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A multimetric macroinvertebrate index for the assessment of the ecological status of Lithuanian rivers

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A R T I C L E I N F O

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A B S T R A C T

We present the design of the Lithuanian River Macroinvertebrate Index (LRMI) – a Water Framework Directive compliant method for assessing the ecological status of Lithuanian rivers that primarily addresses the eutrophication pressure. It is a multimetric index that averages the ecological quality ratios of two conventional metrics – the Danish Stream Fauna Index (DSFI) and the Average Score Per Taxon (ASPT) – as well as the two newly assembled metrics – the taxonomic richness of Diptera, Ephemeroptera, and Plecoptera (#DEP) and the difference between the pooled relative abundances of Ephemeroptera, Heteroptera, and Plecoptera versus Crustacea and Hirudinea (%EHP–%CrHi). The LRMI showed significant correlations with hydrochemical indicators of nutrient enrichment in the national river dataset. Moreover, our analysis showed its potential sensitivity toward hydromorphological alterations of the riverine habitats. Finally, the results indicated that further developments concerning minor type-specific adjustments of the currently set ecological class boundaries may be beneficial to the new method.

1. Introduction

The EU Water Framework Directive (WFD) requires the member states to achieve high or good status for all types of surface waters (EU, 2000). One of the biological elements required to assess the ecological status of surface waters is benthic macroinvertebrates. It is recommended that the taxonomic composition, abundance, the ratio of disturbance sensitive to insensitive taxa, and community diversity, are all considered (EU, 2000). The resulting macroinvertebrate index, thus usually a multimetric one (Hering et al., 2006), is expressed as an ecological quality ratio (EQR), reflecting the deviation of an observed assemblage from one in reference conditions. The EQR is further applied to assign the assessment unit to one of the five ecological quality classes (high, good, moderate, poor, or bad).

Nutrient enrichment, causing eutrophication and consequent deterioration of the aquatic environment, is among the most widespread pressures affecting the European surface waters (Hilton et al., 2006; Solimini et al., 2006). Primarily due to the diffused pollution from intensive agriculture, eutrophication is also a major threat throughout the Lithuanian rivers (LEPA, 2010). Additionally, decades ago a significant part of these rivers suffered significant hydromorphological alterations, mostly channelization of small and medium streams for field amelioration, which resulted in the physical degradation of the riverine habitats preserving and potentially further aggravating the impacts of eutrophication (LEPA, 2010).

For more than a century, stream macroinvertebrate communities are used to indicate the impacts of eutrophication and organic pollution due to their sensitivity to oxygen depletion resulting from the decomposition of the accumulated organic matter. During more than a decade, the ecological status of Lithuanian rivers was assessed using the Danish Stream Fauna Index (DSFI) (Skriver et al., 2000). However, although this indicator taxa-based metric showed adequate relationships with anthropogenic stressors, it only assessed the aspect of taxonomic composition which is insufficient to the WFD. Secondly, the DSFI alone provides values on a discrete scale (from 1 to 7) resulting in a less accurate assessment in comparison to metrics with a continuous variation. Finally, probably due to substantial regional deviations from the Danish riverine macroinvertebrate assemblages that the DSFI was originally based on, it does not always key out to any estimate in Lithuanian rivers (personal obs.).

As different reference communities may be expected in different physical types of lakes or rivers, the WFD also reasonably calls for type-

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specific assessment methods (EU, 2000). The Lithuanian rivers are generally lowland (< 200 m altitude) calcareous rivers. The national typology (Table 1) therefore primarily differentiates these rivers into small, medium, and large, according to the catchment size (by the 100 and 1000 km^3 limits); then it further splits the medium and large rivers into slow and fast -flowing (by the mean slope of 0.7 m km^{-1} for the medium and 0.3 m km^{-1} for large rivers) (LEPA, 2010). As with the macroinvertebrate method for the Lithuanian lakes (Šidagytė et al., 2013), the method for rivers may be initially designed expecting a universal relationship between the selected community aspects and pressures, while if proven needed, the type-specific reference standards may be implemented as further development.

The primary goal of this study was to design a macroinvertebrate-based method for evaluating the ecological status of Lithuanian rivers concerning eutrophication pressure. Based on the national dataset, we aimed at selecting two widely-approved conventional diversity/sensitivity metrics and assembling two new regional compositional/abundance metrics to be incorporated into the WFD-compliant multimetric Lithuanian River Macroinvertebrate Index (LRMI). We validated the relationship between the resulting LRMI and nutrient enrichment and set the initial boundaries for the five ecological classes. Lastly, to assess the potential sensitivity of the method to modification of riverine habitats and to evaluate the need for type-specific class boundary adjustment, we tested the effects of hydromorphological alteration and river types on the LRMI values.

2. Materials and methods

2.1. Macroinvertebrate data

The assessment method was created using the macroinvertebrate data collected during the 2010–2013 period by the Lithuanian Environmental Protection Agency (LEPA) as a result of the national monitoring programme. The quantitative samples were collected using the multihabitat method. Within a site, 10 subsamples (later to be pooled into one sample) were collected from all available microhabitats proportionally to their estimated distribution. Each subsample was collected using the kick method by disturbing the 40 cm length area in front of the standard handnet (25 × 25 cm opening, 0.5 mm mesh size), resulting in 0.1 m^2 of the bottom area sampled per subsample, and 1 m^2 per the whole sample. Additional qualitative samples were collected by searching for macroinvertebrates attached to underwater objects (roots, stones, plants, etc.). The taxa found in these qualitative samples were added to the taxa lists with a quantity of 1 specimen if not discovered in the main quantitative sample.

Operational macroinvertebrate identification levels are provided in Table 2. We mostly employed species-level data, but for some taxonomically complicated groups, such as Coleoptera (genus), Diptera (family), and Oligochaeta (class), higher taxonomic levels were used as a result of taxonomic harmonisation between samples. Some macroinvertebrates (Aranea, Hydracarina, as well as some families of other groups indicated in Table 2) were excluded from the taxa lists before any calculations due to their questionable attribution as fully aquatic or macroscopic animals, potentially leading to the inconsistent collection during the sample sorting procedure. Twelve major macroinvertebrate groups (found in at last 300 samples and in a total abundance of at least 8000 specimens throughout the dataset) were tested for their reaction to nutrient enrichment (see further). To ensure data quality, macroinvertebrate samples containing less than 100 macroinvertebrate specimens were excluded from our study, shrinking our dataset from 949 to 792 samples.

Table 1
National typology of Lithuanian rivers (LEPA, 2010).

