Biotic indices of hydrological variability as tools to inform dynamic ecological status assessments in river ecosystems

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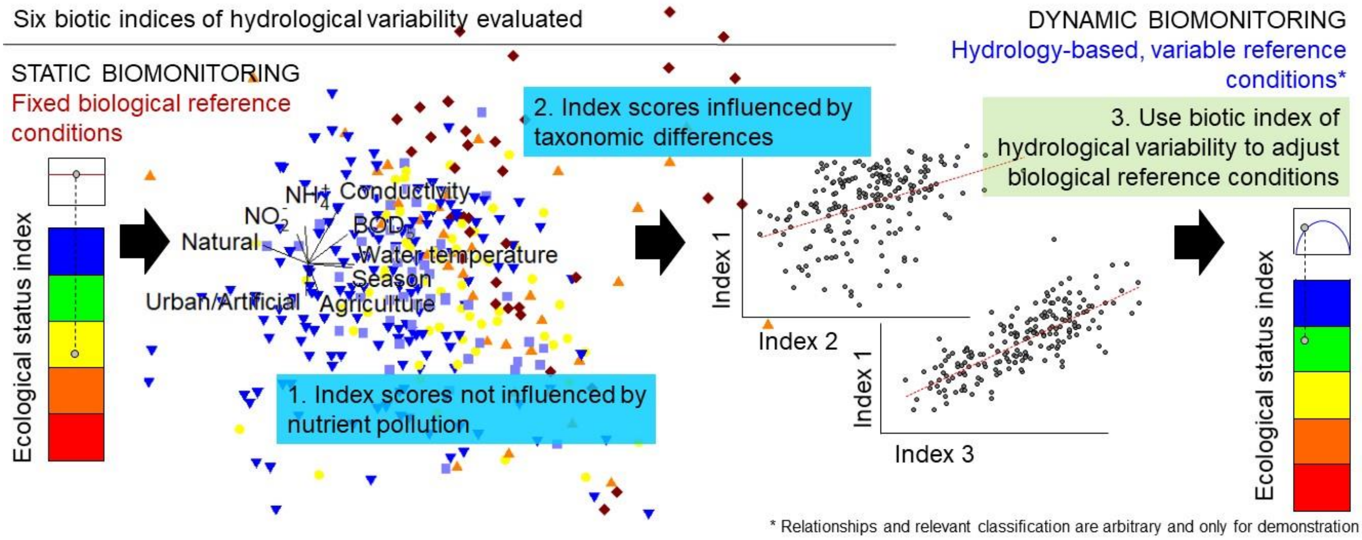
Abstract

River biomonitoring uses biotic indices that assess human-induced degradation, including pollution, by comparison with type-specific static biological reference conditions. However, index scores that indicate pollution may reflect biological responses to natural hydrological variability associated with low flows and drying, leading biomonitoring schemes to misclassify sites as degraded. To address this, dynamic, site-specific adjustments of static biological reference conditions have been proposed, but current biomonitoring tools cannot facilitate implementation of these adjustments. We analyzed 329 samples from mediterranean-climate rivers in Greece, to evaluate the use of six stressor-specific macroinvertebrate-based indices of hydrological variability (CEFI, DEHLI, ELF, LIFE, LIFENZ, MIS-index) as tools to facilitate dynamic adjustments of static biological reference conditions. We examined macroinvertebrate assemblage responses to physicochemical and land use drivers in relation to each sample's hydrological conditions, as assessed by the six indices. We evaluated index performance beyond the region of development by exploring correlations among indices, including correlations with the region-specific Greek ELF index, for which 100% of taxa were represented. We also examined the influence of inorganic nutrient pollution on index performance by comparing index scores from samples with and without nutrient pollution. Season, water temperature, agricultural land use and nutrient pollution were major drivers of macroinvertebrate assemblage composition. Indices were positively correlated but correlation strength varied considerably, driven primarily by taxonomic representation (the proportion of sampled taxa included in each index's calculation), and potentially also by differences in river types, taxonomic resolution and sampling strategies. All indices identified site-specific hydrological conditions both in the presence and absence of nutrient pollution. We recommend the development of region-specific biotic indices of hydrological variability, or regional adaptation of existing indices to represent 100% of the regional taxa pool, and thus enable acceptable performance beyond their region of development. Such indices could inform dynamic adaptation of static biological reference conditions by assessing site-specific hydrological conditions based on a macroinvertebrate assemblage, without the collection of additional, abiotic field data. Application of our proposed approach could prevent misclassification of ecological status, thus avoiding time-demanding and costly mismanagement of rivers and streams.

Keywords: Multiple stressors, ecological status, ecological quality, aquatic invertebrate, bioassessment

Graphical abstract



Highlights

- Rivers are misclassified as degraded due to natural hydrological variability (HV)
- We evaluated the use of HV biotic indices to adjust biological reference conditions
- Taxa representation influenced index performance beyond its region of development
- Nutrient pollution had insufficient influence to compromise index performance
- HV indices could be used as biotic proxies for HV to adjust biomonitoring tools

1. Introduction

Traditionally, river biomonitoring assesses ecological status using biotic indices that compare aquatic communities with type-specific static biological reference conditions established for river types with geographic, climatic and hydromorphological similarities (AQEM Consortium, 2002; Buffagni et al., 2006; Birk et al., 2012). Ecological status is assessed based on the deviation of site-specific biological conditions at the time of sampling from the type-specific biological reference conditions (Nõges et al., 2009; Birk et al., 2012). However, river discharge naturally fluctuates: perennial rivers experience low-flow periods, intermittent rivers dry, and aquatic communities vary over time in response to these changing hydrological conditions (White et al., 2019; Wu et al., 2020). As a result, hydrological variability (i.e. temporal changes in river discharge, potentially including frequent, extreme discharge fluctuations and flow intermittence) significantly affects current biomonitoring tools (Wilding et al., 2018), which cannot distinguish between biological responses to natural hydrological variability and to human-induced hydrological alteration and degradation, resulting in misclassification of ecological status (Buffagni et al., 2020).

