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Assessment of Ecological Status of Standing Water Bodies in Bulgaria: Calibration of a New Multi-Metric for Macroinvertebrates

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Abstract

Reliable assessment of the ecological status of standing waters is key for the effective implementation of the Water Framework Directive and for supporting water management and conservation. Biological quality elements, including benthic macroinvertebrates, provide an integrated measure of ecological condition by reflecting the cumulative impacts of environmental pressures over time. However, assessment methods developed for running waters are not readily applicable to standing waters, highlighting the need for category-specific approaches. We conducted a large-scale survey of representative stagnant water bodies (lakes and reservoirs) on the territory of Bulgaria. The goal was to develop a relevant type-specific methodology for the ecological status assessment of standing waters based on macrozoobenthos. We sampled 79 monitoring lake sites between May and November 2020 through an adapted version of the multi-habitat sampling methodology developed within the European AQEM/STAR projects in accordance with applicable standards. A composite sample was taken from the littoral zone of lakes and reservoirs, representative of the substrates available at each location. The sites represent 19 lake types. Eleven biological indices for lakes and seven for rivers were calculated in order to identify the most suitable metric. For each of the metrics, the pressure–response relationship was estimated. Being the first operational national index based on macroinvertebrates, the proposed Bulgarian multi-metric index for lakes is an adapted version of the Hungarian multi-metric macroinvertebrate index. It consists of three metrics, which have been separately tested in Bulgaria for monitoring purposes as early as 2011–2012 as well as in subsequent scientific research. Ecological quality class boundaries for each type of water body were determined. The compatibility of the BMMI with the existing national monitoring programme would facilitate its future integration into the biological water assessment system.

Keywords: benthic macroinvertebrates; lentic freshwater ecosystems; multi-metric index; ecological status/potential; WFD; Bulgaria

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Key Contribution: Development of a multi-metric index for macrozoobenthos for assessing the ecological status of lakes and reservoirs to be used in hydrobiological monitoring in Bulgaria.

1. Introduction

The adoption of the Water Framework Directive (WFD) (Directive 2000/60/EC) changed the existing paradigm for assessing surface water quality, focusing on comprehensive ecological assessments with a leading role in biological communities not only as indicators of ecosystem health but also as reflections of environmental pressures [1]. The subsequent Common Implementation Strategy (CIS) agreed upon, involving EU member states in 2001 [2], aimed to jointly develop a number of guidance documents, e.g., referring to ecological status classification [3] or the intercalibration of monitoring results [4].

Since bioassessment is based on changes in biological communities resulting from changes in environmental conditions, using aquatic organisms for freshwater ecosystem health assessments is a well-established approach [5]. Various mathematical and ecological indices have been developed worldwide to quantify biological responses. Classical ones focus on community structure without explicitly linking taxa to environmental tolerance (e.g., Shannon–Wiener diversity index (H') [6], Margalef's richness index (d) [7]); others are based on tolerance or sensitivity values for macroinvertebrates such as the biotic index (BI) [8], BMWP (biological monitoring working party), and improved ASPT (average score per taxon) index based on BMWP [9].

Among the biological quality elements (BQEs), benthic invertebrates include several trophic guilds and consumer levels, can be found in most lakes during most of the year and integrate combined and varying pressures [10,11]. They could be used effectively in the assessment of the health of lake ecosystems and for identifying the strength of human disturbance [12,13]. Those were some of the reasons why macrozoobenthos were chosen as one of the BQEs required for the classification of ecological status and the ecological potential of surface water bodies under the Water Framework Directive (WFD) [14–16].

Ecological status assessments in lakes through macroinvertebrate remains challenging because littoral communities are shaped by both natural environmental gradients and anthropogenic pressures. Consequently, river metrics cannot be applied directly to standing waters, making multi-metric approaches essential for reliable ecological assessments. Despite the challenges in their usage, the multi-metric indices of biological conditions have been globally applied as practical tools that incorporate various biological metrics at different levels of ecological organisation [17,18].

In Bulgaria, for the development of a classification system for the assessment of the ecological status/potential of surface water types, several experimental metrics for lakes and reservoirs have been proposed: the biotic index (BI) for slow-flowing waters, the total number of taxa (TNT), an adjusted version of the potamal trophic index (PETI) (part of the feeding type index) and % Oligochaeta [19,20]. Most of them were subsequently tested in several studies [21,22].

This study presents the development and calibration of a Bulgarian multi-metric index (BMMI) for standing water bodies by using macrozoobenthos as biological quality elements, establishing type-specific reference conditions and ecological class boundaries, as well as its applicability for assessing ecological status/potential. We hypothesise that individual biological indicators/indices cannot provide a more unambiguous and sensitive assessment of standing waters in Bulgaria than an integrated multi-metric index could. Furthermore,

we assume that the relationship between pressure and response varies depending on the different lake types, which requires type-specific ecological boundaries.

2. Materials and Methods

The study area covered the whole territory of Bulgaria, where 79 monitoring lake sites were sampled between May and November 2020 (Figure 1, Supplementary Table S1). The sites represent 19 lake types (Table 1) including four types of Black Sea coastal lakes (L07–L10) and covering a total of 23 lakes and 56 reservoirs. The study also included L05a as a subtype of the national lake type L05. The latter one, with Srebarna Lake as the only representative of this type, had been intercalibrated successfully in 2017 [23]. The dataset encompassed natural, artificial and heavily modified water bodies distributed across a broad altitude and geographic range.