Ecoregion	Altitude, m	Geology	Catchment area of stretch, km^2	Slope, m km^{-1}	Type
Baltic	< 200	Calcareous	< 100		1
			100–1000	< 0.7	2
			100–1000	> 0.7	3
			> 1000	< 0.3	4
			> 1000	> 0.3	5

Table 2
Macroinvertebrate groups generally considered when calculating the Lithuanian River Macroinvertebrate Index, with their operational identification levels and excluded families. Note that Aranea and Hydracarina were completely excluded. Asterisks denote major macroinvertebrate groups tested for their reaction toward nutrient enrichment.

Macroinvertebrate group	Identification level	Excluded taxa	Reason for exclusion
Turbellaria	Species		
Oligochaeta*	Class		
Hirudinea*	Species		
Bivalvia*	Species		
Gastropoda*	Species	Succineidae, Zonitidae	Not truly aquatic
Crustacea*	Species	Ostracoda	Not macroscopic
Plecoptera*	Species		
Ephemeroptera*	Species		
Odonata*	Species		
Heteroptera*	Species	Gerridae, Hydrometridae, Mesoveliidae, Veliidae	Not truly aquatic
Megaloptera	Species		
Neuroptera	Species		
Coleoptera*	Genus	Chrysomelidae, Curculionidae	Not truly aquatic
Trichoptera*	Species		
Lepidoptera	Species		
Diptera*	Family		

2.2. Hydrochemical indicators of nutrient enrichment

We used corresponding annual averages of 9 hydrochemical parameters – total phosphorus (Total P), phosphate phosphorus (PO_4 P), total nitrogen (Total N), mineral nitrogen (Mineral N), nitrate nitrogen (NO_3 N), nitrite nitrogen (NO_2 N), ammonium nitrogen (NH_4 N), biochemical oxygen demand for 7 days (BOD_7), and dissolved oxygen (Dissolved O_2) - as indicators of river nutrient enrichment. These data were also collected as a result of the national monitoring programme and are publicly available from the LEPA website (available at https://aaa.lrv.lt/lt/veiklos-sritys/vanduo/upes-ezerai-ir-tvenkiniai/valstybinis-upiu-ezeru-ir-tvenkiniu-monitoringas/upiu-monitoringo-rezultatai). The descriptive statistics of studied trophic gradients are provided in Table 3. For an additional integrated indicator of nutrient enrichment (PCA axis 1), we also extracted the point coordinates on the first axis of the correlation matrix-based Principal Component Analysis

Table 3
Descriptive statistics of hydrochemical indicators of nutrient enrichment within the river study dataset (n = 792): M_e – median, M_u – mean, Q_1 and Q_3 – lower and upper quartiles, Min and Max – lowest and highest values.

Parameter	Min	Q_1	M_e	M_u	Q_2	Max
Total P, mg L^{-1}	0.022	0.055	0.075	0.099	0.111	0.684
PO_4 P, mg L^{-1}	0.005	0.023	0.036	0.054	0.060	0.552
Total N, mg L^{-1}	0.369	1.493	2.289	2.937	4.070	14.680
Mineral N, mg L^{-1}	0.164	0.832	1.367	1.970	2.763	14.034
NO_3 N, mg L^{-1}	0.123	0.749	1.212	1.816	2.564	10.762
NO_2 N, mg L^{-1}	0.003	0.010	0.015	0.020	0.027	0.208
NH_4 N, mg L^{-1}	0.014	0.053	0.077	0.128	0.115	3.850
BOD_7, mg L^{-1}	1.085	1.890	2.400	2.550	2.981	7.075
Dissolved O_2, mg L^{-1}	4.393	8.113	9.001	8.870	9.661	12.375

(PCA) of the 9 hydrochemical parameters.

2.3. Hydromorphological state of habitats

Our main goal for the LRMI was sensitivity toward nutrient enrichment, therefore to avoid confounding effects, only the macroinvertebrate data from hydromorphologically unaltered river habitats, i.e. sites devoid of significant river channel, shore morphology, or natural water flow regime alterations, were used to test (and formally validate) these relationships. Thus, for the design of the LRMI, we shrunk our dataset further down to 539 samples based on expert data. According to such judgement, 205 samples came from hydromorphologically altered habitats, and 48 samples with missing data on hydromorphological habitat state were not included in either group.

2.4. Selection and construction of the core LRMI metrics

We selected the core LRMI metrics based on their relationships with nutrient enrichment tested as Spearman correlations against the hydrochemical indicators of nutrient enrichment within the dataset of hydromorphologically unaltered habitats. We aimed at four metrics of distinct character, each reflecting a unique aspect of the macroinvertebrate community and thereby altogether satisfying all the metric requirements imposed by the WFD. We first chose two out of conventional macroinvertebrate metrics, considering the Simpson, Shannon, Pielou, and Margalef diversity indices; total taxa richness; the previously used DSFI (Danish Stream Fauna Index), (Skriver et al., 2000); the BMWP (Biological Monitoring Working Party score), and its derivative ASPT (Average Score Per Taxon) (Armitage et al., 1983). As for the remaining two metrics, we intended to include the taxa richness and the relative abundance, each pooled across the three major macroinvertebrate groups established as sensitive based on our correlation analyses.

2.5. Reference values and combination of the core LRMI metrics

Each of the four selected macroinvertebrate metrics was then standardised as an EQR using the following formula (Hering et al., 2006):

$$EQR = \frac{\text{observed value - lower anchor}}{\text{reference value - lower anchor}}.$$

While the lower anchors were set as the theoretically lowest possible metric values, the reference values were derived as 90% percentiles from the subdataset coming from the reference sites. The criteria for attribution of a site to reference conditions were as follows: (1) site is natural, without substantial alterations (classification from LEPA), (2) no hydromorphological alterations (expert data as described above), (3) no intensive agriculture in the vicinity (expert data), and (4) high ecological status according to national classification based on hydrochemical indicators (LEPA, 2010), identified at Total P < 0.1 mg L^{-1}, PO_4 P < 0.05 mg L^{-1}, Total N < 2.0 mg L^{-1}, NO_3 N < 1.3 mg L^{-1}, NH_4 N < 0.1 mg L^{-1}, $BOD_7 < 2.3$ mg L^{-1}, Dissolved $O_2 > 8.5$ mg L^{-1} for type 1 and 3-5 and > 7.5 mg L^{-1} for type 2 streams. Within the reference subdataset of 87 samples in total, 19 samples came from type 1, 17 – from type 2, 28 – from type 3, 9 – from type 4, and 14 – from type 5 river sites.

Finally, the four selected core macroinvertebrate metrics were combined into the LRMI by calculating the arithmetic average of the EQRs of these metrics. In cases when the DSFI failed to key out, we averaged only the EQRs of the remaining three metrics. We used Pearson correlations to validate the resulting LRMI against the hydrochemical indicators of nutrient enrichment across the whole sample dataset.

