THE GOULANDRIS NATURAL HISTORY MUSEUM
GREEK BIOTOPE/WETLAND CENTRE

MINISTRY OF ENVIRONMENT & ENERGY

REPORT

ON THE DEVELOPMENT OF THE NATIONAL METHOD FOR THE ASSESSMENT OF ECOLOGICAL STATUS OF NATURAL LAKES IN GREECE, WITH THE USE OF LITTORAL BENTHIC INVERTEBRATES

September 2020

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Ifigeneia Kagalou (Prof., Ecostat national representative) has contributed with comments to this report. Dr. Sebastian Birk has reviewed the report.

List of Contributors

Korina Argiriou: sorting and taxa identification

Maria Bozatzidou: taxa identification

Helena Hadjiharalambous: contribution to analyses and data processing

Eleni Fitoka: contribution to lake littoral habitat analysis

Dimitra Kemitzoglou: contribution to analyses and report writing – up

Efi Mavromati: sorting and identification, statistical analyses, index estimation, report writing - up

Giorgos Nakas: taxa identification

Valentini Navrozidou: sample sorting

Maria Skopa: TP analyst

Maria Tompoulidou: lake littoral habitat analysis

Vasiliki Tsiaoussi: contribution to analyses and report writing – up

Personnel of Greek Biotope/Wetland Centre: Water sampling.

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1. INTRODUCTION

This report discusses the development of a national method for the assessment of ecological status of natural lakes in Greece, based on the Biological Quality Element (BQE) "Benthic Invertabrate Fauna" from the littoral zone, the Hellenic assessment method for Lake Littoral Benthic invertebrate fauna (HeLLBI).

The HeLLBI method consists of metrics indicative of taxonomic composition and abundance, sensitivity/tolerance of taxa and taxa diversity. It addresses morphological alteration and eutrophication pressures.

Most lake assessment methods based on benthic macroinvertebrates to date, evaluate eutrophication and acidification; fewer methods assess morphological pressures on lake ecosystems and they are mostly based on benthic macroinvertebrates from the littoral zone (Poikane et al., 2016).

Greece has a national method for zoobenthos from the profundal zone (GLBiI - Greek Lake Benthic invertebrate Index), that addresses eutrophication, which is included in the 2018 Intercalibration Decision [Commission Decision (EU) 2018/229] (Ntislidou et al., 2018).

The development of the current assessment method, as described in this report, is based on data from the national water monitoring network. In particular, 109 littoral sampling sites in 21 lakes (29 lake years) have been surveyed for benthic invertebrates during the 2015-2018 sampling campaign.

Natural lakes in Greece are grouped into 3 types, according to the mixing regime and depth gradient[1]. As the sampling took place in the littoral zone, data from all lake types were pooled in the dataset.

In order to select the metrics for the national assessment method, relationships of individual metrics with indicators of morphological and eutrophication pressures were carried out (Annex, Table 1).

[1] There is one more inland saline lake, with distinct chemistry, which is considered as a type of its own (not included in the dataset).

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

Greece applies the Hellenic Lake Littoral Benthic Invertebrate Assessment System (HeLLBI) for natural lakes. HeLLBI is composed of 3 parameters, which showed significant relationship with morphological and eutrophication pressures (Annex I, Table 1). These are aggregated in a multimetric index, where all of them have equal weights and are divided by 3. These parameters are the following:

Relative abundance of Odonata (% of abundance classes)	Taxonomic composition and abundance
Average Score per Taxon	Sensitivity / Tolerance
Simpson Diversity index	Diversity

2.1. METHODS AND REQUIRED BQE PARAMETERS

Table 1. Overview of the metrics included in HeLLBI

MS	Taxonomic composition and abundance	Sensitivity/Tolerance	Diversity
GR	RA of Odonata (% of abundance classes)	Average Score Per Taxon (ASPT)	Simpson Diversity Index

The HeLLBI assessment method consists of three metrics as follows:

Relative abundance of Odonata

It is expressed as a percentage of abundance classes, in order to reduce the impact of extreme abundances on the calculated EQR. Seven abundance classes are discerned (AQEM CONSORTIUM 2002)[2] according to German Fauna Index (Bohmer et al., 2014).

