. (2011).

2.5.1. Varpart using linear regression

We automated the *Varpart* using linear regression by developing and applying a parametrized R Markdown document (Allaire et al., 2016). The variable groups comprising physico-chemical and hydromorphological

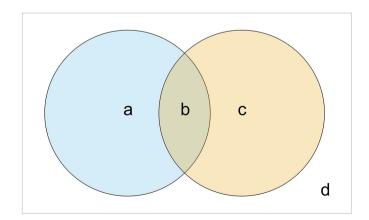


Fig. 2. Schematic Venn diagram resulting from variance partitioning of two different stressor groups. The explained variance of stressor group 1 is represented by fraction [a+b]. The explained variance of stressor group 2 is represented by fraction [b+c]. [a] and [c] represent the individual fractions of variance explained by stressor 1 or stressor 2. [b] is the joint fraction, and [d] is the residual explained variance.

stressors, and the natural variables were included in the analysis of all nine datasets. Within BT4, hydrological variables were added as a separate predictor group.

We used the following criteria, all of which had to be met to identify stressor-specific bioassessment metrics based on the linear Varpart: (i) The explained variance of the full model was at least 25%, (ii) the highest individual fraction was more than twice the second highest individual fraction, (iii) the highest individual fraction was larger than the joint fraction, and (iv) the share of the highest individual fraction to the total explained variance was larger than one-third. For highly correlated metrics (Spearman's rho \geq 0.7), we removed those with lower explained variance of the full model or with lower individual fractions.

2.5.2. Varpart using non-linear regression

BRT models were run for 100 metrics. Model fitting was done using the gbm.step() function and the calibration of several important arguments (Elith et al., 2008; Feld et al., 2016) specified in the following: The gbm.step() function uses k-fold cross validation to provide the optimal number of regression trees. The argument 'family' represents the nature of the error structure and is dependent on the scale of measurement of the response variable. The 'tree complexity' defines the number of nodes of a single tree, determining whether the model is additive or n-way multivariate. The 'learning rate' defines the weight of the individual trees. The last important argument is the 'bag fraction' that defines the size of the subsets used in the cross validation (here: bag,fraction = 0.75). To provide comparable models in our study, a similar number of trees (at least >1000) was computed. The explained variances were calculated for the full model, for the models of individual stressor groups and for all possible combinations of stressor groups by '(mean total deviance — mean residual deviance)/mean total deviance * 100' (Derville et al., 2016).

The explained variances were then fed into the *Varpart* scheme (see Peres-Neto et al., 2006) to calculate all individual and combined fractions. For the simplest case containing two stressor groups (see Fig. 2), three BRT models are needed to calculate the explained variances for the fractions [a + b], [b + c] and [a + b + c]. Following the *Varpart* scheme, four equations are required to calculate the individual fraction [a], [b], [c] and [d]:

$$\begin{aligned} [a] &= [a+b+c] - [b+c] \\ [b] &= [a+b] - [a] \\ [c] &= [a+b+c] - [a+b] \\ [d] &= 1 - [a+b+c] \end{aligned}$$

Stressor-specific biological response metrics were then identified using the same criteria as above. If the biological metrics fulfilled these criteria, we checked significant correlations with the single parameters included in the respective predictor group. Interaction effects between stressor groups were scrutinized using the gbm.interaction() function in the R package *dismo* (Hijmans et al., 2016).

$2.5.3.\ Visualizing\ the\ stressor-specific\ metric\ response$

To visually exemplify the response pattern of stressor-specific metrics, we selected two metrics resulting from the previous linear and non-linear analyses. We split their response range between 'stressed' and 'unstressed' conditions independently of the presence of the other stressors. These conditions were defined by non-biological thresholds for two individual stressors belonging to two separate stressor groups. In our selected cases, the thresholds defined for *chloride* (representing physico-chemical stress) were 200 mg l⁻¹ (OGewV, 2016) and 40 mg l⁻¹ (Halle and Müller, 2014), respectively, while the threshold for hydromorphology was the good-moderate boundary of the parameter *channel planform* and *bank vegetation* of German river habitat quality assessment (i.e. habitat score of 3.4 and 3.6, respectively; Gellert et al., 2014). To compare the stressed

and unstressed metric ranges we used boxplots and tested for significant group difference with the Wilcoxon rank sum test.