2.6. Ecological class boundaries of the LRMI

We established the initial LRMI class boundaries using the mean quartile method – as the means between the lower quartile of the superior class and the higher quartile of the adjacent inferior class based on the established hydrochemical classification (LEPA, 2010) of samples from the hydromorphologically unaltered sites. Using the high/good boundary established using this method as a reference, we also applied the equidistance method to establish the alternative lower class boundaries – as values equally dividing the zero–high/good range into four equal intervals (Johnson et al., 2013). However, for the application in Lithuania, we raised the high/good boundary and established the lower boundaries in between the results of the two discussed methods. The resulting high/good and good/moderate boundaries were supported by the inter-calibration exercise (Šidagytė and Arbačiauskas, 2015). Nevertheless, based on our current analysis of the river type effect, further introduction of slightly diverging type-specific class boundaries may be recommended in the future (see Results and Discussion).

2.7. Hydromorphology effects on the LRMI

To test the effects of hydromorphological alteration and river type on the LRMI, we used a general linear model. As the independent variables we included the integrated hydrochemical indicator of nutrient enrichment (PCA axis 1) as the main covariate (Nutrients – continuous variable), and then the two factors of interest: the hydromorphological state of the habitat (Alteration – 2-level factor: altered/unaltered) and the river type (Type – 5-level factor: type 1 / type 2 / type 3 / type 4 / type 5). Initially, we included the interactions up to the third order and then based on type 3 test results we removed the insignificant interactions following the hierarchical order. We then applied Tukey posthoc testing to investigate the underlying differences among the Type factor levels within the hydromorphologically unaltered habitats as a basis for adjustment of type-specific class boundaries.

2.8. Technical notes regarding statistical analyses

All of the hydrochemical indicators of nutrient enrichment except for Dissolved O_2 were log-transformed before the PCA and Pearson correlations to normalise their distribution and linearise the relationships. All statistical analyses were performed in the R v. 4.0.5 environment (R Core Team, 2021). The PCA was conducted using the packages "FactoMineR" v. 2.4 (Le et al., 2008) and "factoextra" v. 1.0.7 (Kassambara and Mundt, 2020). The posthoc testing was enabled by the package "emmeans" v. 1.7.5 (Lenth, 2022). The correlation matrices were produced using the package "corrplot" v. 0.92 (Wei and Simko, 2021). The partial residual plots of the general linear model were created using the package "visreg" v. 2.7.0 (Breheny and Burchett, 2017).

3. Results

3.1. PCA of hydrochemical indicators

The PCA axis 1 explained 54% of the variance and reflected the gradient between the general nutrient (N and P) enrichment vs. good oxygen conditions (Fig. 1). Point coordinates along the PCA axis 1 were thus further used as an integrated hydrochemical indicator of nutrient enrichment. The PCA axis 2 explained 19% of the variance and appeared to reflect the gradient between the wastewater (BOD_7 and P enrichment) and the agricultural pollution (N enrichment). No clearly pronounced clustering of the samples by site hydromorphological state or river type emerged in the PCA biplots (Fig. 1A–B).

3.2. Selection of the conventional core LRMI metrics – DFSI and ASPT

The correlation matrix used to select the first two metrics of the LRMI is provided in Fig. 2. Surprisingly, the three diversity indices – Simpson, Shannon, and Pielou – barely correlated to any of the hydrochemical

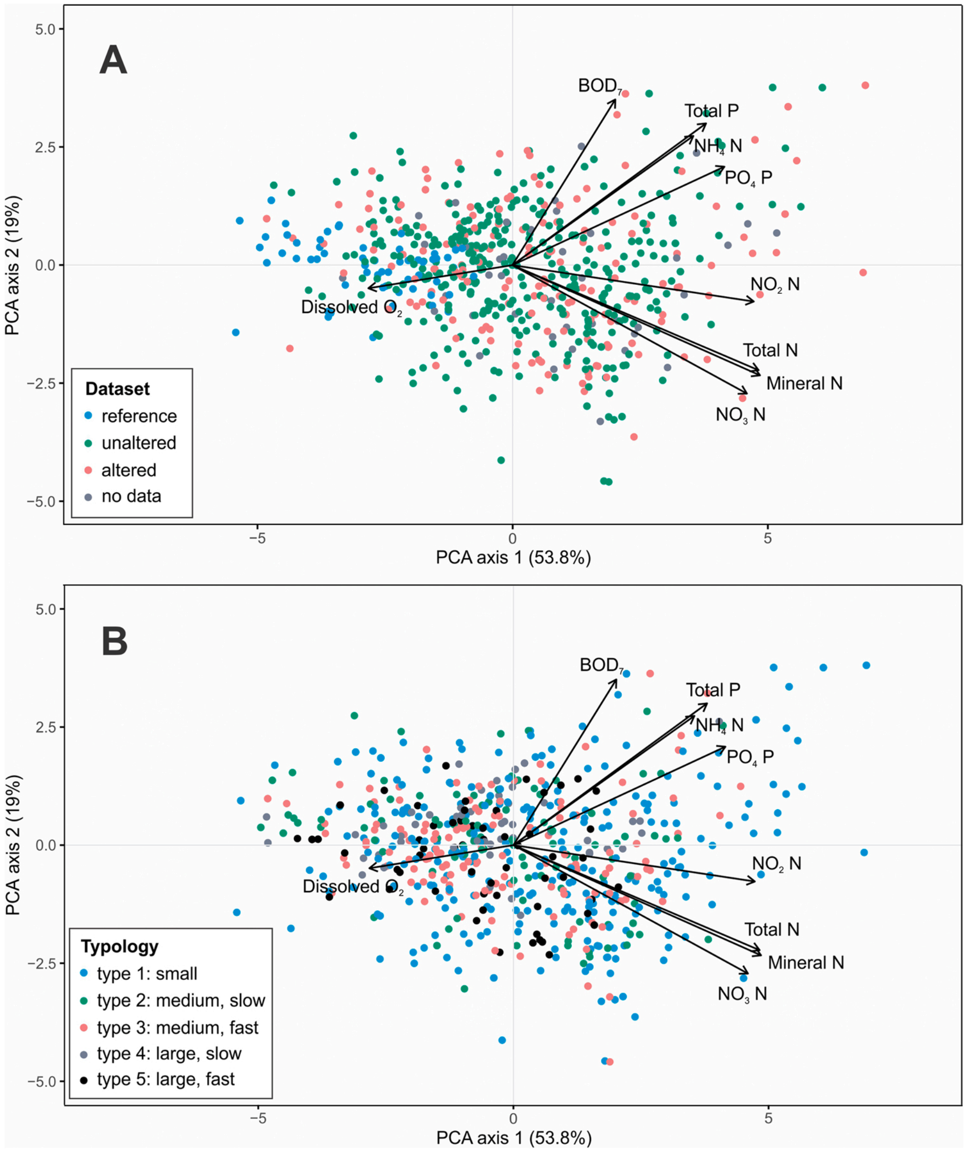

Fig. 1. Biplots of the PCA (axes 1 and 2) of the hydrochemical indicators of nutrient enrichment, showing sample grouping (A) by dataset: samples from reference conditions (blue), non-reference hydromorphologically unaltered (green) and altered (red) habitats, and samples lacking data of site hydromorphological state (grey); (B) by national river type: type 1 (blue), type 2 (green), type 3 (red), type 4 (grey), type 5 (black).