Average Score Per Taxon

Taxa are attributed values, according to their sensitivity/tolerance to pollution, forming the BMWP index, mostly used in rivers. The ASPT equals the average of the scores of all macroinvertebrate taxa found divided by the total number of scoring taxa (Armitage et al., 1983).

Simpson Diversity Index

The metric Simpson Diversity Index is calculated using the following formula:

$D=1-(\Sigma n(n-1)/N(N-1))$

Where n = the number of individuals of a particular taxon
and N = the total number of individuals of all taxa

[2] Class boundaries for abundance classes: max taxa abundance 0-class 0, max taxa abundance 3-class 1, max taxa abundance 10-class 2, max taxa abundance 30-class 3, max taxa abundance 100-class 4, max taxa abundance 300-class 5, max taxa abundance 1000-class 6, taxa abundance>1000-class 7.

WFD compliance

Overall, the Hellenic Lake Littoral Benthic Invertebrate Assessment System for Natural Lakes (HeLLBI) meets the criteria needed for WFD compliance. Parameters for taxonomic composition and abundance, sensitivity / tolerance and diversity are assessed by the metrics described above. The three metrics are converted to Ecological Quality Ratios (EQRs) and the final EQR is calculated as an arithmetic average of the three metric scores, with 5 classes of ecological assessment (High, Good, Moderate, Poor, and Bad).

2.2. SAMPLING AND DATA PROCESSING

Overview

Table 2. Overview of sampling and data processing for HeLLBI assessment method.

Item	Description
Frequency per year	One sampling occasion, in spring (mid-March to Mid-May).
Sampling methods	In order to develop the method, samples were taken from unmodified sites, sites with "soft" anthropogenic modifications and sites with "hard" modifications (concrete walls etc). Sampling is carried out using the three-minute kick / sweep method with standard hand net (500 μm mesh size), at the littoral zone of the lake (up to 1.2 m depth of water). Sampling effort covers proportionally all accessible aquatic habitats within a stretch of 10–20 m, at each sampling site.
Data processing	Samples are sieved on site. Material is preserved in 70% ethanol solution. Sorting, identification and counting are carried out in the lab. Samples are preserved in vials containing 70% ethanol.
Level of identification	Family level. Oligochaetes as a subclass.

2.3. NATIONAL REFERENCE CONDITIONS

The standard approach by applying reference criteria was tried: results showed that although, in terms of morphology, there are un-impacted stretches of lake ecosystems with relatively low TP content, true anthropogenically un-impacted lake ecosystems, for both morphological alteration and eutrophication pressures, hardly exist. Due to the scarcity of resulting reference lakes and the lack of historical data, the reference values were derived from the national dataset (number of samples = 109) at 95th percentile of the distribution of the values of each metric. A similar approach was reported to be followed from the Slovakian method for Benthic Invertebrates of Very Large Rivers (XGIG Milestone 6, 2012). The resulting values were checked for suitability as reference conditions, with other methods (Bohmer et al. 2014).

The upper and lower anchors were set at 95th and 1th percentile respectively of all metric values in the dataset. The quality classes were calculated with equidistant division (Hering et al. 2006).