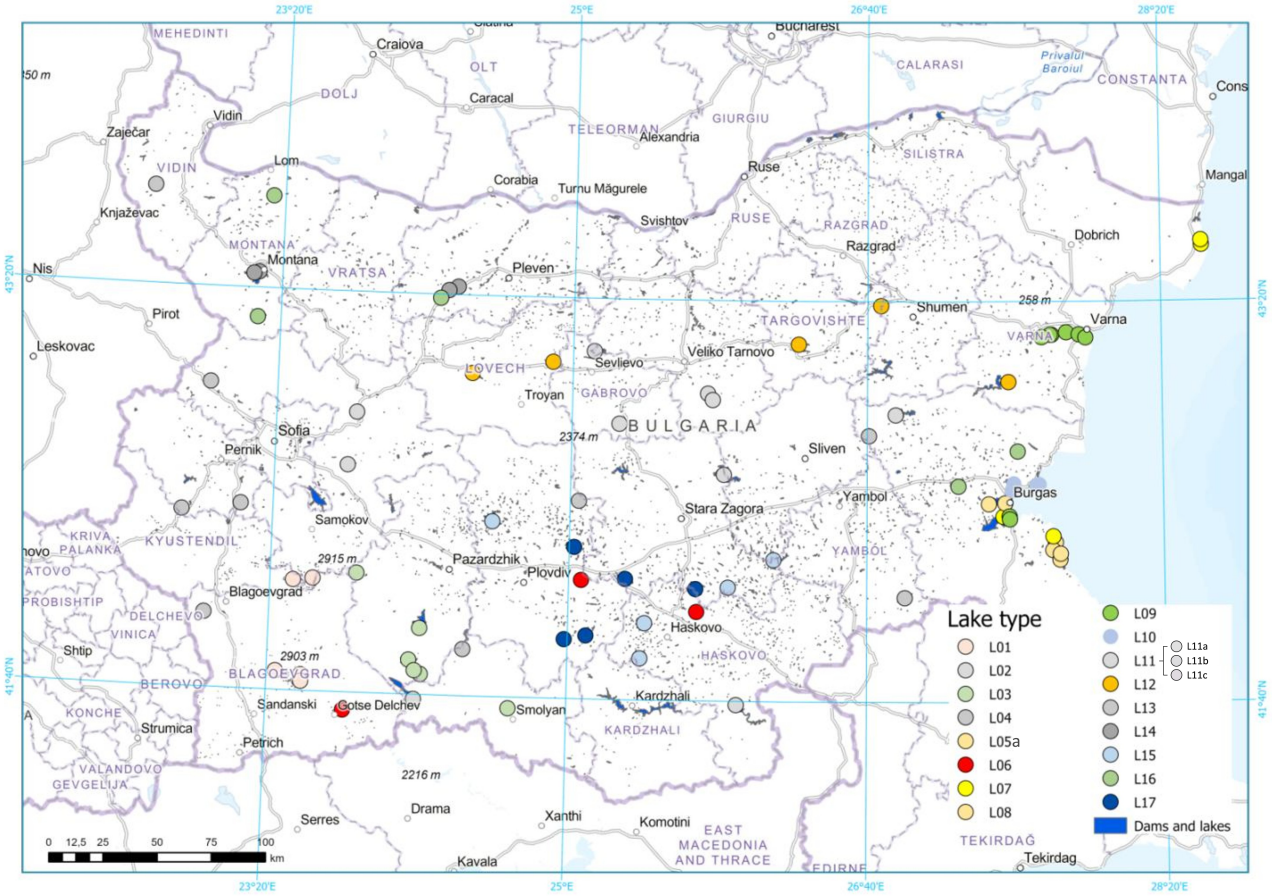


Figure 1. Distributional map of the monitoring lake sites (different colours denote different lake types). Legend: L01—high-mountain glacial/ alpine lakes; L02—mountain lakes and reservoirs in Ecoregion 12; L03—mountain lakes and reservoirs in Ecoregion 7; L04—lowland and semi-mountainous lakes and marshes/swamps; L05a—coastal lakes and swamps in ecoregion ER12 (with eutrophic conditions); L06—riparian wetlands and floodplains; L07—Black Sea coastal freshwater lakes and marshes/swamps; L08—Black Sea coastal oligohaline lakes and marshes/swamps; L09—Black Sea coastal mesohaline lakes and marshes/swamps; L10—Black Sea coastal hyperhaline lakes and marshes/swamps; L11a—large deep reservoirs at 200–800 m a.s.l. in Ecoregion 12; L11b—large deep reservoirs at 200–400 m a.s.l. in Ecoregion 7; L11c—large deep reservoirs above 1000 m a.s.l. in Ecoregion 7; L12—small and medium-sized reservoirs at 200–800 m a.s.l. in Ecoregion 12; L13—small and medium-sized reservoirs usually at 200–800 m a.s.l. in Ecoregion 7; L14—large lowland reservoirs in Ecoregion 12; L15—large lowland reservoirs in Ecoregion 7; L16—small and medium-sized lowland reservoirs in Ecoregion 12; L17—small and medium-sized lowland reservoirs in Ecoregion 7 [24].

Table 1. Number of sampled sites per national lake type, including category of water body (natural, AWB—artificial water body, HMWB—heavily modified water body) and altitude class (in m above sea level) (for lake type codes, see Figure 1).

Lake Type	AWB	HMWB	Natural	<200	200–500	500–800	800–1800	>1800
L01		1	4					5
L02		5			3	2		
L03		5	1				5	1
L04	1	2	1		1	2	1	
L05a			2	2				
L06		1	2	2	1			
L07		1	3	4				
L08		2	2	4				
L09	1	7		8				
L10		4		4				
L11a		2		1	1			
L11b		2		1	1			
L11c		1					1	
L12		5		2	3			
L13		5			1	3	1	
L14		4		4				
L15	1	4		2	3			
L16	1	4		2	3			
L17	1	4		2	3			

The benthic macroinvertebrate collection was performed through an adapted version of the multi-habitat sampling methodology developed within the European AQEM/STAR projects [25,26]. The method was regulated by the applicable Bulgarian water legislation [24]. We sampled the littoral zone of lakes and reservoirs. The sampling method was comparable to the one used in rivers. One composite sample included 10 subsamples collected proportionally from the available substratum types along a 100 m stretch of the wadable zone of the littoral (up to 1 m depth). A hydrobiological net with mesh size of 500 µm was used. The large amounts of bottom substrate collected required pre-screening with standard hydrobiological sieves and primary cleaning of the macrozoobenthos from the substrate on site. The benthic samples were transferred to wide-neck bottles or comparable containers (depending on the amount of material) and preserved in 70% alcohol. The containers were labelled with information about the date and place of sampling. In the laboratory, all macroinvertebrates were separated into systematic groups using a stereomicroscope. For large and/or rich samples, subsamples were taken as the abundance of taxa were later extrapolated according to the portions in the sample. The taxonomic determination followed specialised literature and identification keys [27–31]. The nomenclature of the macroinvertebrates followed Fauna Europaea in general [32]. Benthic organisms were identified at the lowest taxonomic level possible (family or genus level for single specimens; all the others to a species level).

To select the most suitable metric, 11 biological indices for lakes and seven biological indices for rivers were calculated. In most cases, the calculations were carried out with the help of the software Asterics (version 18.0.0) (http://www.aqem.de/mains/products.php, accessed on 5 May 2021) (Table 2).

Table 2. List of the biological indices for lakes with indication of the number of sites for which they were applied in 2020. Abbreviations: Biotic index—BI (when normalised: nBI), see details below; EPT—abundance [%] Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies); RETI3—Rhithral feeding type index [Rhithraler ErnährungsTypen-Index]; ITC—index of trophic completeness [33]; Shannon–Wiener index H'—index of diversity; BMWP—biological monitoring working party; HMMI—Hungarian multi-metric index [34]; ASPT—average score per taxon (=BMWB/ number of families used for the BMWP); PTI—pollution tolerance index [35]. * AMBI was calculated only for lakes of type L09 and L10 [36,37].