indicators of nutrient enrichment. Meanwhile, the negative responses of the Margalef diversity and the total taxa richness were stronger. The DSFI, BMWP, and ASPT had the most robust overall correlations to nutrient enrichment, with the Spearman coefficients ranging between –0.19 and –0.41, and between 0.20 and 0.29 for Dissolved O_2. Thus we opted for the DSFI and the ASPT as the two first core metrics of the LRMI, as the latter metric had the highest overall correlations and the best correlation with the integrated indicator, and the DSFI is an index of a substantially different character.

3.3. Assembly of a new core LRMI metric of taxonomic richness – #DEP

From the taxonomic richness metrics of the major macroinvertebrate groups, the plecopteran species richness showed the strongest negative relationships with nutrient enrichment (from –0.21 to –0.38, and 0.30 for Dissolved O_2), followed by the ephemeropteran species richness (from –0.12 to –0.27, 0.16 for Dissolved O_2), and then by the dipteran family richness (from –0.12 to –0.26, 0.28 for Dissolved O_2) (Fig. 3A). Thus, the newly assembled candidate metric for the LRMI reflecting the pooled taxonomic richness of sensitive groups was denoted the #DEP. Although the pooled #DEP metric showed slightly weaker correlations with the hydrochemical parameters (from –0.19 to –0.36, 0.28 for Dissolved O_2) than the plecopterans alone (Fig. 3A–B), we preferred the composite metric due to its few-fold wider variation (even in reference environments stonefly richness in Lithuanian streams does not exceed 3–5 species).

There were also macroinvertebrate groups with their species richness showing considerable positive relationships with nutrient enrichment (Fig. 3A). Among these were Crustacea (from –0.08 to 0.40, –0.34 for Dissolved O_2) and Hirudinea (from –0.08 to 0.14, –0.14 for Dissolved O_2). We attempted to incorporate this information by subtracting the pooled species richness of these groups from the #DEP metric (#DEP-#CrHi). Such modification resulted in slightly better overall correlations with nutrient enrichment (from –0.17 to –0.41, 0.36 for Dissolved O_2), but weakened the relationships with the Total P and BOD_7 (Fig. 3B). It also only minutely improved the normality of the already numerically-suitable metric distribution (Fig. 3C–D). Taking into account that a similar approach was indeed necessary for the newly assembled relative abundance metric (see further), in the end, we opted for the simpler form of the #DEP, to keep the core metrics of the LRMI as different as

	Total P	PO_4 P	Total N	Mineral N	NO_3 N	NO_2 N	NH_4 N	BOD_7	Dissolved O_2	PCA axis 1
Simpson diversity	−0.01	−0.08	0.03	0.03	0.02	0.10	0.02	0.04	−0.18	0.04
Shannon diversity	−0.05	0.04	−0.06	−0.07	−0.06	−0.14	−0.05	−0.12	0.19	−0.09
Pielou diversity	0.13	0.18	0.04	0.06	0.06	−0.01	0.04	0.12	0.12	0.07
Margalef diversity	−0.26	−0.15	−0.09	−0.13	−0.12	−0.17	−0.15	−0.38	0.07	−0.19
Taxa richness	−0.22	−0.14	−0.17	−0.2	−0.18	−0.25	−0.15	−0.35	0.19	−0.25
DSFI	−0.22	−0.19	−0.25	−0.24	−0.23	−0.31	−0.22	−0.36	0.20	−0.31
BMWP	−0.25	−0.20	−0.26	−0.28	−0.26	−0.32	−0.23	−0.41	0.21	−0.34
ASPT	−0.24	−0.23	−0.34	−0.34	−0.33	−0.35	−0.25	−0.32	0.29	−0.39

Fig. 2. Coefficients of Spearman correlations between the conventional macroinvertebrate metrics and the hydrochemical indicators of nutrient enrichment within the dataset of samples from the hydromorphologically unaltered habitats ($n = 539$). Significant coefficients ($p < 0.05$) are indicated by colour: red – negative, blue – positive. Colour intensity reflects correlation strength.

possible.

Interestingly, the trichopteran species richness, as well as the coleopteran genera richness, which are both conventionally considered as rather sensitive groups, did not correlate too well with most indicators apart from the BOD_7 (Fig. 3A). The species richness of Heteroptera and Odonata, however, showed some potential as sensitive groups, while no clear patterns emerged for the Oligochaeta presence or the species richness of Bivalvia and Gastropoda.

3.4. *Assembly of a new core LRMI metric of relative abundance – %EHP-%CrHi*

Similarly to the taxonomic richness analysis, among the relative abundances of the major groups, Plecoptera showed the strongest negative relationships with nutrient enrichment (from –0.22 to –0.37, and 0.29 for Dissolved O_2) (Fig. 3E). The next two groups with strongest negative reactions were Heteroptera (from –0.15 to –0.26, 0.22 for Dissolved O_2) and Ephemeroptera (from –0.11 to –0.22, 0.11 for Dissolved O_2). Therefore, we initially termed the newly assembled candidate metric reflecting the pooled relative abundance of sensitive groups as the %EHP. Again, the pooled metric correlated with the hydrochemical indicators a little weaker (from –0.18 to –0.30, 0.19 for Dissolved O_2) than Plecoptera alone (Fig. 3E–F), and the distribution of the metric was still not numerically suitable (Fig. 3G) (usually these taxa comprise only a small proportion in the macroinvertebrate assemblage).

We thus modified the initial %EHP metric by subtracting the pooled relative abundance of groups showing significant positive reactions to nutrient enrichment, namely again Crustacea (from –0.01 to 0.43, –0.38 for Dissolved O_2) and Hirudinea (from 0.03 to 0.26, –0.22 for Dissolved O_2) (Fig. 3E), to obtain the final core metric %EHP-%CrHi. This resulted in the strengthening of the correlation to the hydrochemical indicators (from –0.15 to –0.41, 0.30 for Dissolved O_2) (Fig. 3F), as well as extensively improved the normality of the metric distribution (Fig. 3G–H).