Conversion to Ecological Quality Ratios (EQRs)

The raw values of each metric were converted to Ecological Quality Ratio as follows (Eq. 1):

Equation 1

$$EQRi = \frac{LAKEi}{REF}$$

Normalization of EQRs

At this step each metric's Ecological Quality Ratio is converted to a normalized scale with equal class widths and standardized class boundaries, where the H/G, G/M, M/P and P/B boundaries are 0.8, 0.6, 0.4 and 0.2, respectively. This normalization is based on a linear interpolation between each class's upper and lower boundaries (Eq. 2):

Equation 2

$$\text{If} \quad EQRi \geq 1 \quad : \quad nEQRi = 1$$

$$1 \geq EQRi \geq EQR_{H/G} \quad : \quad nEQRi = \frac{(EQRi - EQR_{H/G})}{(1 - EQR_{H/G})} x0.2 + 0.8$$

$$EQR_{H/G} \geq EQRi \geq EQR_{G/M} \quad : \quad nEQRi = \frac{(EQRi - EQR_{G/M})}{(EQR_{H/G} - EQR_{G/M})} x0.2 + 0.6$$

$$EQR_{G/M} \geq EQRi \geq EQR_{M/P} \quad : \quad nEQRi = \frac{(EQRi - EQR_{M/P})}{(EQR_{G/M} - EQR_{M/P})} x0.2 + 0.4$$

$$EQR_{M/P} \geq EQRi \geq EQR_{P/B} \quad : \quad nEQRi = \frac{(EQRi - EQR_{P/B})}{(EQR_{M/P} - EQR_{P/B})} x0.2 + 0.2$$

$$EQR_{P/B} \geq EQRi \geq 0 \quad : \quad nEQRi = \frac{EQRi}{EQR_{P/B}} x0.2$$

EQR_i: Ecological Quality Ratio value for each metric, as calculated for a lake i;
$EQR_{H/G \text{ or } G/M \text{ etc.}}$: EQR values for the corresponding boundaries, as calculated during boundary setting;
$nEQR_i$: Normalized EQR value for the corresponding EQR value of each metric of lake i.

Rule of combination to a final score

The final value of the HeLLBI assessment method, is calculated as an arithmetic average of the normalized EQRs of the above three metrics (Eq.3):

Equation 3

$$HeLLBI = \frac{nEQR_{ASPT} + nEQR_{SIMPSON} + nEQR_{ODONATA}}{3}$$

$HeLLBI_i$:	Final value of HeLLBI assessment method, which is a normalized EQR for the assessment of lake i;
$nEQR_{ODONATA}$:	Normalized EQR value of % Odonata (% of abundance classes) for lake i.
$nEQR_{ASPT}$:	Normalized EQR value of ASPT for lake i;
$nEQR_{SIMPSON}$:	Normalized EQR value of Simpson Divesity Index for lake i.

As a result, the final score of HeLLBI can be assigned to an ecological status class according to Table 3.

Table 3. Final boundary values of HeLLBI assessment method.

Quality classes boundaries	Quality classes
[1-0.8)	High
[0.8-0.6)	Good
[0.6-0.4)	Moderate
[0.4-0.2)	Poor
[0.2-0]	Bad

2.5. PRESSURES ADDRESSED

Most assessment methods address eutrophication pressure. The HeLLBI assessment method, as already mentioned, addresses both morphological alteration and eutrophication pressures in Greek natural lakes.

In order to evaluate the performance of the method in assessing morphological alteration, the percentage of lake artificial shoreline was used as morphological alteration index. Lake shorelines were initially generated by the Corine Landuse Landcover (CLC) thematic layer (version 18, classes 4.1.1 and 5.1.2). Particular landcover/landuse types of special interest, such as human modifications and alterations, agricultural surfaces and natural areas, were discriminated and delineated through user photointerpretation and digitization using the Google hybrid basemap. The QGIS software was employed for the specific task. A linear regression model between HeLLBI and Artificial Alteration (percentage) was applied and the resulting coefficient of determination (R^2), Pearson's correlation coefficient (r) and p value (p) were estimated.

In order to evaluate the performance of the method in assessing eutrophication, total phosphorus concentration (Annual mean; TP) is used as the main proxy. A linear regression model was applied and the resulting coefficient of determination (R^2), Pearson's correlation coefficient (r) and p value (p) were estimated.