Using such biomonitoring tools, approximately 40% of European watercourses have been assessed as failing to reach the Water Framework Directive (WFD) 2000/60/EC target of ‘good’ ecological status (EEC, 2000) due to hydromorphological alteration; such rivers are officially considered water-stressed (EEA, 2012, 2018). However, this 40% may include responses to natural hydrological variability that have been incorrectly identified as human-induced deviations from static biological reference conditions (Stubbington et al., 2018). To prevent such misclassifications, recent research has developed dynamic biomonitoring frameworks designed to enable seasonal or site-specific adjustments of static biological reference conditions in dynamic rivers (Nõges et al., 2009; Cid et al., 2020). In addition, new approaches have used hydromorphological and other habitat information collected at the time of sampling to adjust the biological reference conditions against which ecological status is assessed (Buffagni et al., 2020). Indices that characterize biotic responses to site-specific current and antecedent hydrological conditions (e.g. ‘LIFE’; Extence et al., 1999) have the potential to facilitate such dynamic adjustments without the need for additional hydrological information, but their performance—including an index’s applicability beyond its region of development—has not been evaluated.

Worldwide, six stressor-specific macroinvertebrate-based indices assess biological responses to hydrological variability, including both natural fluctuations and human-induced alterations. These indices (hereafter, HC indices) characterize assemblages in relation to site-specific hydrological conditions during—and in the months preceding—sampling, and thus provide a biotic proxy of these conditions (Dunbar et al., 2010). Three HC indices score taxa based on their flow velocity preferences: the LIFE index, developed in the UK (Extence et al., 1999), and the New Zealand LIFENZ index (Greenwood et al., 2016) and Canadian CEFI index (Armanini et al., 2011), which are both based on LIFE. The Greek multimetric ELF index (Theodoropoulos et al., 2020) is also based on metrics describing flow velocity tolerances and preferences, whereas the UK DEHLI index (Chadd et al., 2017) classifies taxa based on their association with habitats lost at key stages of riverbed drying, and the UK MIS-index (England et al., 2019) characterizes the responses of aquatic, semi-aquatic and terrestrial taxa to changing habitat availability in intermittent rivers. These indices score macroinvertebrate taxa based on their environmental preferences, taxon scores are weighted and/or summed, the result is often divided by the number of taxa, other metrics and/or traits may be incorporated, and the final score indicates the biological response to hydrological conditions, potentially including natural and/or human-induced variability.

Studies comparing HC indices have considered few river types, namely UK lowland chalk streams (England et al., 2019; Sarremejane et al., 2019; White et al., 2019) and lowland rivers in New Zealand (Greenwood et al., 2016). It therefore remains unclear how index performance varies in relation to regional variability in macroinvertebrate assemblages, and whether HC indices can be applied beyond their region of development. In addition, co-occurring gradients of hydrological variability (including drying in intermittent rivers) and pollution may interact to influence macroinvertebrate assemblage composition (Kalogianni et al., 2017), but HC index performance may remain stable despite these compositional changes (Kath et al., 2018). It is thus also unclear how HC indices perform when hydrological dynamism, which characterizes mediterranean-climate rivers, interacts with anthropogenic stressors such as nutrient pollution.

We examined the potential of HC indices for use (1) beyond the region of development and (2) in the presence of nutrient pollution, to facilitate dynamic adjustments of the static biological reference conditions that underpin current regulatory biomonitoring, by enabling inference of site-specific hydrological conditions at and prior to the time of sampling in the absence of hydrological data. We a priori accepted the ability of HC indices to act as biotic proxies for site-specific hydrological conditions and, using a dataset comprising macroinvertebrate samples from Greek rivers, we tested two hypotheses. Our first hypothesis (H1) was that HC index performance declines beyond its region of development due to taxonomic differences. If H1 is accepted, HC indices would require regional adaptation or region-specific indices should be developed. Our second hypothesis (H2) was that HC index scores are not sufficiently influenced by nutrient pollution to compromise their applicability. If H2 is accepted, HC indices can reflect hydrological conditions at both nutrient polluted and unpolluted sites.

2. Materials and methods

2.1. Macroinvertebrate dataset

We analyzed a regulatory dataset comprising 329 macroinvertebrate samples collected from 174 perennial and 22 intermittent sites (which dry in summer), in small (10–100 km^2) to large (>1000 km^2) Greek river basins, in spring (April to late May) and summer/early autumn (late June to early September) 2018 (Fig. 1). Macroinvertebrates were sampled from all microhabitats in proportion to their occurrence using a 3-min kick-and-sweep method (ISO, 2012; 0.25 × 0.25 m^2, 500-µm-mesh net). Samples were preserved in 96% ethanol, transferred to the laboratory, and all individuals identified to family level (except for Oligochaeta, which were not identified further) and counted.