Regarding the other groups, as opposed to the taxonomic richness analysis, the dipteran relative abundance did not indicate any significant reaction to nutrient enrichment. Lastly, the relative abundances of Trichoptera, Coleoptera, and Odonata, traditionally regarded as sensitive groups, as well as Oligochaeta, Bivalvia, and Gastropoda, showed barely any responses.

3.5. *The LRMI, its response to nutrient enrichment, and class boundaries*

The reference values of the four core metrics of the LRMI suggested by our analyses – the DSFI, ASPT, #DEP, and the %EHP-%CrHi – were derived as 90% percentiles from the reference sample dataset, and their lower anchors were established as the theoretically possible lowest values (Table 4). This allowed for calculating the EQR of each metric and then averaging them into the LRMI. The resulting multimetric index demonstrated significant responses to nutrient enrichment, validated across the whole dataset as the Pearson correlations against the hydrochemical indicators (Fig. 4). Including only the samples from the hydromorphologically unaltered habitats resulted in slightly different correlation coefficients: –0.27 for Total P, –0.25 for PO_4 P, –0.38 for Total N, –0.37 for Mineral N, –0.34 for NO_3 N, –0.40 for NO_2 N, –0.30 for NH_4 N, –0.35 for BOD_7, 0.33 for Dissolved O_2, and –0.44 for the PCA axis 1).

The distributions of the LRMI values across the hydrochemical status classes using the data only from the hydromorphologically unaltered habitats and also across the whole dataset are provided in Fig. 5. The class boundaries established using the mean quartile and the equidistance methods, as well as the currently used values modified based on expert judgement, are provided in Table 5.

3.6. *Effects of hydromorphology on the LRMI*

After removing the insignificant interaction terms from our linear model testing for the effects of nutrient enrichment, hydromorphological alteration and river-type effects on the LRMI values, we obtained the results provided in Table 6. All the main effects were significant, and the only remaining significant interaction term was Alteration × Type. Such results firstly indicated that the LRMI may be substantially sensitive not only to nutrient enrichment but also to the physical alteration of the riverine habitat. Secondly, there may be type-specific offsets to the LRMI relationship with nutrients. And lastly, the pattern of these offsets may be different within the physically altered and unaltered habitats (Fig. 6). Interestingly, while generally, the LRMI values were lower in the altered habitats, the opposite pattern was observed in type 5 (large, fast-flowing) rivers.

The least-square means within the hydromorphologically unaltered habitat data were as follows: 0.65 for type 1, 0.64 for type 2, 0.70 for type 3, 0.60 for type 4, and 0.63 for type 5, and posthoc tests indicated a significant exclusion of type 3 (medium, fast-flowing) rivers ($p \leq 0.01$). When considering the deviations from the general mean (0.65), which was well-reflected by the means of type 1–2 rivers, modifications for the LRMI class boundaries for type 3–5 rivers could be considered. Type-specific boundaries roughly proposed according to these results (offsets 0.05 and –0.05 for type 3 and types 4–5, respectively) are appended to Table 5.

4. Discussion

In this work, we present the design of the WFD-compliant macroinvertebrate-based method for assessing the ecological status of Lithuanian rivers, aimed at the regionally most relevant eutrophication pressure. As broadly recognised, it contains an inherently flexible multimetric index bearing the ability to reflect different aspects of the community (Solimini et al., 2006; Trigal et al., 2009; Gabriels et al., 2010; Menetrey et al., 2011). To this point, the new Lithuanian River Macroinvertebrate Index (LRMI) averages the EQRs of four equally weighted metrics: the widely-approved Average Score Per Taxon (ASPT) (Armitage et al., 1983) and the Danish Stream Fauna Index (DSFI), (Skriver et al., 2000) as well as the two new metrics assembled using our regional dataset, namely the taxonomic richness of Diptera, Ephemeroptera, and Plecoptera (#DEP) and the difference between the pooled relative abundances of Ephemeroptera, Heteroptera, and Plecoptera versus Crustacea and Hirudinea (%EHP-%CrHi). Across the national