Biological Index	Lakes	Software
Number of taxa	79 (79)	Asterics
BI	79 (79)	Excel
EPT abundance [%]	-	Asterics
RETI3	-	Asterics
ITC	73 (79)	MaTros
Shannon diversity index H'	79 (79)	Asterics
Number of families	79 (79)	Asterics
BMWP	79 (79)	Asterics
HMMI	79 (79)	Excel + Asterics
ASPT	76 (79)	Asterics
Oligochaete abundance [%]	78 (79)	Asterics
PTI	61 (79)	Asterics
AMBI	12 *	AMBI and M-AMBI

The biotic index, based on the Irish biotic index (Q-scheme), [38] is the national method used for the intercalibrated river types R2, R4, R7 and R8. The index calculation is based on two metrics: the total number of taxa and the taxa richness of indicator groups A, B, C, D and E (very sensitive to very tolerant) [26]. The individuals are counted and the abundance of individual taxa is related to one of the abundance classes: few (1–5), present (6–20), common (21–50), abundant (51–100), dominant (100+). The BI is calculated from the relative proportions of the tolerance groups. Depending on additional criteria, the calculated value can be downgraded by expert judgement.

For AMBI, the software from https://ambi.azti.es/ (AMBI version 6.0, accessed on 11 September 2021) was used. The index ITC was calculated using the software MaTros (http://www.macro.nemi-ekb.ru/index.php?r=site/login&lang=en, accessed on 13 May 2021).

In addition to the biological data, information on physical and chemical water parameters was collected from most sampling dates or from dates close to the date of the biological sampling. The data included the sampling date, water temperature, electrical conductivity, oxygen concentration and saturation, pH, total phosphorus (TP) and orthophosphate (as soluble reactive phosphorus), total nitrogen (TN), ammonium (NH_4-N), nitrate (NO_3-N) and nitrite (NO_2-N) and biological oxygen demand (BOD_5) (Supplementary Table S2).

The pressure index included the following parameters: Corine landcover, hydromorphological pressure (HabAlt), aquaculture, and chemical parameters (TP, NH_4-N with double weight). The pressure index was calculated as the sum of the normalised values of the stressors. The data were normalised within the range of the 10th and the 90th % percentiles, which present the lower and the upper anchor, respectively (Table 3). For TP, type-specific anchor points were used as defined for the BQE phytoplankton [39]. Prior to normalisation, the hydromorphological pressure value for HabAlt (which can vary between 0 and 3) was divided by 3 to achieve the range of 0–1. The Corine landcover (CLC) data was extracted from https://land.copernicus.eu/en/products/corine-land-cover (accessed on 2 September 2026) and overlaid with the watersheds of each water body in GIS, using the CLC class 1 (artificial) (CLC1). The double weighting of NH_4 was based on the pressure–

response analyses, which showed a stronger correlation between the overall pressure index and the BMMI when NH_4 was assigned a higher weight.

Table 3. The anchor points of the normalised values of stressors.

Presurre Component	LA = 10th Perc.	UA = 90th Perc.
CLC1	0	0.17
NH_4 (mg/L)	0.01	0.237
TP (mg/L)	0.01	0.212
HabAlt	0	3
Aquaculture	0	3

It should be noted that the analyses of individual pressure parameters in relation to benthic invertebrate community composition and the development of the overall pressure index followed different approaches. While the former aimed to identify pressures associated with changes in community composition, the latter focused on identifying a combination of pressures showing a strong relationship with the BMMI. Consequently, pressure parameters significantly related to community composition were not necessarily included in the final pressure index, as changes in community composition are not always reflected to the same extent in the BMMI.

$$PI = CLC1^* + HabAlt + Aquaculture + TP^* + 2{\cdot}NH_4{}^*$$

Correlation analysis was used to explore relationships among biotic metrics and between biotic metrics and environmental variables. The analysis served primarily to identify potential collinearity among metrics and to assess their responsiveness to environmental factors. Correlation coefficients and their statistical significance were used to evaluate the strength and relevance of these relationships, while correlation matrices were used for the visual exploration of the overall patterns.

A non-metric multidimensional scaling (nMDS) method was carried out to represent the similarity between species in communities. We also used the ANOSIM test and the Mantel test (both from R package vegan, version 2.5-7) [40], as well as an indicator species analysis. The Mantel test was carried out to find suitable biological indices for the subsequent evaluation method (pressure—response relationship). The normalised values were thus calculated as follows:

$$x^* = \frac{LA - x}{LA - UA}$$

x* = normalised value
x = raw value
LA, UA = lower/upper anchor point

Reference sites were identified and heavily modified water bodies were excluded from the reference dataset. Ecological class boundaries were established following the three-step boundary setting procedure [41]. Where the number of reference sites was insufficient to derive statistically robust reference values and class boundaries, expert judgement was used to support the interpretation of the available data and the establishment of provisional reference conditions.

3. Results

3.1. Descriptive Analyses of the Biological Community

The species communities were analysed in terms of region (River Basin Directorate), lake types (freshwater vs. transitional waters, natural vs. heavily modified water bodies) and altitude classes. Although the results of the overall ANOSIM test (Table 4) revealed significant results, the R values were generally low.

Table 4. Similarity of communities in terms of different characteristics with the R value and the significance values. HMWB denotes heavily modified water bodies.

Grouping	R	*p*	Significance
River basin district	0.1389	<0.001	***
Type	0.5069	<0.001	***
Freshwater/Transitional	0.5011	<0.001	***
Natural/HMWB	0.1325	<0.05	*
Altitude class	0.0362	0.2347	ns

Significance levels: * $p < 0.05$, *** $p < 0.001$, ns—non-significant.

An R value close to 1 suggests dissimilarity between groups while an R value close to 0 suggests an even distribution. Higher R values were only found in the grouping by lake types and especially among the transitional waters belonging to the national lake types L7 to L10. These oligo- to polyhaline lakes separated clearly from freshwater lakes and reservoirs (Figures 2 and 3).

Although the ANOSIMAnosim statistics had a low R value, high-altitude lakes (>1800 m and 801–1800 m a.s.l.) could be clearly separated from low- to medium altitude lakes (the remaining altitude classes), which in turn showed a significant overlap among each other (Figure 4).