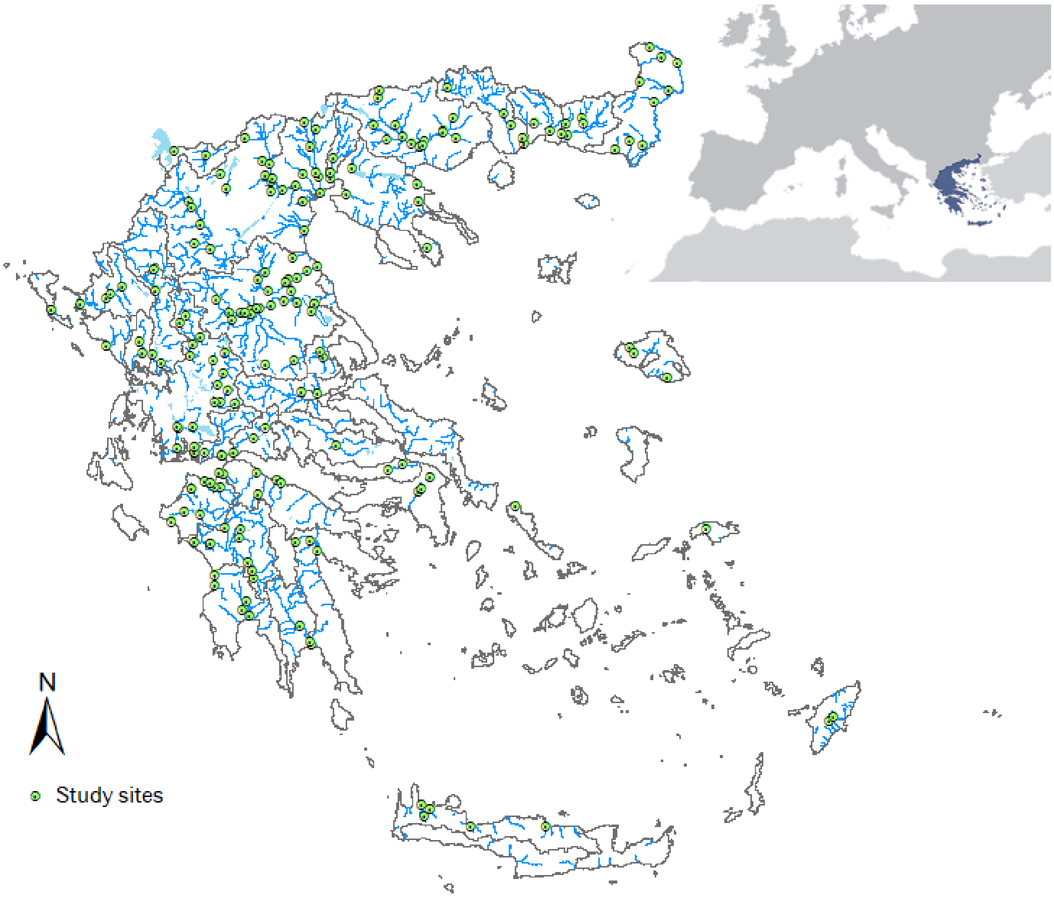


Fig. 1. Map of the study area in Greece, indicating the locations of the 196 sampling sites.

2.2. Environmental dataset

Electrical conductivity (µS/cm), total dissolved solids (TDS; mg/L), dissolved oxygen (DO; mg/L) and oxygen saturation (%) were measured at each site using an AQUAREAD AP2000 multiparameter probe, prior to macroinvertebrate sampling. Water samples were collected, stored at 4°C, transferred to the laboratory and analyzed (mg/L) for five-day biological oxygen demand (BOD_5), chloride, silicates, nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), orthophosphates (PO_4^{3-}), total nitrogen (TN) and total phosphorus (TP) using a MERCK Pharo 300 spectrophotometer. CORINE Land Cover 2012 data (https://land.copernicus.eu/pan-european/corine-land-cover) were used to estimate the % agricultural, natural and urban/artificial land uses for the catchment upstream of each site using GIS-based analysis (ArcGIS 10.1; ESRI, 2012). Chemical status was assessed using the Nutrient Classification System (NCS; Skoulikidis et al., 2006), in which NO_2^-, NO_3^-, NH_4^+ and PO_4^{3-} concentrations are categorized into WFD-based classes (bad, poor, moderate, good, high). Samples of bad, poor or moderate chemical status (i.e. those exceeding the lower threshold of the good status class for at least one nutrient) were considered nutrient-polluted, and samples with good or high NCS status were classified as unpolluted (Fig. S1).

2.3. Calculation of stressor-specific biotic indices of hydrological variability

The macroinvertebrate taxa list included 131 aquatic families; no terrestrial or semi-aquatic taxa were identified. The families in each sample were used to calculate (1) the Lotic-invertebrate Index for Flow Evaluation (LIFE; Extence et al., 1999), (2) the Drought Effect of Habitat Loss on Invertebrates index (DEHLI; Chadd et al., 2017), (3) the Monitoring Intermittent Streams index (MIS-index; England et al., 2019), (4) the Canadian Ecological Flow Index (CEFI; Armanini et al., 2011), (5) the LIFE New Zealand index (LIFENZ; Greenwood et al., 2016) and (6) the Hellenic Flow Index (ELF; Theodoropoulos et al., 2020). Although four of these indices were primarily developed based on taxon-specific flow velocity preferences, they can effectively represent community responses to short and long-term hydrological variability (Dunbar et al., 2010; Worrall et al., 2014). Each index was developed using its own taxa list and thus, from the 131 families, 131 (100%) were included in the calculation of ELF, 100 (76%) in LIFE, 86 (66%) in DEHLI, 41 (31%) in MIS-index and CEFI, and 38 (29%) in LIFENZ. The indices were calculated as follows:

(1) LIFE: Each family was assigned to a flow group (I: rapid, II: moderate/fast, III: slow/sluggish, IV: flowing/standing, V: standing, VI: drought resistant) and an abundance category (A: 1–9, B: 10–99, C: 100–999, D: 1000–9999, E: > 10,000), which were used to determine a flow score ranging from 1 to 12. LIFE was then calculated in ASTERICS 3.1.1 software as:

$$\mathrm{LIFE} = \frac{\sum \mathrm{fs}}{\mathrm{n}}$$

where $\sum \mathrm{fs}$ is the sum of the individual family flow scores for a sample and n is the number of families used to calculate $\sum \mathrm{fs}$.

(2) DEHLI: Each family was assigned a drought intolerance score (DIS) ranging from 1 (for drought-tolerant taxa) to 10 (for taxa that need fast-flowing, well-oxygenated water). Families assigned to more than one DIS group (Chadd et al., 2017) were assigned the highest group score. DEHLI was then calculated as:

$$\mathrm{DEHLI} = \frac{\sum \mathrm{DIS}}{\mathrm{n}}$$

where $\sum \mathrm{DIS}$ is the sum of the individual family DIS for a sample and n is the number of families used to calculate $\sum \mathrm{DIS}$.

(3) MIS-index: Each family was assigned to a MIS-group (lotic [fast], lotic, generalist or lentic) indicating its association with different habitat conditions; no taxa were assigned to the semi-aquatic or terrestrial MIS-groups. A weighting factor was assigned to each family based on its MIS-group and the season in which the sample was collected (either spring or summer/early autumn). The MIS-index was then calculated using:

$$\mathrm{MIS_j} = \frac{\sum_{i=1}^{6} \mathrm{M_{si} T_{ij}}}{\sum \mathrm{T_j}}$$

where i = 1 to 6 denotes one of six MIS-groups, s is the season, $\mathrm{M_{si}}$ is the weighting factor for MIS-group i in season s, $\mathrm{T_{ij}}$ is the number of families in group si in sample j and $\mathrm{T_j}$ is the total number of families in sample j.

(4) CEFI: Each family was assigned a flow velocity preference optimum value and an indicator weight score (the weighted standard deviation of optimum values) and CEFI was calculated as:

$$\mathrm{CEFI} = \frac{\sum_{i=1}^{n} \mathrm{F_i R_i V_i W_i}}{\sum_{i=1}^{n} \mathrm{F_i R_i W_i}}$$

where, for the ith family, $\mathrm{F_i}$ is its relative frequency class, $\mathrm{R_i}$ is its relative abundance, $\mathrm{V_i}$ is its flow velocity optimum, and $\mathrm{W_i}$ is its indicator weight score.