3. Results

3.1. Varpart using linear regression

Table 2 shows the stressor-specific metrics resulting from the linear *Varpart*. We identified a total of 16 metrics meeting our criteria, from which twelve metrics yielded stressor-specific responses for the medium to large lowland rivers (BT4). Especially macrophyte- and fish-based metrics were relevant for this river type, with most of the metrics specifically responding to hydromorphological stress. Three invertebrate-based metrics fulfilled our criteria with many others failing to meet *criterion iii* (i.e. the highest individual fraction was not larger than the joint fraction). This result was different for the geographically and methodologically more homogeneous data subset that we analyzed in addition: eight metrics responding specifically to physico-chemical stressors were identified (Table 2).

On average, the stressor-specific response (i.e. the highest individual fraction) amounted to 18% across all river types and organism groups, irrespective of the stressor group. Hydromorphological stressors yielded a specific response of 19% of explained variance. The two metrics responding to hydrological stress showed an average of 14%, while the physico-chemical stressors resulted in 13%. The latter increased to an average of 22% for the BT9 invertebrate data subset. Average stressor-specific explained variances per organism group were 16% (macrophytes), 18% (fish) and 20% (benthic invertebrates, incl. data subset).

The explained variances of the full models increased from macrophytes (31%) to fishes (34%) to benthic invertebrates (37%, incl. data subset). The joint fractions, indicating a non-stressor-specific response, were low for macrophytes and fishes (6% and 5%, respectively) and high for benthic invertebrates (14%, incl. the results based on a data subset). The natural variables (altitude, catchment size) explained an average variance of 4% (macrophytes), 1% (benthic invertebrates, incl. data subset) and 12% (fishes).

The three stressor-specific metrics performing best in the linear *Varpart* were: *German Saprobic Index* for the BT9 data subset (physicochemical stressors, individual fraction: 27%), *Relative abundance of eurytopic fish species* for BT4 (hydromorphological stressors, individual fraction: 26%) and *Relative abundance of species tolerant to water quality deterioration* (hydromorphological stressors, individual fraction: 25%).

3.2. Varpart using non-linear regression

We calculated 100 non-linear *Varpart* models covering 23 macrophyte metrics, 48 invertebrate metrics and 29 fish metrics, respectively (see Table S4). The overall mean explained variance of the full models was 77%. The mean individual fraction was 15%, while the mean joint fraction was 61%. None of the metrics identified in the linear *Varpart* could be confirmed in the non-linear *Varpart*, except for the *Croatian Saprobic Index*. This metric, however, differed in stressor-specificity between linear and non-linear *Varpart* (physico-chemical versus hydromorphological stressors). For all other metrics, the joint fractions notably exceeded all individual fractions, rendering these responses non-stressor-specific. Three metrics fulfilled our selection criteria established for the linear *Varpart*, featuring an average explained variance of 43% for the highest individual fractions (Table 3).

The Index of Biocoenotic Region showed significant Spearman correlations with the hydromorphological parameters flow diversity (R=-0.20) and substrate diversity (R=-0.23). The Relative abundance of alien invertebrate species revealed significant correlations with the physico-chemical parameters oxygen concentration (R=-0.26), chloride (R=0.36), water temperature (R=0.34) and total phosphorus (R=0.26). The hybrid-response of the Croatian Saprobic Index was characterized by significant correlations with

Table 2Results of the linear Varpart. The table specifies the adjusted R² values of the different fractions and the full model resulting from the Varpart. The highest individual fraction is bold. BT = Broad Type.