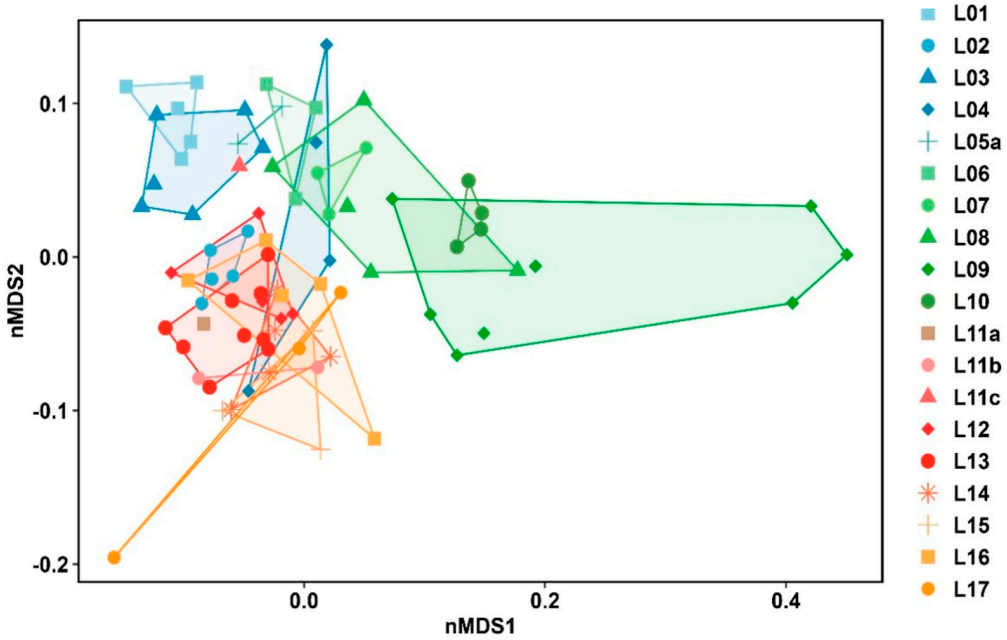


Figure 2. NMDS plot based on macroinvertebrate abundance data and grouped by lake types, stress = 0.205. For lake type codes, see Figure 1.

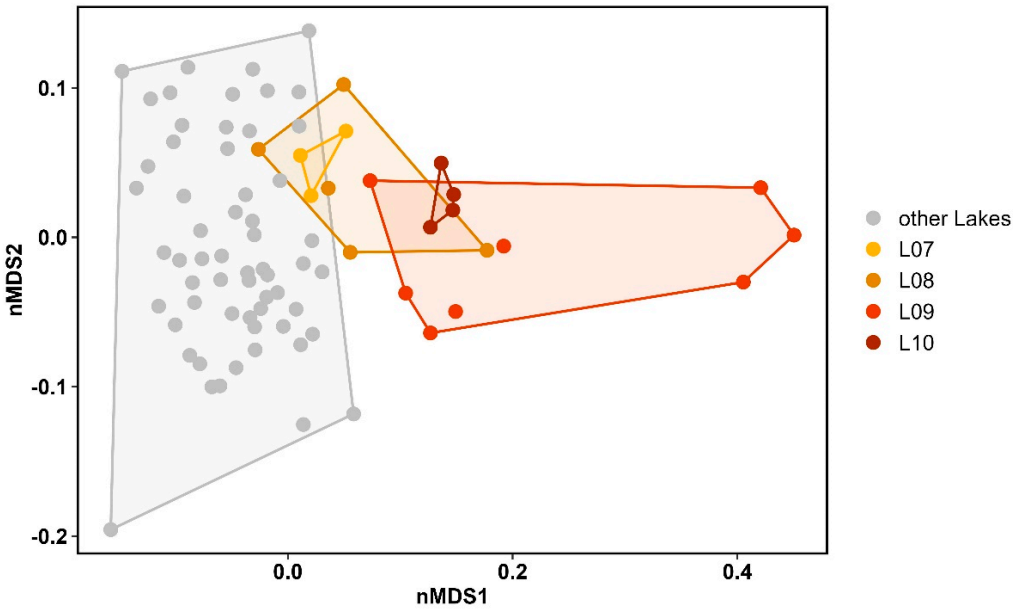


Figure 3. NMDS plot based on macroinvertebrate abundance data and grouped by lake types (transitional waters L07 to L10 against others) (for lake type codes, see Figure 1).

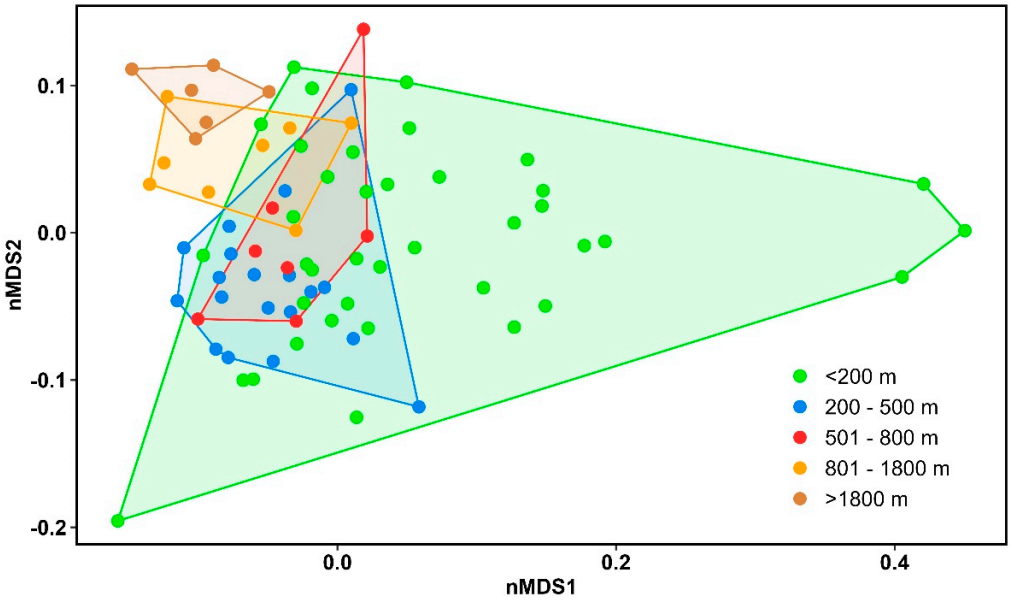


Figure 4. NMDS plot based on macroinvertebrate abundance data and grouped by altitude (m a.s.l.).

3.2. Relationship Between Biological Indices

The tested biotic metrics (normalised values) were strongly correlated with each other (Figure 5).

The correlation between used biological indices and metrics of the macroinvertebrate community (number of taxa and abundance) was not highly significant in any of the tested metrics and showed only a low R value. Significant results were achieved for the BI, ASPT, number of families, and Shannon–Wiener diversity H′ (Table 5). Two of them (no. of families and H′) are also included in the Hungarian multi-metric index HMMI.