Table 4 shows the relationships between HeLLBI and a) Artificial Shoreline and b) TP, for Greek natural lakes. The respective pressure-response curves are given in Figures 1 and 2. Both relationships are statistically significant.

Table 4. Overview of the relationships between nEQR values and pressure indicator values (Artificial Shoreline and TP), according to linear regression.

Relationship	**n**	**r**	**R^2**	**p**	**Regression Equation**
HeLLBI~Artificial Shoreline	21	-0.54	0.29	0.01	y = -0.3724x + 0.6031
HeLLBI~TP	29	-0.56	0.32	0.00	y = -0.2225x + 0.928

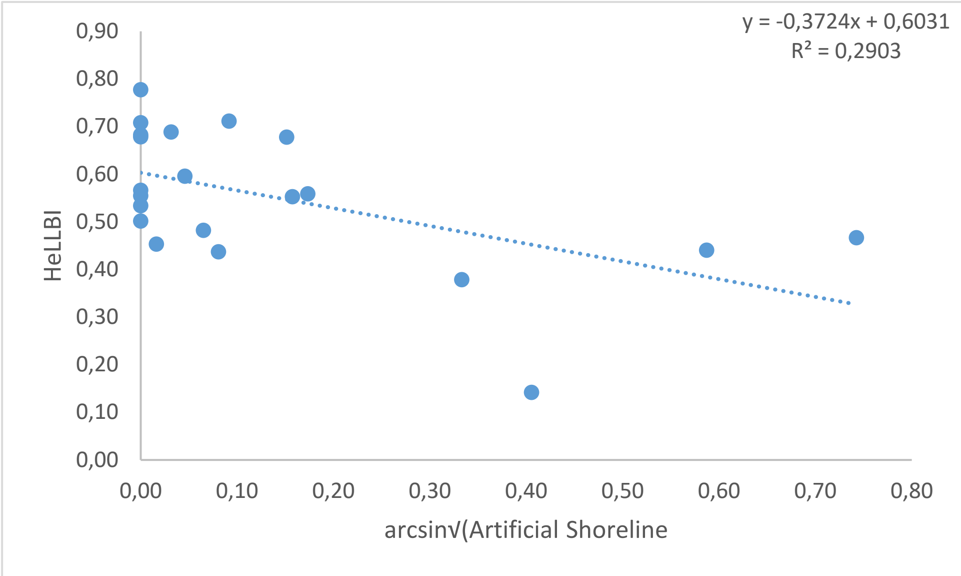

Figure 1. Pressure-response curve of HeLLBI in relation to Artificial Shoreline (percentage).