(5) LIFENZ: Each family was assigned to a flow group and to an abundance category and these values used to determine a flow score ranging from 2 to 12. LIFENZ was then calculated as:

$$\mathrm{LIFENZ} = \frac{\sum_{i=1}^{s} \mathrm{fs}}{\mathrm{S}}$$

where, fs is the flow score of each family and S is the total number of families in the sample.

(6) ELF: Each family was assigned to one of seven flow-sensitivity classes (highly generalist, generalist, meso-generalist, intermediate, meso-specialist, specialist, highly specialist) and a multimetric approach applied, in which the following metrics were calculated and categorized in a 0–1 five-class system: (i) the relative abundance of highly generalist, generalist, meso-generalist and intermediate families (CR_A); (ii) the relative richness of these four classes (CR_R); (iii) the degree of deviation of the mean macroinvertebrate assemblage discharge-per-unit-width (q) preference from the optimal q preference (Doq); (iv) the relative abundance of rheobiont–rheophilic–rheolimnophilic families ($F1_A$), based on the EUROLIMPACS database (Schmidt-Kloiber and Hering, 2015, 2021); (v) the relative abundance of limnobiont–limnophilic–limnorheophilic families ($F2_A$); and (vi)–(vii) the relative richness of the $F1_A$ and $F2_A$ families, respectively ($F1_R$ and $F2_R$). Metrics i–vii were combined based on the site's NCS classification, and ELF calculated using:

ELF = MIN (CR_R, $F1_A$, $F2_A$, $F1_R$, $F2_R$) for polluted sites, and
ELF = MAJAV (CR_A, Doq, $F1_A$, $F2_A$, $F2_R$) for unpolluted sites

where MIN is the minimum value of the metrics and MAJAV denotes a majority-vote combination (i.e. the index class indicated by ≥ 3 of 5 metrics), unless all metric classes were different, in which case the mean was calculated.

2.4. Data analysis

2.4.1. Identifying physicochemical and land use drivers of macroinvertebrate composition

To identify the environmental drivers that influenced macroinvertebrate assemblage composition at sites with and without nutrient pollution (the main anthropogenic stressor in our study area), two datasets were developed, one incorporating all (polluted and unpolluted) samples (*n* = 329) and one including only unpolluted samples (*n* = 107; identified by the NCS system; Fig. S1). For each dataset, environmental (ENV) and macroinvertebrate (BIO) distance matrices were created using Euclidean and Bray-Curtis distances, respectively. The ENV matrix included season (spring, summer) and all physicochemical and land use variables (see section 2.2), and the BIO matrix included raw abundance data. Variance inflation factors (VIF) were calculated for each variable in the ENV matrix, and variables with the highest VIFs (i.e. TN, TP, DO, TDS, pH, Cl^- and PO_4^{3-}) were sequentially removed until all retained variables had VIF < 3 (Zuur et al., 2010). The ENV and BIO matrices were then correlated using Spearman's correlation coefficients (rho).

Two-dimensional non-metric multidimensional scaling (NMDS) ordinations were produced to visualize the distribution of macroinvertebrate assemblages in relation to environmental drivers. To assess the relative contribution of each physicochemical and land use variable to assemblage composition, distance-based linear models (LM) were developed to identify relationships between the BIO and ENV matrices using a stepwise selection procedure, with corrected Akaike information criteria (AIC) used to identify the most parsimonious model.

2.4.2. H1: Regional vs. wider index performance

To test H1, we (i) calculated taxonomic representation (TR; the number of taxa used to calculate index scores as a % of the number of taxa sampled) for each HC index; (ii) used linear regression to calculate R^2 coefficients describing correlations among indices for the all-sample and unpolluted datasets; and (iii) compared R^2 values among indices, using ELF as a benchmark of 100% TR, after applying polynomial regression (selected based on the lowest AIC) to extrapolate R^2 values to the 0–100% TR range. Moderate ($0.5 \leq R^2 < 0.75$) or weak ($R^2 < 0.5$) relationships among indices with low TR and stronger relationships for indices with higher TR would support H1. MIS-index R^2 values were calculated separately for spring and summer/early autumn samples using seasonal weighting factors from England et al. (2019).

2.4.3. H2: The effects of nutrient pollution on index performance

To test H2, all indices were normalized to a 0–1 scale as follows:

$$\mathrm{x_n} = \frac{\mathrm{x_i} - \mathrm{x_{min}}}{\mathrm{x_{max}} - \mathrm{x_{min}}}$$

where $\mathrm{x_n}$, $\mathrm{x_i}$, $\mathrm{x_{min}}$ and $\mathrm{x_{max}}$ are the normalized, non-normalized, minimum and maximum index values in the dataset, respectively. Normalized values were assigned to one of five equal-sized hydrological classes from 0–0.2 to 0.8–1, which represent assemblages associated with relative hydrological and associated habitat conditions from the lowest to the highest flow conditions, respectively (i.e. lentic, slow-flowing, intermediate, flowing, fast-flowing), with field observations validating $\mathrm{x_{max}}$ as the highest flow conditions that typically occur at the site. For both datasets and for each index, NMDS-ordinated samples were colour-coded with the five (independently classified) hydrological classes to enable visualization, and their distribution (as NMDS1 and 2 scores) related to season, nutrient pollution and land use using Spearman's rho. Permutational multivariate analysis of variance (PERMANOVA) of the BIO distance matrix was used to compare macroinvertebrate assemblage composition between each pair of hydrological classes, as assessed by each HC index, for the all-sample and unpolluted datasets. Differences were identified using the pseudo-*t* statistic (i.e. the square root of pseudo-*F*, which is applicable for pairwise comparisons; Anderson et al., 2008), with 999 unrestricted permutations of raw data. For a given pair of an index's hydrological classes, statistically comparable PERMANOVA *t* and *p* values for the all-sample and unpolluted datasets would indicate that index values are not influenced by nutrient pollution, supporting H2.

All analyses were conducted in PRIMER 6+ software (methods PERMANOVA, RELATE and distLM; Clarke and Gorley, 2006), except for linear/polynomial regression and VIF analysis, which were performed using the car package (Fox and Weisberg, 2019) in R version 3.5.1 (R Core Team, 2018).