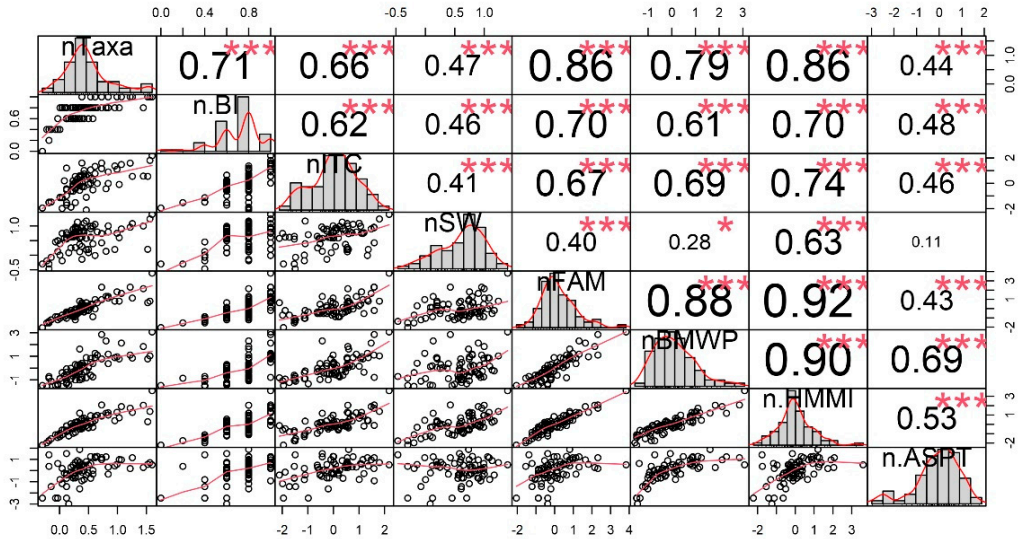


Figure 5. Correlations among selected biological indices quantified with the Spearman correlation coefficient. The level of significance is indicated by red stars. Abbreviations: nTaxa—number of recorded taxa per site; nBI—normalised biotic index; nITC—normalised index of trophic completeness; nSW—normalised Shannon–Wiener index of diversity; nFAM—normalised number of families; nBMWP—normalised biological monitoring working party; nHMMI—normalised Hungarian multi-metric index; nASPT—normalised average score per taxon). Significance levels: * $p < 0.05$, *** $p < 0.001$.

Table 5. Results of the Mantel tests between macroinvertebrate community composition (based on taxonomic composition and abundance) and biological indices. Mantel correlations were based on Spearman rank correlation. BI—biotic index; ITC—index of trophic completeness; BMWP—biological monitoring working party; ASPT—average score per taxon (=BMWB/number of families used for the BMWP); Shannon–Wiener Index H'– index of diversity. Significance levels: * $p < 0.05$, ** $p < 0.01$.

Indices	Correlation R	*p*	Significance
BI	0.124	<0.01	**
ITC	0.089	<0.1	
BMWP	0.111	<0.1	
ASPT	0.132	<0.01	**
Number of families	0.109	<0.1	*
Shannon–Wiener diversity H'	0.084	<0.05	*

Mantel tests based on Spearman rank correlations showed significant relationships between macroinvertebrate community composition and several of the environmental variables considered, including nutrient, hydromorphological and land-use variables (Table 6), although most associations were relatively weak. Electrical conductivity showed the strongest association with macroinvertebrate community composition. However, conductivity is influenced not only by anthropogenic pressures but also by natural differences among lake types, particularly in coastal and transitional waters, and should therefore not be interpreted as an unequivocal indicator of anthropogenic pressure.

Table 6. Results of Mantel tests between macroinvertebrate community composition and environmental pressure variables. Mantel correlations were based on Spearman rank correlation. Abbreviations: EC—electrical conductivity; TP—total phosphorus; VegRip—alteration in riparian vegetation at the sampling site; HabAlt—habitat alteration at the sampling site; CLC1—CORINE landcover class 1 (artificial); CLC_AgrIntensive—intensive and extensive agriculture in the catchment area; For details, see Section 2. Significance levels: ** $p < 0.01$, *** $p < 0.001$.

Pressure variable	Correlation R	*p*	Significance
Physicochemical parameters			
EC	0.54	<0.001	***
TP	0.19	<0.01	**
Hydromorphological pressures			
VegRip	0.14	<0.001	***
HabAlt	0.17	<0.001	***
Water level fluctuations	0.12	<0.01	**
Anthropogenic pressure—landscape	0.09	<0.01	**
Sum of all pressures	0.14	<0.001	***
CORINE landcover			
CLC1	0.34	<0.001	***
CLC_AgrIntensive	0.12	<0.01	**

3.3. Proposal of a Multi-Metric Index for Bulgarian Lakes (BMMI)

The relationships between the individual biological metrics and macroinvertebrate community composition were used to support the selection of suitable metrics for the BMMI. The Mantel analysis was not intended as an independent validation of the candidate metrics but to assess to what extent individual metrics reflect multivariate variation in the overall macroinvertebrate community. Based on these results, Shannon diversity and number of families were selected as component metrics. Although ASPT showed a stronger relationship with community composition than BMWP, BMWP was selected as the third metric because it had already been established in the Bulgarian assessment system and its inclusion was consistent with the structure of the Hungarian HMMI, which served as the methodological basis for the BMMI. Number of families and BMWP partly overlap in their information content, as BMWP inherently includes a richness component. However, BMWP additionally accounts for differences in taxon sensitivity and therefore provides information beyond family richness alone. The three-component metrics were normalised using the 10th and 90th percentiles as lower and upper anchor points (Table 7), respectively, and combined with equal weighting. Equal weighting was chosen as a simple and transparent approach and should not be interpreted as an empirically optimised weighting scheme. As percentiles rather than the observed minimum and maximum were used as anchor points, the influence of extreme values is reduced and values below the 10th percentile or above the 90th percentile may result in normalised values below 0 or above 1. Most metric values, however, fell within this range. The BMMI is then calculated as follows:

$$BMMI = (H^* + No.fam^* + BMWP^*)/3$$

Table 7. Anchor points used for the normalisation of biological metrics.

Metric	Lower Anchor	Ri Upper Anchor
Shannon–Wiener index (H′)	0.8748	2.4632
Number of families (No.fam)	7	20
Biological Monitoring Working Party (BMWP)	12.7	73.6

3.4. Pressure—Response Relationship

The linear regression model (p-value: 0.0008327) between the pressure index and the Bulgarian multi-metric index revealed very low (0.14) and below threshold R^2, which was considered as a prerequisite in the Intercalibration Guidance documents (0.25) (Figure 6). Following the standard practice and established practice in intercalibration exercises [42], linear regression was retained to ensure methodological consistency and comparability. Alternative non-linear models provided only moderately better fits and likewise did not reach the R^2 threshold of 0.25, without changing the general evidence for a significant pressure–response relationship.