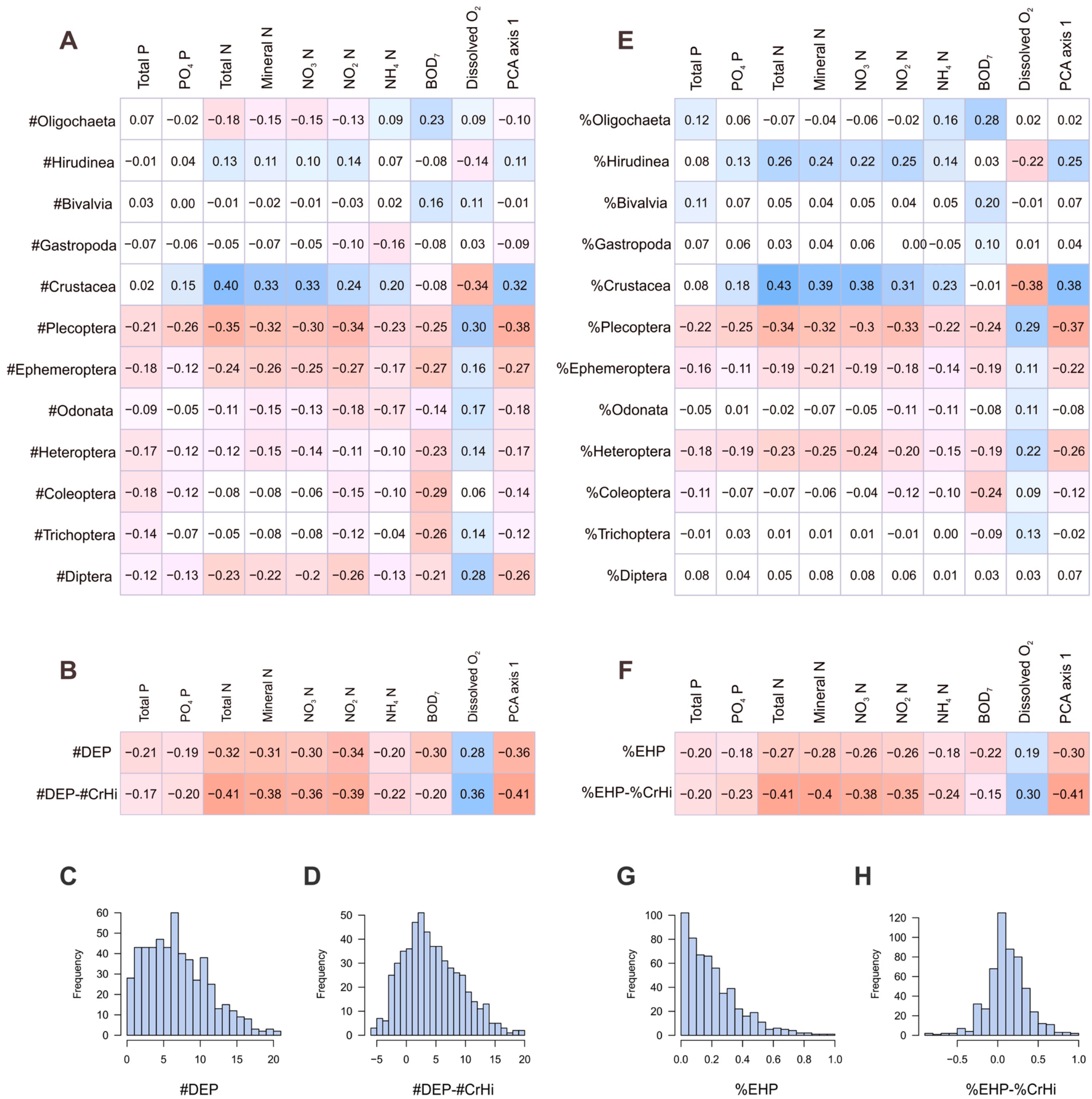

Fig. 3. Assembly and selection of the pooled taxonomic richness and relative abundance macroinvertebrate metrics within the dataset of samples from the hydromorphologically unaltered habitats (n = 539): coefficients of Spearman correlations for the taxonomic richness (A) and relative abundances (E) of the major macroinvertebrate groups, and the assembled candidate metrics of pooled taxonomic richness (B) and relative abundance (F) against the hydrochemical indicators of nutrient enrichment; distributions of the assembled candidate metrics of pooled taxonomic richness (C–D) and relative abundances (G–H). Significant coefficients ($p < 0.05$) are indicated by colour: red – negative, blue – positive. Colour intensity reflects correlation strength.

dataset, the LRMI demonstrated significant correlations with hydrochemical indicators of nutrient enrichment, thus we deem it applicable for the detection of the eutrophication stress in the Lithuanian rivers.

4.1. The core metrics of the LRMI

One of the interesting findings of our study was the ineffectiveness of the various conventional diversity indices to detect the eutrophication stress across Lithuanian rivers. The Shannon Diversity in the mathematical form of the effective number of taxa, showing relatively well-defined relationships with nutrient enrichment, was selected as one of the four core metrics for the Lithuanian Lake Macroinvertebrate Index (Šidagytė et al., 2013). However, the current study illustrated that the macroinvertebrate community may relate to the stress gradients very differently depending on the type of aquatic ecosystem. Thus, to reflect the diversity aspect of the macroinvertebrate assemblage within the LRMI, we opted for the DSFI metric, which showed one of the most decent correlations to nutrient enrichment. This was as a logical progression given its strict intention for river assemblages and previous use as the only measure of the ecological status of Lithuanian rivers (Višinskienė and Bernotienė, 2012). On the other hand, the ASPT, showing correlations with nutrient enrichment of similar levels in both types of ecosystems, appeared to be a rather universal metric, now selected for use in both, the Lithuanian lake and river methods, to reflect the assemblage sensitivity aspect (Šidagytė et al., 2013).

Our novel #DEP metric is a modification of the conventional #EPT metric, with the replacement of the trichopteran species with the dipteran family richness. Despite the rather vast species diversity in Lithuanian waters (Višinskiene, 2009), the trichopteran species richness (as well as the relative abundance), did not show good correlations with

Table 4
The core metrics within the Lithuaninan River Macroinvertebrate Index, and their reference values.

Abbreviation	Description	Reference value	Lower anchor
DSFI	Danish Stream Fauna Index (Skriver et al., 2000)	7	1
ASPT	Average Score Per Taxon of original BMWP system (Armitage et al., 1983)	7.0	1.0
#DEP	Taxa richness of Diptera (families), Ephemeroptera and Plecoptera (species)	15	0
%EHP–%CrHi	Difference between the pooled relative abundance of Ephemeroptera, Heteroptera, and Plecoptera vs. Crustacea and Hirudinea	0.6	–1.0

nutrient enrichment in lakes as well (Šidagytė et al., 2013). On the other hand, the Lithuanian lake method uses the analogous #CEP metric, which instead of Diptera families considers the diversity of Coleoptera genera as this group reacted the strongest to eutrophication (Šidagytė et al., 2013). However, while the Coleoptera genera richness showed a weak reaction to nutrient enrichment in the current study, the assembly process of the lake analogue did not even involve testing Diptera. Thus, it remains to be answered if the dipteran family richness could be a better indicator of deterioration in lakes as well.

Perhaps the most intriguing result of this study is the assembly of the %EHP-%CrHi metric. While the use of the relative abundances of the Ephemeroptera and Plecoptera, universally perceived as sensitive groups in aquatic biomonitoring, is not unheard of, the discovered sensitivity of Heteroptera, as well as the tolerance of Crustacea and Hirudinea deserves some elaboration. Firstly, it has to be reminded that the Heteroptera here refers only to the Nepomorpha subdivision (divers) as the Gerromorpha families (striders) were removed from taxa lists (see Table 2). Even despite this exclusion, it appeared that the discovered relationships were driven by the separate families within these groups, as logically reflected by their sensitivity scoring in the BMWP system. Among the most common and abundant true bugs in our dataset, the Aphelocheiridae (10 BMWP points; Armitage et al., 1983) noticeably dominated, further followed by the Corixidae (5 points). In the case of the crustaceans, it was mostly Asellidae (3 points) and Gammaridae (6 points), while among leaches Erpobdellidae and Glossiphoniidae (both 3 points) stood out. However, fully understanding the responses of these groups, and also the potential role of alien crustaceans within these responses (Arbačiauskas et al., 2011a, 2011b), warrants a separate analysis on a finer taxonomic resolution (family and/or species).

Regarding the mathematical form of the %EHP-%CrHi, we were aware that instead of counterbalancing the relative abundance of sensitive taxa by subtracting the same for the tolerant taxa to achieve better numerical suitability, it is probably more often chosen to use the ratio of the two groups or to simply log-transform the relative abundance of the sensitive groups. However, both of these commonly applied procedures are technically more complicated. For example, if, theoretically, no tolerant taxa are encountered, the ratio will result in division by zero; or, if log-transformation is applied, the added arbitrary constant has to be decided upon.