Organism group	BT	Metric	Individual fractions					Full
			Physico-chemistry Hydro-morpho		Hydrology	Natural variables	fraction	Model
Macrophytes ^a	4	Total abundance of taxa with plant life-form: Hemicryptophytes	0.146	0.062	0.027	0.011	0.111	0.357
		Total abundance of taxa with plant life-form: Geophytes	0.032	0.160	0.070	0.047	0.038	0.347
		Total number of taxa with aerenchyma	0.030	0.155	0.042	0.050	0.050	0.327
		Total abundance of free-floating taxa	0.004	0.164	0.059	0.052	0.075	0.269
		Total number of taxa with growth form: Hydrocharids	0.025	0.157	0.023	0.046	0.003	0.254
Benthic	4	German Fauna Index (D03)	0.008	0.036	0.119	0.032	0.071	0.266
invertebrates ^b	5	Croatian Saprobic Index	0.124	0.062	_	0.026	0.104	0.316
	9	Relative abundance of taxa with reproduction by cemented isolated eggs	0.165	0.000	-	0.020	0.139	0.324
	9	German Saprobic Index	0.276	0.007	_	0.003	0.225	0.511
	(subset)	Relative abundance of EPT taxa	0.243	0.018	_	0.002	0.206	0.469
		SPEAR index	0.235	0.000	_	0.014	0.168	0.417
		Average Score Per Taxon	0.216	0.006	_	0.007	0.177	0.406
		German Fauna Index (German type 5)	0.194	0.012	_	0.001	0.182	0.389
		Rheoindex	0.202	0.008	_	0.000	0.153	0.363
		Relative abundance of taxa with locomotion type swimming or diving	0.164	0.003	-	0.031	0.137	0.335
		Relative abundance of alien invertebrate species	0.205	0.000	_	0.023	0.024	0.252
ish ^c	4	Relative abundance of species with life span >15 years	0.020	0.117	0.054	0.209	0.064	0.352
		Relative abundance of species intolerant to habitat degradation	0.000	0.202	0.025	0.119	0.087	0.335
		Relative abundance of species tolerant to water quality deterioration	0.029	0.249	0.030	0.011	0.008	0.327
		Relative abundance of species with females maturing before/at age of 4 or 5 years	0.017	0.019	0.154	0.138	0.030	0.358
		Relative abundance of species tolerant to habitat degradation	0.000	0.245	0.049	0.048	0.104	0.446
	5	Average relative abundance of species fecundity scores	0.136	0.051	_	0.157	0.004	0.279
	9	Relative abundance of species intolerant to habitat degradation	0.131	0.019	-	0.082	0.088	0.354
		Shannon Wiener Index	0.097	0.000	-	0.189	0.037	0.272

^a Autecological information based on Willby et al. (2000) and Baattrup-Pedersen et al. (2016).

the physico-chemical and hydromorphological parameters chloride (R=0.27), nitrate (R=0.21), oxygen concentration (R=-0.31), total phosphorus (R=0.31), water temperature (R=0.31), width variability (R=-0.33), riparian buffer strip (R=-0.34), transverse bars (R=-0.28), channel planform (R=-0.28), artificial barriers (R=-0.15), flow diversity (R=-0.22), substrate diversity (R=-0.19) and bank features (R=-0.41). We did not observe strong interaction effects between stressor groups in the full BRT models.

3.3. Visualizing the stressor-specific metric response

The metric selected from the *linear Varpart* to visualize the stressor-specific response ranges between stressed and unstressed conditions was the macrophyte metric *Total number of taxa with aerenchyma* resulting from the analysis of the medium to large lowland river data (BT4). The Wilcoxon rank sum test showed a significant difference between stressed and unstressed conditions in relation to *channel planform*, but not to *chloride* (Fig. 3A).

Mostly located in intensively farmed agricultural landscapes, the altered planform of these rivers is characterized by straightened and widened channels for flood protection. These conditions favor macrophytes such as *Potamogeton* or *Lemna* that feature aerenchyma, i.e. spongy tissue forming air channels enhancing the gas exchange within the plant. Our results reveal that the metric does not respond to water quality issues indicated by chloride.

The metric selected from the *non-linear Varpart* was the invertebrate metric *Relative abundance of alien invertebrate species* resulting from the analysis of the small mid-altitude river data (BT9). The Wilcoxon rank sum test showed a significant difference between stressed and unstressed conditions in relation to *chloride*, but not to *bank vegetation* (Fig. 3B). These findings are discussed later in the paper.

4. Discussion

The aim of our study was to identify bioassessment metrics that respond specifically to different (groups of) stressors. Based on our criteria

Results of the non-linear Varpart. The table specifies the adjusted R² values of the different fractions and the full model resulting from the Varpart. Highest individual fraction is bold. BT = Broad Type.

Organism group	Broad type	Metric	Individual fractions	Joint fraction	Full model		
			Physico-chemistry	Hydromorphology	Hydrology		
Benthic invertebrates	4	Index of Biocoenotic Region	0.026	0.594	0.000	0.225	0.845
	5	Croatian Saprobic Index	0.114	0.341	_	0.325	0.780
	9	Relative abundance of alien invertebrate species	0.329	0.029	_	0.073	0.431
	9 (data subset)	Relative abundance of alien invertebrate species	0.448	0.000	-	0.000	0.448

b Autecological information based on Schmidt-Kloiber and Hering (2015) and Tachet et al. (2010). Metrics were calculated with ASTERICS Version 4.0.4 (Meier et al., 2006).

^c Autecological information based on Holzer (2008).