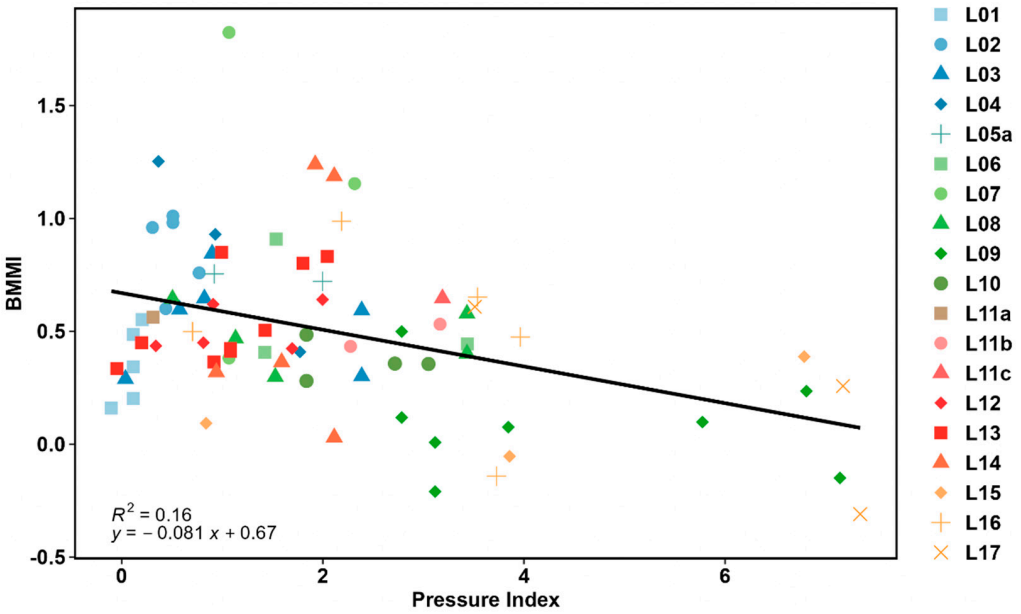


Figure 6. Correlation between the pressure index and the Bulgarian multi-metric index BMMI. For lake type codes, see Figure 1.

The high variability among lakes with comparable pressure indices is also partly due to the methodology (only one sample also from large lakes) and type-specific differences. An additional source of variability may be the relatively broad sampling period from May to November. Although current Bulgarian monitoring regulations do not prescribe a narrower sampling period for benthic macroinvertebrates in lakes, seasonal changes in community composition may contribute to variability in the assessment. Future refinement of the method should therefore consider whether a more narrowly defined sampling period could reduce this variability and improve the precision of the assessment.

The statistical approach recommended [42] is a linear mixed model to combine sites from different types and calculate type-specific offsets. This method assumes comparable (same slope) but shifted (different intercept) regression lines in single-type models. The analysis, however, made clear that the sample sizes are too small to give reasonable results. The outcome of this model was thus not directly used but adapted by expert judgement to define plausible type-specific offsets (Table 8). Given the limited number of sites per type, these offsets should be regarded as provisional rather than as independently validated type-specific corrections. They provide a pragmatic approach based on the available data and should be reassessed when additional monitoring data becomes available.

Table 8. Type-specific offsets defined to calculate the benchmarked values of the Bulgarian multi-metric index (cbBMMI) in lake types. For lake typology, see Figure 1.

Type	Offset	Type	Offset	Type	Offset
L01	–0.3	L08	0	L13	0.1
L02	0.4	L09	0	L14	0.3
L03	0.1	L10	0.1	L15	0
L04	0.3	L11a	0	L16	0.2
L05a	0.2	L11b	0.2	L17	0.3
L06	0.3	L11c	0.2		
L07	0.5	L12	0		

They were used to calculate benchmarked values of the Bulgarian multi-metric index, thereafter named cbBMMI. The linear regression model (p-value: 9.381×10^{-6}) between the pressure index and cbBMMI (Figure 7) showed a slightly higher fit with $R^2 = 0.23$ compared to 0.16 in the model with the uncorrected BMMI (Figure 6). Although statistically significant, the pressure–response relationship remained relatively weak, with the unadjusted model explaining only about 16% of the variation and the type-adjusted model 23%. The latter value remains slightly below the threshold of $R^2 = 0.25$ used as an orientation in this study. The results therefore indicate a pressure-related biological response but should not be interpreted as evidence of a strong pressure–response relationship.

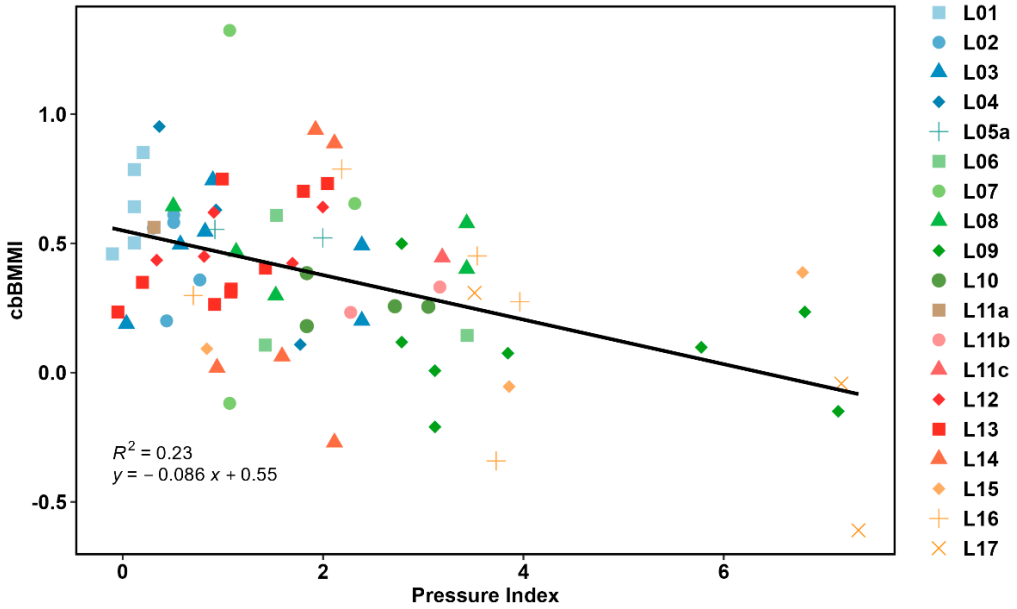


Figure 7. Correlation between the pressure index and the benchmarked Bulgarian multi-metric index (cbBMMI). For lake type codes, see Figure 1.

3.5. Reference Conditions and Class Boundaries for the Ecological Status

3.5.1. Reference Values

We defined a combination of two reference criteria:

- no heavily modified water bodies
- high or good ecological status as classified using the BQE phytoplankton

Applying these criteria the number of potential reference sites shrunk to six, covering only two lake types. In addition, Lake Shabla was excluded due to an extremely low BMMI value, which did not match the pressure situation (according to field data, 70% of the lake bottom was covered by *Cladophora*, which significantly affected the benthic community).