3. Results

3.1. Physicochemical and land use drivers of macroinvertebrate composition

A total of 304,358 macroinvertebrates belonging to 131 taxa were identified from 329 samples. In the all-sample analysis, environmental variables explained 21% of the variation in assemblage composition (ENV–BIO Spearman's rho = 0.21). Season (LM $F = 5.26$; $p = 0.001$), water temperature ($F = 5.71$; $p = 0.001$), % natural land use ($F = 6.59$; $p = 0.001$), % agricultural land use ($F = 4.78$; $p = 0.001$), % urban/artificial land use ($F = 2.16$; $p = 0.004$), oxygen saturation ($F = 3.70$; $p = 0.001$), BOD_5 ($F = 6.91$; $p = 0.001$) and NO_3^- concentrations ($F = 2.48$; $p = 0.001$) influenced macroinvertebrate assemblage composition, as indicated by their inclusion in the best LM. In the analysis of unpolluted samples ($n = 107$), environmental variables explained 11% of the variation in assemblage composition

(ENV–BIO Spearman's rho = 0.11), which was primarily influenced by season (*F* = 4.13; *p* = 0.001) and % agricultural land use (*F* = 4.20; *p* = 0.001), which were included in the best LM.

3.2. H1: Index performance in relation to taxonomic representation

In the all-sample analysis, almost all correlations among hydrological indices were significant and positive (Table 1; Figs. S2–S3). Correlation strength varied considerably among index pairs and, except for a moderate correlation between LIFE and DEHLI (R^2 = 0.539), all relationships were weak (i.e. R^2 < 0.5), with R^2 values ranging from 0.019 (LIFENZ–CEFI) to 0.435 (DEHLI–MIS[Summer]). Ten correlations exceeded R^2 = 0.2, LIFE was the only index for which correlations with all other indices were R^2 > 0.2, and R^2 for DEHLI and MIS-index were 0.321–0.435 (Table 1). The benchmark ELF index, which included all 131 macroinvertebrate taxa (100% TR), was most strongly correlated with LIFE (100 taxa), and correlation strength then declined with TR, from DEHLI (86 taxa) to CEFI (41 taxa), MIS[summer] (41 taxa), LIFENZ (38 taxa) and MIS[spring] (41 taxa). At 100% TR (in the hypothetical case of all taxa in a dataset being included in the calculation of an index other than ELF), correlation strength reached R^2 > 0.7 (Fig. 2).

In the analysis of unpolluted samples, all correlations were again significant and positive, except for LIFENZ–CEFI (Table 1; Figs. S4–S5), and correlation strength again varied considerably among index pairs. Thirteen R^2 values were higher than in the all-sample analysis, four were comparable, and three lower (Table 1). Again, all correlations except for LIFE–DEHLI (R^2 = 0.539) were weak, with R^2 values ranging from 0.013 (LIFENZ–CEFI) to 0.484 (LIFE–MIS[summer]). The ELF index was most strongly correlated with LIFE (100 taxa), DEHLI (86 taxa) and LIFENZ (38 taxa), and had weaker relationships with CEFI (41 taxa) and with both MIS[summer] and MIS[spring] (41 taxa). At 100% TR, correlation strength reached R^2 > 0.7 (Fig. 2).

Table 1. R^2 values describing the correlation between macroinvertebrate-based biotic indices of hydrological variability, in all samples and in unpolluted samples. R^2 values that are higher in the analysis of unpolluted samples compared to the all-sample analysis are in bold.

		LIFE	LIFENZ	DEHLI	CEFI	MIS[spring]	MIS[summer]
All samples	ELF	0.286**	0.115**	0.208**	0.132**	0.032*	0.157**
	LIFE		0.412**	0.539**	0.205**	0.307**	0.292**
	LIFENZ			0.329**	0.019*	0.153*	0.170**
	DEHLI				0.101**	0.321**	0.435**
	CEFI					0.101*	0.137*
Unpolluted samples	ELF	**0.464****	**0.378****	**0.392****	**0.259****	**0.134***	**0.302****
	LIFE		0.323**	**0.609****	**0.320****	0.307**	**0.484****
	LIFENZ			0.263**	0.013	0.140**	**0.207****
	DEHLI				**0.182****	**0.326****	0.346**
	CEFI					0.098*	**0.175****

** and * indicate significant correlations at the p<0.01 and p<0.05 levels, respectively

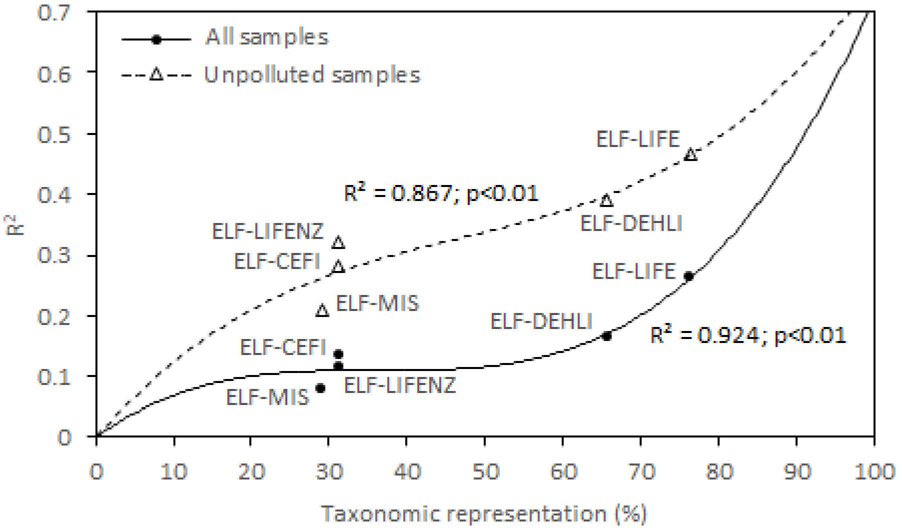


Fig. 2. R^2 values between the ELF index (as a benchmark of 100% taxonomic representation; TR) and the CEFI, DEHLI, LIFE, LIFENZ and MIS indices, plotted against each pair's TR for the all-sample and unpolluted sample datasets. Polynomial regression was used to extrapolate correlation strength to the 0–100% TR range. Abbreviations are defined in the text.

3.3. H2: The effects of nutrient pollution on index performance

In the all-sample ordination, NMDS1 was correlated with season (Spearman's rho = 0.19), water temperature (0.19), % natural land use (-0.19), NO_3^- (-0.16), BOD_5 (0.16) and conductivity (0.13). NMDS2 was correlated with conductivity (0.24), % agricultural land use (-0.13), % urban/artificial land use (-0.13), NH_4^+ (0.15), NO_2^- (0.12) and BOD_5 (0.12) (Figs. 3a; S6i). In the analysis of unpolluted samples, NMDS1 was correlated with season (0.41), water temperature (0.51), % agricultural land use (0.40) and % urban/artificial land use (0.34), and NMDS2 was correlated with season (0.18) (Figs. 3b; S6ii).