4.2. Macroinvertebrate assessment of other pressures in Lithuanian rivers

Our analysis of the effect of hydromorphological alteration on the LRMI indicated that this metric was affected by physical habitat degradation, usually negatively. However, in type 5 (large, fast-flowing) rivers, the samples from such degraded habitats even got higher LRMI estimates than ones from unaffected habitats. This could be related to the heterogeneous character of available hydromorphological alterations. The significant interaction between typology and hydromorphological alteration effect on the LRMI may suggest that the new index has the potential to also indicate the channelization pressure, which is more characteristic of the small and medium (type 1–3) rivers (LEPA, 2010). However, its ability to indicate community disturbance by hydropeaking, which often affects larger (type 3–5) rivers, may be limited and require another assessment method. An elaborated analysis is warranted to clarify this matter.

In addition to eutrophication and hydromorphological alterations, rivers, especially the large ones, may also be heavily impacted by alien macroinvertebrate species, or biocontamination (Arbačiauskas et al., 2008, 2011a). Non-indigenous macroinvertebrate species (NIMS) can heavily transform the resident macroinvertebrate assemblages and consequently distort the estimates of ecological status (Kelly et al., 2006; Arbačiauskas et al., 2008, 2011a; MacNeil et al., 2010). Differentiation of pressures between eutrophication, hydromorphological alteration, and biocontamination is generally approved by most EU member states (Cardoso and Free, 2008; Vandekerkhove and Cardoso, 2010). Therefore, the presence of NIMS is to be assessed in parallel to ecological status, for which the Fauna Autochthony Index (FAI) developed for the evaluation of Lithuanian lakes (Šidagytė et al., 2013) may be easily applied. It remains to be investigated whether and how the LRMI is affected by NIMS presence. It was not the case with the multimetric Lithuanian lake method (Šidagytė et al., 2013), however, the lakes are expected to be significantly less biocontaminated than the rivers.

4.3. Type-specific prospects of the LRMI

After establishing the LRMI ecological class boundaries using both mean quartile and then equidistance methods, primarily based on the hydrochemical classification of sites, we used expert judgement to obtain a more natural distribution of the Lithuanian samples. This has resulted in even more elevated high/good and good/moderate class boundaries, as well as moderate/bad and bad/poor boundaries in between the values given by the two methods. Currently, the LRMI method with the resulting 0.8/0.6/0.4/0.3 class boundaries is applied to all Lithuanian rivers uniformly, and the highest boundaries are already intercalibrated with the other countries of the Central-Baltic region (Šidagytė and Arbačiauskas, 2015).

Although the WFD requires setting diverging standards for different types of rivers (EU, 2000), our analysis showed that, if such modifications to class boundaries were to be made, they would be minor. Our current analysis indicated that the boundaries for types 1 and 2 (small and medium slow-flowing rivers) reflect the general mean and would not need to be adjusted. According to the differences in the predicted means, all the boundaries for type 3 (medium, fast-flowing) rivers could be raised by 0.05, while for types 4 and 5 (all large rivers) they could be by 0.05 lower. Such a pattern of highest community standards in the medium fast-flowing rivers may be expected as these rivers combine the widest range of available physical habitats (which could be considered as an ecotone between small and large rivers) with the best oxygen conditions. This is in line with the River Continuum Concept (Vannote et al., 1980) and generalisation of field studies suggesting that the highest macroinvertebrate richness is typical of mid-order streams (Clarke et al., 2008).

5. Conclusion

Our newly developed multimetric LRMI is already applied for the national monitoring programme of Lithuanian rivers, and it is expected to be a more robust and informative indicator of the eutrophication stress than the previously applied method of the single DSFI metric. Being a flexible multimetric index, the LRMI can be easily updated in line with further accumulating data. It can be developed by adjusting the ecological class boundaries for different river types as suggested by this study, by including new types of metrics, especially those based on