The remaining potential reference sites were Chernoto, Gergiisko, Bezbog, Kremensko and Ezeretsko lakes.

The calculated benchmarked values of the Bulgarian multi-metric index of these fixed sites ranged from 0.50 to 1.32 (median 0.786). Based on this small sample of potential reference sites, a reference value for the continuous-benchmarked BMMI at 0.8 was defined using expert judgement.

3.5.2. Class Boundaries

The H/G boundary was usually derived by defining a percentile in the reference sites as the boundary. As the number of reference sites in the case of BG lakes is low, this approach is not reasonable. Therefore, we recommend using the equal-distance approach not only for the G/M to P/B boundaries [41] but also for the H/G boundary. This results in EQR (ecological quality ratio) values at 0.8, 0.6, 0.4 and 0.2 (hence, identical to the nEQR as calculated in a separate step for rivers). Given the small number of potential reference sites, both the reference value and the resulting class boundaries should be regarded as provisional. They represent a pragmatic classification scheme based on the currently available data rather than statistically validated type-specific thresholds. Further validation and, where necessary, adjustment will be required when additional monitoring data becomes available. The outcome of this final calculation is summarised in Table 9.

Table 9. Type-specific values for the Bulgarian multi-metric index for all types of lakes/reservoirs (except L05 national type). cbBMMI—calculated benchmarked values of the Bulgarian multi-metric index BMMI; EQR—ecological quality ratio; H/G—high to good boundary; G/M—good to moderate boundary; M/P—moderate to poor boundary; P/B—poor to bad boundary. Values are presented for completeness for all types included in the analysis. For L09 and L10, the calculated values are not recommended for ecological assessment because the freshwater-based BMMI is not considered applicable to these mesohaline and hyperhaline types. Applicability to the oligohaline type L08 requires further validation. For heavily modified and artificial water bodies, the values shown do not represent directly applicable ecological status boundaries; their use in the assessment of ecological potential is discussed in Section 3.5.3.

Lake Type	BMMI				
	Ref	H/G	G/M	M/P	P/B
L01	0.50	0.40	0.30	0.20	0.10
L02	1.20	0.96	0.72	0.48	0.24
L03	0.90	0.72	0.54	0.36	0.18
L04	1.10	0.88	0.66	0.44	0.22
L05a	1.00	0.80	0.60	0.40	0.20
L06	1.10	0.88	0.66	0.44	0.22
L07	1.30	1.04	0.78	0.52	0.26
L08	0.80	0.64	0.48	0.32	0.16
L09	0.80	0.64	0.48	0.32	0.16
L10	0.90	0.72	0.54	0.36	0.18
L11a	0.80	0.64	0.48	0.32	0.16
L11b	1.00	0.80	0.60	0.40	0.20
L11c	1.00	0.80	0.60	0.40	0.20
L12	0.80	0.64	0.48	0.32	0.16
L13	0.90	0.72	0.54	0.36	0.18
L14	1.10	0.88	0.66	0.44	0.22
L15	0.80	0.64	0.48	0.32	0.16
L16	1.00	0.80	0.60	0.40	0.20
L17	1.10	0.88	0.66	0.44	0.22

For completeness, calculated values for L09 and L10 are retained in Table 9 because these types were included in the analytical dataset. However, they should not be interpreted as recommended classification boundaries. The freshwater BMMI is not considered appropriate for the transitional mesohaline and hyperhaline types L09 and L10, as their benthic invertebrate assemblages differ substantially from those of freshwater lakes and freshwater-based metrics such as BMWP are not appropriate for these communities. Applicability to the oligohaline type L08 remains uncertain and requires further validation.

For heavily modified and artificial water bodies, the values presented in Table 9 should not be interpreted as ecological status class boundaries. These water bodies require assessment of ecological potential, for which the BMMI may provide supporting biological information but cannot be applied using the reference-based status boundaries without further consideration.

3.5.3. Ecological Potential

The reference approach is not recommended for the classification of ecological potential based on benthic macroinvertebrates in lakes. Even the pragmatic approach of defining it as one class below ecological status (e.g., high status = good potential), as proposed for rivers, is not justified.

In the mitigation measures or Prague approach, good ecological potential is defined by removing measures only leading to slight changes in the biological conditions. For benthic invertebrates, the following set of mitigation measures is considered as most relevant and necessary to achieve the **maximum ecological potential**:

- Enhancement of shore/shallow habitats (especially in the littoral zone)
- Creation of secondary habitats
- Where possible, management of lake level by reducing the water level fluctuations and limiting the rate of lowering the water level (to allow large and mobile animals to follow the water)
- Mitigation of physicochemical conditions
- Management of lake use/ designation of protected areas
- Beneficial use of dredged material

Under **good ecological potential**, we propose the following set of mitigation measures:

- Enhancement of shore/shallow habitats (especially in the littoral zone)
- Creation of secondary habitats (at smaller scale than under maximum ecological potential)
- Where possible, management of lake level by limiting the rate of lowering the water level
- Mitigation of physicochemical conditions
- Management of lake use/designation of protected areas

4. Discussion

The large-scale and comprehensive study of macrozoobenthos in lakes and reservoirs in Bulgaria led to the development and validation of an appropriate, type-specific methodology for assessing the ecological status of standing water bodies. The applied procedures and stages followed the recommended steps at the European level [41,42]. Three metrics and indices were selected and combined into a Bulgarian multi-metric index, reflecting the specificity and typological diversity of lakes in Bulgaria. Such a tool for assessing benthic invertebrates is an essential contribution to the overall assessment of lake ecosystems, simultaneously considering 1. the diversity of macrozoobenthos in the littoral zone and 2. their response to the effects of combined pressures [10,12].

According to the EU WFD, macroinvertebrate-based assessment methods are required to reflect changes in the diversity, ratio of disturbance sensitive to less sensitive taxa and the abundance and taxonomic composition of benthic communities in rivers, lakes and transitional and coastal waters [1]. The significant relationship between the Bulgarian multi-metric index BMMI and the composite pressure index indicates that macroinvertebrate assemblages integrate the cumulative effects of multiple environmental pressures rather than responding to a single stressor. The multi-metric approach provides a pragmatic means of integrating different aspects of macroinvertebrate community structure into a single assessment value. However, the present analysis does not demonstrate statistical superiority of the BMMI over its individual component metrics.

Despite the relatively good applicability of some of the analysed metrics (Table 2), current research has shown various limitations and the impossibility of an objective and relevant assessment of standing water bodies' ecological state. The above made it necessary to apply an approach in which the advantages of several metrics are combined in one multi-metric.