In the all-sample analysis, most indices indicated a gradient of decreasing flow along NMDS1, which was mostly correlated with season and water temperature (Figs. 3a; S6i). ELF and CEFI indicated fast-flowing and flowing conditions for most spring samples (which had lower NMDS1 scores); LIFE, LIFENZ and DEHLI indicated flowing and intermediate conditions; and MIS-index mostly indicated flowing to slow-flowing conditions. All indices indicated intermediate, slow-flowing and lentic conditions for most summer samples (higher NMDS1 scores). CEFI, DEHLI, ELF and LIFE indicated a range of flow conditions, from fast-flowing and flowing to intermediate and slow-flowing for sites with high % agricultural land use (lower NMDS2 scores), and for nutrient-polluted samples (higher NMDS2 scores; Fig. S6a–d). LIFENZ and MIS-index mostly indicated intermediate to lentic conditions for both sites in agricultural land uses and nutrient-polluted sites (Fig. S6e–f). In the analysis of unpolluted samples, all indices except MIS-index indicated fast-flowing and flowing conditions for spring samples from sites adjacent to natural land uses (lower NMDS1 scores), and intermediate to lentic conditions for summer samples next to agricultural land (high NMDS1 scores) (Figs. 3b; S6ii). MIS-index identified intermediate to slow-flowing conditions for spring samples and mostly lentic conditions for summer samples.

In the all-sample analysis, macroinvertebrate assemblage composition differed in 9 of 10 pairs of hydrological classes for the ELF, DEHLI, LIFE and CEFI indices, 8 of 10 for LIFENZ and 7 of 10 for MIS-index (PERMANOVA $p < 0.05$; Table 2). PERMANOVA t statistics indicated that the greatest compositional differences were between the slow-flowing and fast-flowing classes of LIFE, DEHLI and LIFENZ, the lentic and fast-flowing ELF classes, the intermediate and fast-flowing CEFI classes, and the lentic and slow-flowing MIS-index classes. Patterns were similar in the analysis of unpolluted samples: all differences between classes remained significant, with minor differences in t-values (mean t-value difference: 17 ± 11%) between the same pairwise comparisons (PERMANOVA; Table 2). Significant differences were observed in 9 pairwise comparisons of hydrological classes for LIFE and CEFI; 8 for ELF, DEHLI and LIFENZ; and

6 for MIS-index (Table 2). The greatest differences in assemblage composition were between the intermediate and fast-flowing CEFI classes, the lentic and flowing ELF classes, the intermediate and flowing DEHLI classes, the slow-flowing and flowing LIFE classes, the slow-flowing and fast-flowing LIFENZ classes, and the lentic and slow-flowing MIS-index classes.

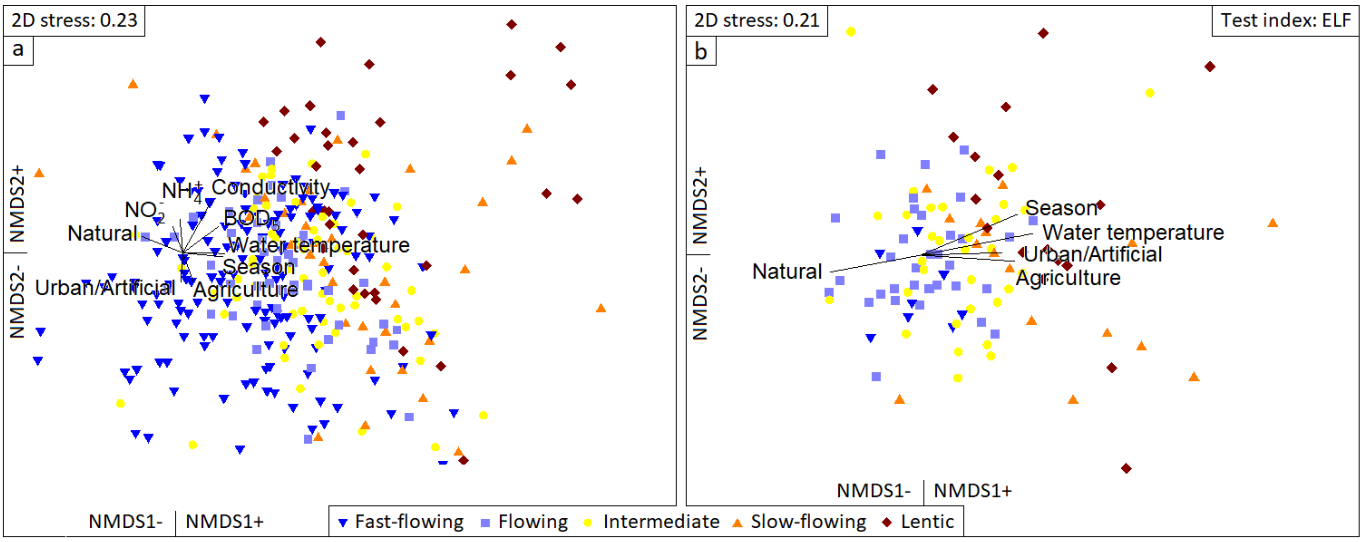


Fig. 3. Non-metric multidimensional scaling (NMDS) ordination of macroinvertebrate samples, coded by the hydrological class assigned to each sample by the ELF index (as an example; Fig. S6 shows other HC indices): (a) all samples (n = 329); (b) unpolluted samples (n = 107). Natural, urban/artificial and agriculture vectors represent land use percentages.

Table 2. PERMANOVA *t* statistics indicating differences between pairs of hydrological classes as categorized by each macroinvertebrate-based index of hydrological variability, for all samples and for unpolluted samples. 1: lentic, 2: slow-flowing, 3: intermediate, 4: flowing, 5: fast-flowing. Values in bold indicate significant differences.