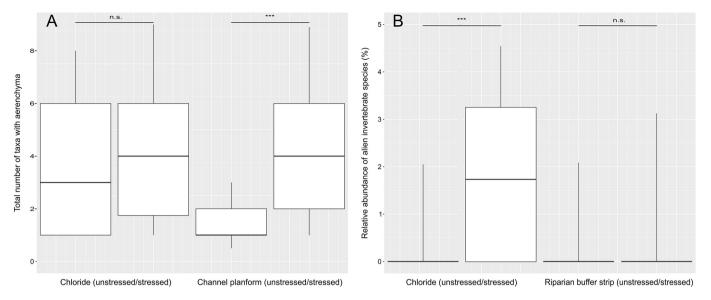


Fig. 3. (A) Value ranges of the metric 'Total number of macrophyte taxa with aerenchyma' across sampling sites of the medium to large lowland rivers of calcareous or mixed catchment geology (BT4) in stressed and unstressed conditions for the stressors 'chloride' (n = 168 surveys unstressed) and 'channel planform' (n = 16 surveys unstressed). Total number of surveys = 188. (B) Value ranges of the metric 'Relative abundance of alien invertebrate species (%)' across sampling sites of the small mid-altitude rivers of siliceous catchment geology (BT9) in stressed and unstressed conditions for the stressors 'chloride' (n = 237 surveys unstressed) and 'bank vegetation' (n = 121 surveys unstressed). Total number of surveys = 283. *** = significant difference (Wilcoxon test) between metric values of stressed and unstressed with $p \le 0.001$; n.s. = no significant difference. The whiskers in the plot represent the 10th and 90th percentile values.

for selecting stressor-specific bioassessment metrics, we identified 24 and three metrics from the linear and non-linear models, respectively.

The results gained from both analyses, however, were hardly comparable. Only one metric was commonly identified by both types of models, yet with ambiguous results. These incomparable results point at fundamental differences between the two analytical techniques. The power of the non-linear *Varpart* to explain the full model and joint fraction variances was up to ten times higher than for the linear *Varpart*. However, the mean explanatory power of the individual fractions was comparable among the analytical techniques. This suggests that the highly efficient BRT algorithm inflates the joint explanatory power of all stressor groups while overruling the signal of individual stressor group effects. We observed this pattern for most of the metrics except for those three presented in Table 3.

Following this particularly strong evidence of individual fractions' explanatory power revealed in the non-linear *Varpart*, we consider two of the three metrics identified in this analysis as genuinely stressor-specific:

- 1. The *Index of Biocoenotic Region*, which represents the invertebrate community related to the longitudinal river zonation (Schmidt-Kloiber and Hering, 2015), responds almost exclusively to hydromorphological stressors in the medium to large lowland rivers (BT4). Its negative relationship with *flow diversity* and *substrate diversity* demonstrates the community shift due to potamalisation effects (Jungwirth et al., 1995).
- 2. The *Relative abundance of alien invertebrate species* seems an ideal stressor-specific metric for the small mid-altitude rivers (BT9): With a high individual fraction and the other fractions being (almost) zero, this metric responds exclusively to physico-chemical impairment. This finding is in line with Früh et al. (2012a, 2016) who described the important role of water quality parameters (esp. *chloride, oxygen concentration* and *water temperature*) to explain the presence of alien species. For medium to large rivers (BT4), however, the occurrence of alien species also relates to hydromorphological stressors (Früh et al., 2012b). This highlights that the ability of bioassessment metrics to provide stressor-specific responses differs among river types. Moreover, stressor-specific bioassessment benefits from a stringent river type definition, as shown by the better performance of the BT9 data subset (see also Von der Ohe and Goedkoop, 2013).

The stressor-specific response of the third metric resulting from the non-linear *Varpart*, the *Croatian Saprobic Index* (Wegl, 1983; Birk and Schmedtje, 2005), is more unclear. Though meeting our selection criteria, the values for the different fractions demonstrate strong effects of all stressor groups on the metric variance. This is confirmed by significant correlations with the single physico-chemical and hydromorphological parameters. Designed to indicate water quality deterioration, the Saprobic Index also responds to hydromorphological stressors because (i) these stressors often entail issues in water quality (e.g. Shields et al., 2010) and (ii) the index refers to taxa being sensitive to oxygen depletion (like Ephemeroptera, Plecoptera and Trichoptera) that are affected by other 'modes of action' as well.