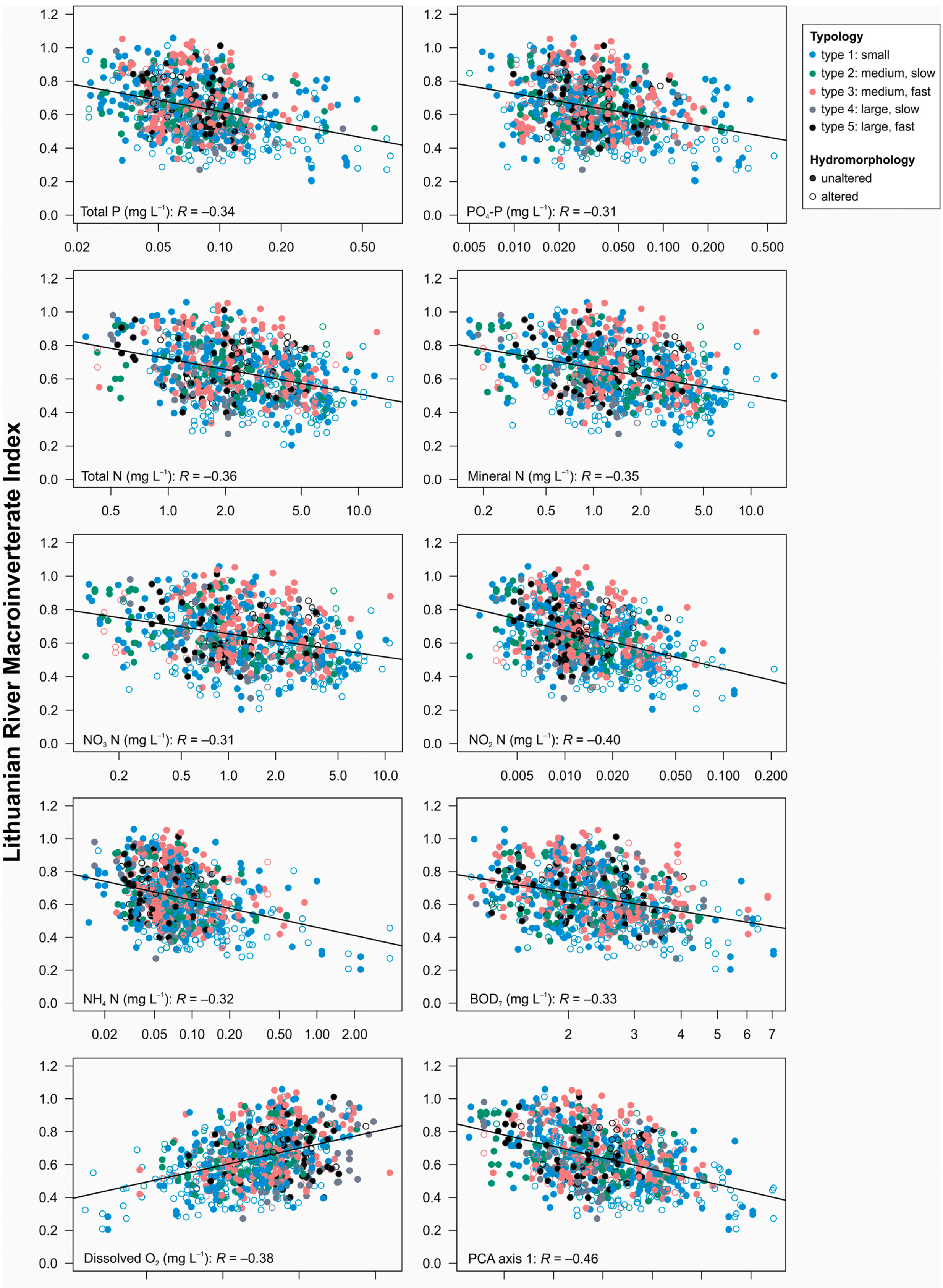

Fig. 4. Relationships between the Lithuanian River Macroinvertebrate Index and the hydrochemical indicators of nutrient enrichment (n = 792). Note the log-transformation of the x-axis in most plots. The shown Pearson correlation coefficients (*R*) are all significant at $p < 0.001$. Point colours reflect the national typology (see Table 1): type 1 (blue), type 2 (green), type 3 (red), type 4 (grey), type 5 (black). Different point symbols denote sample grouping by habitat hydromorphological state: unaltered (closed) and altered (open).

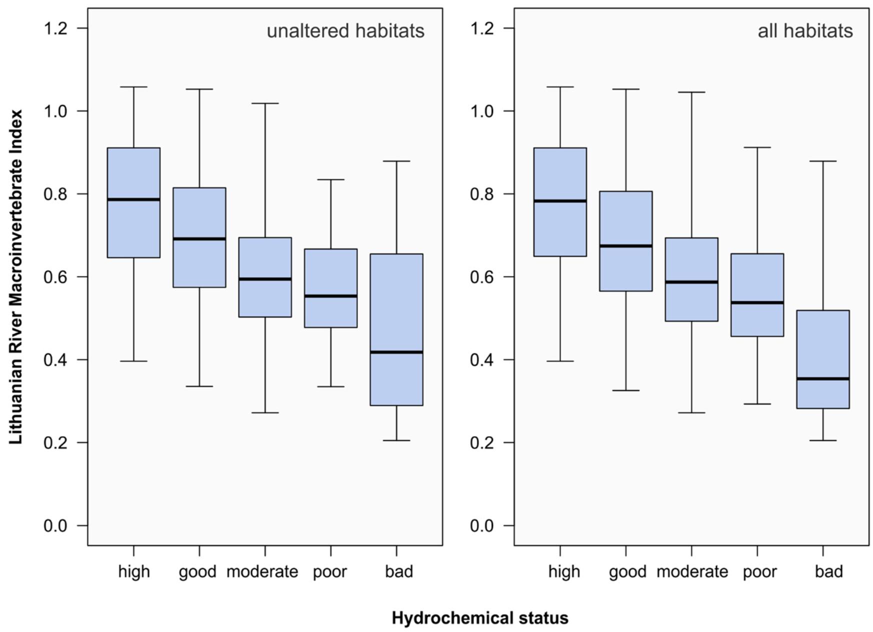

Fig. 5. Variation of the Lithuanian River Macroinvertebrate Index (median, quartiles, minimum-maximum) by hydrochemical status class within the dataset of samples from the hydromorphologically unaltered habitats (left: n = 539) and within the whole dataset (right: n = 792).

Table 5
Ecological class boundaries of the Lithuanian River Macroinvertebrate Index established using the mean quartile method, the equidistance method (within the dataset of hydromorphologically unaltered habitats), modified by expert judgement and currently used (in the bold script), and proposed type-specific adjustments based on our analysis (in the italic script). Asterisks denote intercalibrated boundary values.

Source \ Boundary	High / Good	Good / Moderate	Moderate / Poor	Poor / Bad
Mean quartile	0.73	0.63	0.59	0.56
Equidistance	0.73	0.55	0.37	0.18
Current	**0.80***	**0.60***	**0.40**	**0.30**
Proposed, types 1–2	*0.80*	*0.60*	*0.40*	*0.30*
Proposed, type 3	*0.85*	*0.65*	*0.45*	*0.35*
Proposed, types 4–5	*0.75*	*0.55*	*0.35*	*0.25*

Table 6
Analysis of variance results (type 1 tests) for the reduced general linear model testing for the effects of nutrient enrichment (Nutrients), hydromorphological alteration of habitat (Alteration) and river type (Type) on the values of the Lithuanian River Macroinvertebrate Index (insignificant interaction terms were removed).

Term	*df*	*F*	*p*
Nutrients	1	215.8	< 0.0001
Alteration	1	11.8	< 0.001
Type	4	6.8	< 0.0001
Alteration × Type	4	3.7	0.005
Residuals	735		

macroinvertebrate functional traits (e.g. Usseglio-Polatera et al., 2000; Bady et al., 2005), or by differentiating the weights of different metrics for indication of more relevant pressure of each river type.

Declaration of Competing Interest

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

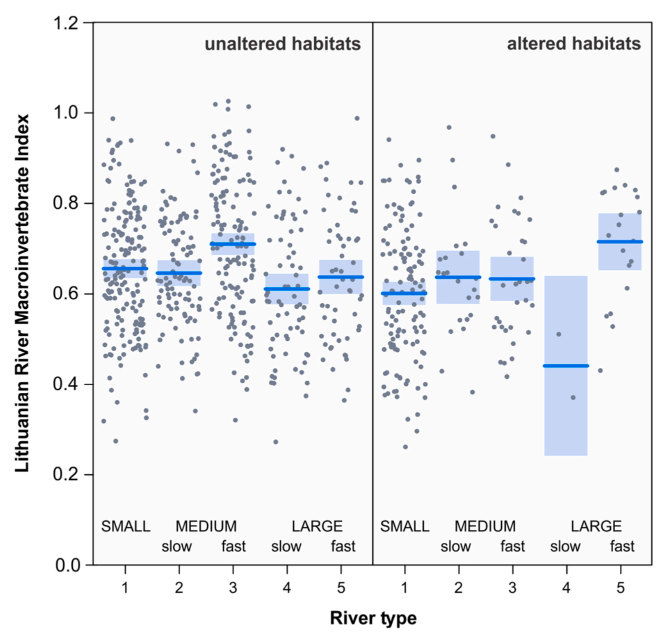

Fig. 6. Partial residuals plots (estimated means with 95% confidence bands) from a linear model (see Table 6) showing the interacting conditional effects of hydromorphological habitat alteration and river type (see Table 1 for typology description) on the Lithuanian River Macroinvertebrate Index.

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