ELF	1–2	1–3	1–4	1–5	2–3	2–4	2–5	3–4	3–5	4–5
All	1.153	**1.933****	**2.453****	**2.699****	**1.348****	**1.948****	**2.233****	**1.255***	**2.254****	**2.124****
Unpolluted	1.115	**1.834****	**2.528****	**2.128****	**1.469****	**2.126****	**1.710****	**1.474****	**1.422***	1.072
DEHLI	1–2	1–3	1–4	1–5	2–3	2–4	2–5	3–4	3–5	4–5
All	**1.271***	**1.697****	**2.040****	**1.815****	**2.224****	**3.166****	**2.120****	**2.934****	**1.867****	0.927
Unpolluted	1.232	**1.470****	**1.751****	**1.604***	**1.208***	**1.974****	**1.744****	**2.170****	**1.627****	0.857
CEFI	1–2	1–3	1–4	1–5	2–3	2–4	2–5	3–4	3–5	4–5
All	1.225	**2.067****	**1.364****	**2.785****	**1.535****	**1.968****	**2.729****	**1.867****	**3.548****	**2.876****
Unpolluted	0.952	**1.401***	**1.768****	**2.213****	**1.402***	**1.880****	**2.566****	**1.691****	**2.668****	**2.079****
LIFE	1–2	1–3	1–4	1–5	2–3	2–4	2–5	3–4	3–5	4–5
All	**1.340***	**1.893****	**2.725****	**2.243****	**2.100****	**3.868****	**2.663****	**2.384****	**2.051****	1.125
Unpolluted	**1.320***	**1.726****	**2.071****	**2.234****	**1.607****	**2.578****	**2.341****	**1.889****	**2.203****	1.163
LIFENZ	1–2	1–3	1–4	1–5	2–3	2–4	2–5	3–4	3–5	4–5
All	1.097	1.221	**1.805****	**1.955****	**1.583****	**3.127****	**2.704****	**2.408****	**2.294****	**1.238****
Unpolluted	1.147	1.133	**1.450****	**1.644****	**1.424****	**2.264****	**2.448****	**1.681****	**2.145****	**1.326***
MIS	1–2	1–3	1–4	1–5	2–3	2–4	2–5	3–4	3–5	4–5
All	**2.547****	**1.823****	**2.203****	**2.136***	1.134	**1.698***	**1.570****	**1.331***	1.074	1.051
Unpolluted	**1.895****	**1.534****	**1.774****	**1.664****	0.997	**1.362***	**1.359***	1.042	0.924	1.052

** and * indicate differences significant at the $p<0.01$ and $p<0.05$ levels, respectively.

4. Discussion

We evaluated the ability of stressor-specific macroinvertebrate-based indices of hydrological variability—which can act as biotic proxies for site-specific hydrological conditions at and preceding the time of sampling—to inform dynamic adjustments of biological reference conditions underpinning current biomonitoring. We found that stressor-specific indices could characterize biotic responses to hydrological variability in both the presence and absence of nutrient pollution. Almost all indices were correlated, but the strength of these correlations varied considerably, which largely reflected variability in the proportion of sampled taxa included in the calculation of indices developed in different regions (i.e. taxonomic representation; TR). In addition, climatic and geomorphological differences among river types, the taxonomic resolution to which organisms are identified, and sampling strategies may have contributed to the observed variability. To maximize index performance, region-specific indices should thus be developed, but adaptation of available indices to achieve 100% TR could also enable acceptable index performance beyond the region of development. Nutrient pollution did not influence HC index scores sufficiently to compromise their performance, and these indices could therefore facilitate adaptation of the static biomonitoring tools currently used to assess the ecological status across a breadth of freshwater ecosystems, including those exposed to considerable hydrological variability.

4.1. H1: Index performance beyond the region of development declined with decreasing taxonomic representation

Our first hypothesis was supported: weakening correlations among indices as TR decreased indicated declining index performance beyond the region of development, and this trend was exacerbated by nutrient pollution. This demonstrates TR as a major factor limiting the wider applicability of an index beyond its region of origin, especially in regions including sites affected by nutrient pollution. Compared to the region-specific benchmark of the Greek ELF index (100% TR), LIFE (76% TR) performed best, followed by DEHLI (66%), CEFI (31%), MIS-index (31%) and LIFENZ (29%). However, index performance was high at 100% TR ($R^2 > 0.7$) both in the all-sample and unpolluted sample analyses (Fig. 2), suggesting that performance beyond the region of development could be improved by regional index adaptation to increase TR to 100%. However, even if all taxa in a dataset were included in an index's calculation, performance beyond its region of development would not reach 100% due to variation among index scores not accounted for by TR. This non-taxonomic variation may relate to differences among river types (Verdonschot and Nijboer, 2004; Aroviita et al., 2009), taxonomic identification levels (Monaghan, 2016), and sampling strategies (Spitale, 2017).

Climatic and geomorphological conditions are among the key causes of variability in macroinvertebrate distribution among different river types (Verdonschot and Nijboer, 2004), with assemblages varying between river types due to differing taxon-specific habitat requirements. Of the indices developed in the cool, wet temperate UK, DEHLI—developed from calcareous sites in lowland areas of south and east England—included 86 taxa in our family-level Mediterranean-river dataset, whereas LIFE—based on a wider dataset encompassing five geographically and geologically distinct river types—included 100 taxa, and had the strongest correlation with ELF; comparison with the MIS-index is compromised by its taxonomic level of operation. CEFI and LIFENZ, developed in rivers of various geomorphological conditions across subarctic to temperate Canada (CEFI) and temperate to tropical New Zealand (LIFENZ) included 41 and 38 taxa, respectively. Moreover, one family may have different scores in two indices due to the contrasting habitat preferences of their constituent genera and species in different regions. For example, Elmidae are characterized as extremely versatile lotic taxa by DEHLI, are associated with moderate flows in LIFENZ, and with moderate to fast flows in LIFE. Ceratopogonidae are characterized as highly tolerant lentic taxa by DEHLI, are associated with moderate flows in LIFENZ, and are unclassified in LIFE. This variation in TR and in taxon-specific scores from different regions might have introduced variability that influenced index scores and thus performance beyond the region of development.

Our dataset included macroinvertebrate families, but only CEFI, DEHLI and ELF operate exclusively or primarily at family level, whereas LIFE uses family to species-level information, LIFENZ works largely at genus level, and MIS-index operates mainly at genus-to-species level. Index performance may therefore have been affected by variability in taxonomic resolution. Genera within one family and species within one genus may have contrasting habitat requirements, and thus a family may be indicative of contrasting hydrological conditions due to variation in its constituent taxa (Armanini et al., 2011). In the family-level LIFE index, for example, Limnephilidae is associated with flowing/standing waters, but individual *Limnephilus* species are associated with moderate/fast, slow/sluggish, flowing/standing and standing waters (Extence et al., 1999). Using region-specific species-level data might thus increase index performance, but would also alter the efficiency of regulatory biomonitoring.