The Croatian Saprobic Index exemplifies the challenge of unveiling stressor-specific bioassessment: We almost always found high joint fractions in our Varpart models, suggesting that the biological community responds to stressors in rather an integrative than a specific way. This applies in particular to the taxonomically and functionally diverse group of benthic invertebrates, the metrics of which showed the highest joint factions in our analyses. In general, two main reasons may provide explanations for our findings:

- 1. Metric design: All metrics are calculated with biological community data. The metrics either directly process the information on the taxonomical composition of the sampled organisms or refer to autecological information assigned to each taxonomic unit identified from the samples. In both cases, species (or lower taxonomic ranks) form the basic 'entities' for bioassessment. The traits of these entities, which imply specific stressor sensitivity, however, are not independent features, but co-occur and associate with other traits (Verberk et al., 2013). This suggests a limited ability of trait-metrics calculated based on biological community data for stressor-specific response, particularly demonstrated for benthic invertebrates (e.g. Pilière et al., 2016; Berger et al., 2018). In this regard, Mondy et al. (2016) point at further investigating into trait combinations and associations to elucidate stressor-specific sensitivities. This may also include calculating trait-metrics not based on the entire biological community (which implies averaging out specific responses) but based on selected taxa only.
- Stressor data: Empirically testing our concept of stressor-specific responses related to independent 'modes of action' would require the

modeling of biological responses to single direct stressors (e.g. *chloride concentration* and *water temperature*). From this perspective, the 'stressor groups' we defined represented heterogeneous amalgamations of different environmental factors modified by anthropogenic activities. Especially hydromorphology comprises a multitude of factors, each with several 'modes of action' affecting different aspects of the ecological niches (e.g. decreasing *flow diversity* entails changes in hydraulic stress and oxygen supply). Truncated stressor gradients resulting from the ubiquitous human influence (especially relevant for the region investigated in our study) and possible spatiotemporal mismatches between the stressor and response data further blur stressor-specific responses.

Despite these fundamental conceptual and pragmatic issues, studies such as Statzner and Bêche (2010), Von Der Ohe and Goedkoop (2013) or Baattrup-Pedersen et al. (2016) provide convincing accounts for stressor-specific biological metrics relevant for aquatic ecosystem assessment. The existence of such metrics across different organism groups and ecosystems was affirmed by the comprehensive approach using linear and non-linear modeling, as well as the metric visualization. Detecting these metrics, however, did not only depend on the criteria defined for stressor-specific response, but also on the analytical (statistical) technique used to unveil this response. While we deem variance partitioning generally appropriate, the choice of the underlying regression approach seems decisive. The high explanatory power yielded from the non-linear analysis is compelling, but the simple linear approach also reveals convincing outcomes (see Fig. 3). The visualization approach thus represents an indispensable auxiliary to ultimately confirm stressor-specificity.

5. Management implications

Stressor-specific bioassessment metrics hold a promise for diagnosing single stressor effects under multi-stressor conditions. Ideally, their response flags the presence of a particular stressor acting on the biology, irrespective of other stressors being active or not. For environmental management this can provide evidence to guide appropriate mitigation measures.

With stipulating the use of different aquatic organism groups in ecological status assessment, the Water Framework Directive implies that each group reacts to different stressors, e.g. autotrophs to nutrient enrichment and heterotrophs to oxygen depletion caused by organic pollution. This view is corroborated by the use of multimetric indices that aim at capturing stressor-specific biological impairment (e.g. Karr and Chu, 1997; Hering et al., 2006b). However, this rather presumptive notion is rarely evidenced, but frequently pursued in support of integrative environmental assessment (Baattrup-Pedersen et al., 2017). Our empirical analysis targeted for stressor-specific metrics suggests two things: (1) Genuinely stressor-specific metrics are more the exception than the norm, and (2) their applicability depends on data-specific features attributable to the biogeographical context (e.g. type and length of stressor gradients, type of river). The latter conclusion is backed by the fact that the findings of Von der Ohe and Goedkoop (2013) and Baattrup-Pedersen et al. (2016) could not be reproduced despite including their metrics in our study.

To support evidence-based, diagnostic decision making in environmental management we recommend identifying suitable stressor-specific bioassessment metrics to be included in routine monitoring programs. These metrics can be determined based on available monitoring data using space-for-time substitution (as done in our study) or time series of adequate length, but their applicability is restricted to specific regions (e.g. river basin districts) reflecting the case-specific circumstance to which these metrics are conditioned.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.09.157.

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