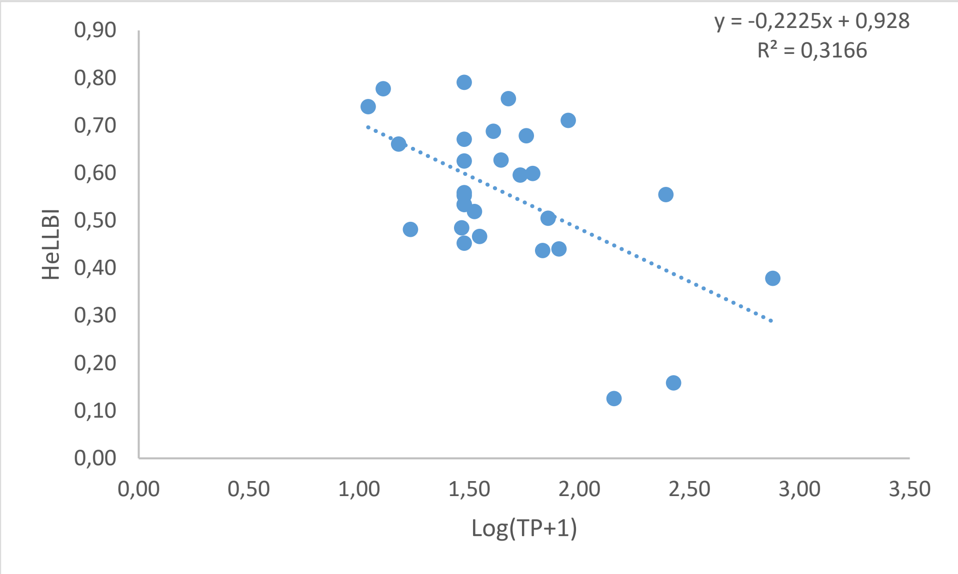

Figure 2. Pressure-response curve of HeLLBI in relation to Total Phosphorus.

3. WFD COMPLIANCE CHECKING

Table 5 summarizes the aspects of WFD compliance of HeLLBI assessment method.

Table 5. List of the WFD compliance criteria and the WFD compliance checking process and results of HeLLBI assessment method.

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES (Table 3)
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	YES (Section 2.4)
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	YES (Section 2.1)
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	NO, there are no intercalibration common types for MED-GIG natural lakes
The water body is assessed against **type-specific near-natural reference conditions**	The 95th percentile of the whole dataset was used (Section 2.3)
Assessment results are expressed as **EQRs**	YES (Table 3)
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES (Section 2.2)

All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES (Section 2.2)
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES (Section 2.2)

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.

There are no common intercalibration types for MED-GIG natural lakes.

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

The HeLLBI assessment method is significantly correlated with morphological alteration and eutrophication. The Spanish Invertebrates Index for Lakes, IBCAEL, is significantly correlated with eutrophication, organic enrichment and pollutants (Spain Lakes Benthic Fauna, IBCAEL, 2019). The Spanish Invertebrates Index for Ponds (Catalan region) responds to eutrophication and pollution by organic matter (Boix et al., 2005). The Benthic Quality Index for Italian Lakes addresses eutrophication (Boggero et al., 2017).

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods.

National assessment concepts in the Mediterranean are shown in the table below.

Table 6. Assessment concepts of Mediterranean methods.

Method	**Assessment concept**	**Remarks**
Method GR	%Odonata, ASPT, Simpson Diversity	Sampling in spring, from the lake littoral zone, using the three-minute kick / sweep method with standard hand net (500 μm mesh size), covering all representative aquatic habitats at each sampling site.
Method SP	IBACAEL index for Lakes	One sampling per year during summer for permanent lakes, during flooded season for temporary wetlands; in each sampling site, two complementary composite samples, one for crustacean abundance data for the ABCO index and one for the RIC index. In shallow lakes (≤1m) samples are taken from coastal and inner area, in lakes with depth≥1m, samples are from littoral zone.
Method SP-Catalan region	$QAELS_{2010}$ index for temporary and permanent shallow ponds	Sample collection twice a year at late winter (March) and at the end of spring (June) for permanent ponds and at the beginning of hydrologic period and before drying up for temporary shallow ponds, with a dip-net in the littoral zone.
Method IT	Benthic Quality Index for Italian Lakes	Biannual sampling; February to April and September to October by Ekman grab on soft bottoms. Each site is located along a transect in 3 different sampled areas (littoral, sublittoral and profundal).

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

During the Intercalibration exercise, MED-GIG countries had tried to find a statistically robust set of natural lakes belonging to the same type, but failed to do so. Thus, currently we are not able to check HeLLBI assessment method for its intercalibration feasibility.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