Variability among sampling strategies may also have affected index performance. CEFI, DEHLI, ELF, LIFE and MIS-index were developed from 3-min kick samples collected using 0.4–1-mm-mesh hand nets and sought to represent all aquatic habitats, and MIS-index also included thorough sampling of marginal habitats to collect semi-aquatic and terrestrial taxa. Datasets including aquatic, semi-aquatic and terrestrial taxa might thus improve MIS-index performance beyond UK chalks streams. LIFENZ was developed from pooled Surber samples (250-µm mesh) collected from the single, dominant microhabitat type, which likely reduced the number of macroinvertebrate taxa included in LIFENZ compared to other indices, thus influencing index scores (Haase et al., 2004) and limiting index performance.

4.2. H2: Index performance was not significantly influenced by nutrient pollution

Pollution and hydrological variability are key influences on macroinvertebrate distribution, and covariation in taxon-specific responses to these drivers can make indices developed to detect pollution responsive to hydrological variability (Monk et al., 2006; Buffagni et al., 2009). Despite this, our second hypothesis was supported: nutrient pollution had insufficient influence on index performance to prevent indices from assessing the hydrological conditions of a site. All indices classified differences of comparable strength and significance between hydrological class pairs for the all-sample and unpolluted-sample datasets. This suggests that differences among hydrological classes were largely due to hydrological variability, and that nutrient pollution did not significantly alter the ability of indices to characterize hydrological conditions. Thus, our results suggest that nutrient pollution should not hamper the use of HC indices for the development of dynamic adjustments to static biomonitoring tools.

4.3. Towards the use of stressor-specific biotic indices of hydrological variability in dynamic biomonitoring

Physicochemical and land use variables explained 21% of the compositional variability in macroinvertebrate assemblages. When nutrient-polluted samples were removed from the analysis, season, water temperature and agricultural land use still influenced macroinvertebrate composition, but nutrient concentrations did not. Assemblage composition in the absence of nutrient pollution was thus mostly related to natural (seasonal, thermal) and human-induced (agricultural but not nutrient-related) habitat variability, most of which was not represented by our environmental variables. This supports previous research highlighting macroinvertebrate assemblages in highly dynamic mediterranean-climate rivers—including perennial and intermittent systems—as shaped by seasonal hydrological variability (Menció and Boix, 2018; Buffagni et al., 2020), including dry and wet years (Munné and Prat, 2011), streambed drying (Acuña et al., 2005), high flows (Theodoropoulos et al., 2017) and floods (Piniewski et al., 2017). This variability compromises the ecological suitability of static biomonitoring schemes for such dynamic systems, even where indices of ecological status have been demonstrated as effective, for example after long flowing phases in intermittent rivers (Munné and Prat, 2009; Stubbington et al., 2018).

The need for dynamic adaptation of static biomonitoring approaches has previously been acknowledged for intermittent rivers (Stubbington et al., 2018; Cid et al., 2020). Our results indicate that adapted approaches are also required in dynamic perennial rivers, such as those in our mediterranean-climate study region, because their communities are also influenced by natural low flows and partial riverbed drying in summer (Dewson et al., 2006; Dallas, 2013). Dynamic adjustments of static reference conditions have recently been suggested based on hydromorphological and habitat information recorded at the time of sampling, for example, flow, substrate and aquatic vegetation types (Buffagni et al., 2009; 2020). Our study indicates that HC indices, which represent the hydrological conditions of a site based on its macroinvertebrate assemblage, could be key tools to enable such adjustments without the need for abiotic information.

A dynamic biomonitoring scheme would require two phases. First, to characterize dynamic biological reference conditions (i.e. to assess how communities vary in response to natural hydrological and wider environmental variability), standard protocols would be followed to collect macroinvertebrate samples from unimpacted reference sites, then to calculate both the official, static index of ecological status, and the HC index. Relationships between the two indices would then be analyzed to inform adjustment of the reference values for the index of ecological status based on the hydrological conditions indicated by the HC index, i.e. dynamic adjustment of biological reference conditions. Second, to assess ecological status based on dynamic biological reference conditions, macroinvertebrate samples would be collected from sites of unknown ecological status, both indices calculated, and the ecological status index value adjusted, based on the dynamically adjusted reference value corresponding to each site's hydrological conditions indicated by the HC index; Buffagni et al. (2020) outline a similar approach using hydromorphological and other habitat information instead of HC indices. Alternatively, hydrological data could enable characterization of dynamic biological reference conditions, and the HC indices could then enable assessment of the hydrological conditions at samples of unknown ecological status after calibrating against hydrological data.

5. Conclusion

Ideal biomonitoring tools should (a) assess ecological status, (b) identify potential degradation, (c) distinguish between natural biological variability and human-induced degradation, (d) discriminate among various causes of human-induced degradation and (e) quantify the relative contribution of multiple stressors (Menezes et al., 2010). In freshwater ecosystems, current biomonitoring programmes successfully address 'a' and 'b' and we suggest how stressor-specific biotic indices of hydrological variability could be used to address 'c', but further research is needed. Our results suggest that biotic indices developed to characterize site-specific hydrological conditions during and preceding sampling are fit for purpose, given TR-based adaptations to enable application beyond the region of development. HC indices could provide cost-effective and time-efficient alternatives to direct measurement of hydrological conditions in dynamic biomonitoring schemes encompassing both unpolluted and nutrient polluted sites. Dynamic biomonitoring could then be enhanced by locally available hydrological data that characterize a site's antecedent hydrological history, including continuous gauged data and/or frequent observational data (Sefton et al., 2019). However, macroinvertebrate-based HC indices—which also reflect both current and recent site-specific hydrological conditions—have much greater potential for widespread use due to their lower resource requirements.

As climate change increases the hydrological dynamism of global river flow regimes, we call for region-specific development or adaptation of biotic indices of hydrological variability. Using these indices to assess a site's hydrological conditions could inform dynamic adjustments of biological reference conditions that recognize natural biological variability, thus increasing the accuracy of ecological status assessments and supporting management actions that seek to protect biodiversity within functional river ecosystems as they adapt to global change.

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Conflict of interest

The authors declare no conflict of interest.

Data availability statement

The data that support the findings of this study are available by the corresponding author upon request.

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