The use of multi-metric indices in biological assessment has been globally applied as they incorporate various biological metrics at the different levels of ecological organisation and provide integrative assessments of ecological integrity. An additional challenge in creating and implementing multi-metric indices is the necessity of distinguishing natural variability from anthropogenic impacts [17,43]. Most of the known multi-metrics are taxonomic-based but some attempts to also incorporate the functional diversity in adjusted ones clearly distinguished least-disturbed sites from most-disturbed ones, exhibited high precision and low bias and showed a significant negative response to land uses, e.g., [44].

Up to now, a common practice in research studies in Bulgaria was for the BI to be used mostly for the assessment of rivers along with a supporting application of the metric TNT and RETI/PETI index. The mentioned metrics were also used for the purposes of the national monitoring of standing waters and the reporting of the status/potential of lakes and reservoirs at the European level, but a version for slow-flowing river stretches of BI was applied. The applicability of these indices in the present study was justified based on the correspondence of the results with those from prior studies for both BI and TNT (projects reports) or for the trophic index PETI [26,45]. The ecological status/potential evaluation of some of the biggest standing water bodies in Bulgaria based on macrozoobenthos has shown that further long-term studies are needed to allow the collection of data, which will help to develop an appropriate multi-metric assessment system for standing water bodies. An important condition for the ecological status/potential evaluation is to consider the type-specific characteristics of the studied lakes and reservoirs. The ecological assessment was based on % Oligochaeta, an adapted version of the Irish Q-scheme [35,38], PETI [26], an adjusted variant of Feeding type index [45] and TNT. EQR scales relevant to the metrics used were developed for the purposes of the national classification system [46,47]. Due to the varying salinity of the studied sites, a set of metrics that characterised both freshwater and coastal waters were used: TNT; adapted BI [8,48]; Shannon–Weaver diversity index calculated based on data from environmental assessment of coastal waters database; AMBI (a biotic index based on species sensitivity/tolerance, with diversity and richness according to the requirements of the WFD) and M-AMBI (a multi-metric index for assessing the ecological status of marine and transitional waters, integrating AMBI) [36,37].

Until 2020, TNT, the BI for slow-flowing river stretches, percentage of Oligochaeta, and PETI were applied to assess the ecological status of the studied water bodies [26]. The biotic index was defined as leading in the evaluation while the other metrics had a supportive role. Study on seasonal effects on the ecological status/potential of representative lentic water bodies, assessed through macrozoobenthos, (lakes and reservoirs in Ecoregion 7

Eastern Balkans, South Bulgaria) showed that the ecological status/potential evaluation depended on the type of standing water body (natural/heavily modified/artificial), environmental factors and different anthropogenic pressure, which caused changes in the aquatic ecosystems [49]. The lack of type-specific scales of the indices used, insufficiently long ranges of datasets on which to test the methods, as well as the anthropogenically induced fluctuations at the water level which affects the distribution of macrozoobenthos in the sampling littoral zone were the main difficulties for a more precise assessment of the ecological status/potential of standing water bodies in Bulgaria. The assessment systems need to be adapted to reflect environmental changes brought about by the prevalent stressors [50]. Most of the known assessment methods for benthic invertebrates not only adequately consider the main types of pressures (e.g., acidification, eutrophication or hydromorphological changes) but also their combinations, which are a prerequisite for effective comparison and harmonisation of class- boundaries [15].

During the intercalibration of the Eastern Continental lakes, the HMMI developed in Hungary was applied and accepted for Srebarna Lake (national type L5). It is a multimetric index calculated as the arithmetic mean of the metrics number of families, species diversity index (H′) and BMWP—all of them calculated as type-specific EQRs [51]. Hence, it makes little sense to directly use the HMMI for other lakes, even if it showed a significant correlation in the Mantel test. Different combinations of the BI, BMWP, ASPT and diversity/richness metrics did not clearly suggest a certain multi-metric index. Therefore, we proposed using the same combination of metrics as defined and intercalibrated for Lake Srebarna. However, the HMMI cannot be directly applied to the other lakes but must be calculated separately. In addition, we propose not calculating the multi-metric index as the arithmetic mean of the EQR values but rather as the means of the normalised metrics and calculating the EQR at the end. The other option has been used in the intercalibration and was accepted by the ECOSTAT group. A typical example in this regard is the Greek Lake Benthic macroinvertebrate index—GLBiI—which is expressed as the mean of the EQRs of its metrics [52]. Nevertheless, the determination of an EQR at a final step is more common among European classification methods and easier to calculate.

Even though conceptually based on the Hungarian HMMI, the Bulgarian multi-metric index BMMI was adapted and calibrated using Bulgarian monitoring data, making it suitable for national application while remaining consistent with European intercalibration principles. Since 2023, the Bulgarian multi-metric index has been legally regulated [24,53] and is currently applied in monitoring practices. An additional advantage of the proposed BMMI is its compatibility with the existing national monitoring programme. Since the component metrics are already routinely calculated by monitoring laboratories, implementation of the multi-metric approach would not require substantial changes in field or laboratory protocols, facilitating its future integration into the national ecological assessment system.

5. Conclusions

Despite the limitations described, the newly developed national multi-metric is compatible with the WFD and is recommended for use in assessing the ecological status of lakes and reservoirs in Bulgaria. A critical point in the proposed method was the insufficient dataset. Therefore, the application of the Bulgarian multi-metric index BMMI on a larger number of macrozoobenthos samples collected from all types of standing water bodies identified in Bulgaria is of key importance. This process has already started with the implementation of the annual national monitoring of standing waters. Thus, a strategy for "spatial and temporal averaging" could make the evaluation more reliable.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fishes11110612/s1: Table S1: Study sites with indicated geographic coordinates, altitude and lake type sorted by types of water body (artificial, heavily modified and natural) and altitude; Table S2: Values of the physicochemical parameters for the lakes during the sampling campaign. Abbreviations: water temperature (WT), electric conductivity (EC), oxygen concentration (DO) and saturation (O_2-sat), pH, total phosphorus (TP), orthophosphate (SRP), total nitrogen (TN), ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N) and biological oxygen demand (BOD_5).

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Abbreviations

The following abbreviations are used in this manuscript:

BQE	Biological quality element
WFD	Water Framework Directive
BI	Biotic index
TNT	Total number of taxa
PETI	Potamal trophic index
RETI	Rhithral trophic index
BMMI	Bulgarian multi-metric index
BMWP	Biological monitoring working party
ASPT	Average score per taxon
EPT	Ephemeroptera, Plecoptera, Trichoptera
ITC	Index of trophic completeness
HMMI	Hungarian multi-metric index
PTI	Pollution tolerance index
AMBI	AZTI's marine biotic index
TP	Total phosphorus
TN	Total nitrogen
NH_4-N	Ammonium
NO_3-N	Nitrate
NO_2-N	Nitrite

BOD_5	Biological oxygen demand
CLC	Corine landcover
EQR	Ecological quality ratio

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