In order to describe the biological communities of benthic invertebrates in lakes of different ecological status, the national dataset with the monitoring data (109 sampling sites) was used. The ratio of sensitive to tolerant taxa was calculated (Annex, Table 2); taxa scores are defined for Greek rivers by Lazaridou et al. (2018) (Table A.5., Supplementary Information) for the calculation of Hellenic Evaluation Score (HES score). In particular, taxa are grouped to three categories based on their sensitivity, i.e. sensitive, medium tolerant and tolerant and attributed a score; sensitive taxa have the highest scores, followed by medium-tolerant ones and by tolerant taxa. Scores are further refined according to the relative abundance of taxa, at intervals of 0–1% (present), 1–10% (common), >10% (abundant). Scores are increased with respect to the relative abundance of sensitive taxa or decreased with respect to the relevant abundance of tolerant taxa.The sum of taxa scores was plotted against HeLLBI (Annex, Fig. 1). Moreover, a Simper Analysis was undertaken in order to tabulate taxa contributions to the average similarity of sampling sites within each ecological status (Clarke and Gorley, 2015) (Annex, Table 3).

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

Benthic macroinvertebrate communities at high status exhibit high diversity of taxa, including sensitive ones (e.g. from Odonata Libellulidae). Chironomidae are very abundant, followed by Oligochaeta, Gammaridae and Coenagrionidae.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

Benthic macroinvertebrate communities at good status slightly differ from undisturbed communities in composition and abundance. The number of families belonging to the Ephemeroptera and Odonata orders are still high, but the proportion of sensitive families to tolerant ones is now lower. Libellulidae are still present but in lower abundancies. Chironomidae are very abundant, followed by Corixidae and Caenidae.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

Benthic macroinvertebrate communities at moderate status are mainly composed of high numbers of Chironomidae, Oligochaeta, Gammaridae and Corixidae. The proportion of sensitive taxa to tolerant ones is now much lower.

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XGIG Large River Intercalibration Exercise WFD Intercalibration Phase 2: Milestone 6 report. 2012.

ANNEX

Table 1. Overview of the statistically significant relationships between metric values and pressure indicator values, according to linear regression.

Relationship	n	r	R^2	p
%Odonata classes~TP	29	-0.44	0.19	0.02
ASPT~TP	29	-0.41	0.17	0.03
ASPT ~Artificial Shoreline	21	-0.55	0.31	0.01
Simpson~TP	29	-0.47	0.23	0.01
Shannon~TP	29	-0.44	0.20	0.02
ETO~TP	29	-0.38	0.15	0.04
Gastropoda ~LUL Catchment[1]	21	0.47	0.22	0.03
Bivalvia~TP	29	-0.40	0.16	0.03

[1] LUL-catchment:Land-use index from the % of land uses in the lake catchment (1 × % extensive agriculture + 2 × % intensive agriculture + 4 × % urban areas) according to Poikane et al., 2016.

Table 2. Ratio of sensitive to tolerant taxa per quality class. Scores for sensitive and tolerant taxa are taken from Lazaridou et al. (2018) (Supplementary Information, Table A.5.).

No of samples	STATUS	Ratio Sensitive/Tolerant taxa
14	HIGH	**0.81**
34	GOOD	**0.57**
35	MODERATE	**0.25**
19	POOR	**0.04**
7	BAD	**0.00**

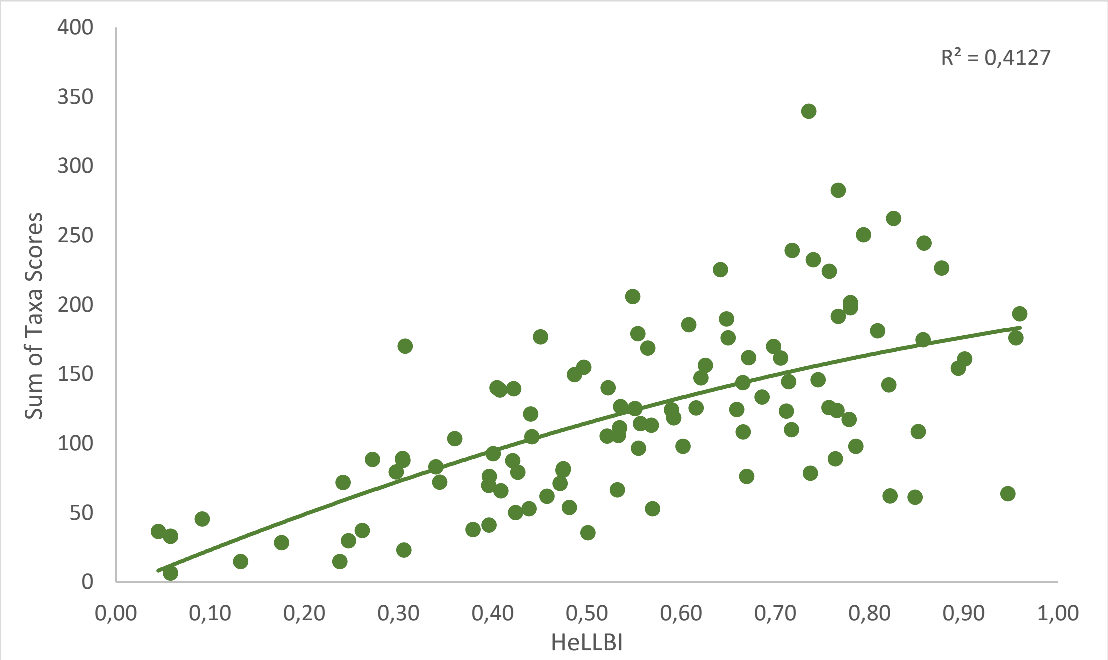

Fig. 1. Sum of taxa scores at lake sampling stations in relation to HeLLBI EQR values. Taxa scores are provided in Lazaridou et al. (2018) (Supplementary Information, Table A.5.).

Table 3. Summary table of the SIMPER results for benthic taxa contribution to similarity between sites (PRIMER 7 Software).

Ecological status: High, n=14 (Similarity: 35.92%)			Ecological status: Good, n=34 (Similarity: 44.92%)			Ecological status: Moderate, n=35 (Similarity: 39.33%)			Ecological status: Poor, n=19 (Similarity: 40.13%)			Ecological status: Bad, n=7 (Similarity: 69.84%)		
Taxa	%	%Cum.	Taxa	%	%Cum.	Taxa	%	%Cum.	Taxa	%	%Cum.	Taxa	%	%Cum.
Chironomidae	26.97	26.97	Chironomidae	26.79	26.79	Chironomidae	33.50	33.50	Chironomidae	46.10	46.10	Chironomidae	97.13	97.13
Oligochaeta	13.25	40.22	Corixidae	16.16	42.95	Oligochaeta	17.99	51.49	Oligochaeta	16.15	62.26			
Gammaridae	11.18	51.41	Caenidae	12.70	55.65	Gammaridae	16.95	68.43	Corixidae	15.75	78.01			
Coenagrionidae	8.96	60.37	Coenagrionidae	9.75	65.40	Corixidae	14.73	83.17	Gammaridae	11.93	89.93			
Corixidae	8.57	68.94	Gammaridae	8.17	73.57	Caenidae	6.22	89.39	Caenidae	3.41	93.35			
Caenidae	8.40	77.34	Oligochaeta	7.73	81.29	Asellidae	1.69	91.08	Ceratopogonidae	2.05	95.40			
Libellulidae	6.14	83.47	Atyidae	4.18	85.48	Erpobdellidae	1.54	92.62						
Atyidae	4.88	88.35	Baetidae	3.02	88.49	Hydracarina	1.27	93.89						
Dreissenidae	1.98	90.33	Asellidae	2.13	90.62	Atyidae	1.16	95.05						
Baetidae	1.87	92.20	Ceratopogonidae	1.52	92.15									
Asellidae	1.63	93.83	Lymnaeidae	1.38	93.53									
Physidae	1.22	95.05	Libellulidae	1.09	94.63									
			Dreissenidae	0.97	95.60									