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Intercalibrating the national classifications of ecological status for Eastern Continental lakes

Biological Quality Element: Benthic invertebrates

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Abstract

The European Water Framework Directive (WFD) requires the national classifications of good ecological status to be harmonised through an intercalibration exercise. In this exercise, significant differences in status classification among Member States are harmonized by comparing and, if necessary, adjusting the good status boundaries of the national assessment methods.

Intercalibration is performed for rivers, lakes, coastal and transitional waters, focusing on selected types of water bodies (intercalibration types), anthropogenic pressures and Biological Quality Elements. Intercalibration exercises were carried out in Geographical Intercalibration Groups - larger geographical units including Member States with similar water body types - and followed the procedure described in the WFD Common Implementation Strategy Guidance document on the intercalibration process (European Commission, 2011).

The Technical reports are organized in volumes according to the water category (rivers, lakes, coastal and transitional waters), Biological Quality Element and Geographical Intercalibration group. This volume addresses the intercalibration of the Eastern Continental Lake GIG Benthic invertebrate ecological assessment methods.

Three countries (Bulgaria, Hungary, Romania) participated in the intercalibration exercise and harmonised their lake benthic invertebrate systems. The results were approved by the WG ECOSTAT and included in the EC Decision on intercalibration (European Commission, 2018).

1. Overview of methods to be intercalibrated

Detailed method descriptions can be found in annex 1 for RO and annex 2 for HU.

Table 1. National benthic invertebrate assessment methods

Member State	Method	Status
BG	HU method adopted	Finalised formally agreed national method (Annex 2)
HU	Hungarian Macrozoobenton Multimetric Index for lakes ($HMMI_{\_lakes}$)	Finalised formally agreed national method (Annex 2)
RO	ECO-NL-BENT Romanian **eco**logical status assessment system for **n**atural **l**akes using **bent**hic invertebrates	Finalised formally agreed national method (Annex 1)

2. Checking of compliance of national assessment methods with the WFD requirements

All methods were evaluated as WFD-compliant (Table 2)

Table 2. National benthic invertebrate assessment methods

Compliance criteria	**Compliance checking conclusions**
1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	HU/BG: Yes RO: Yes
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	HU/BG: High-good boundary derived from metric variability at alternative benchmark sites (lower quartile). Good - moderate boundary derived from metric variability at full database sites (upper quartiles). The boundary setting was done on biological parameter level. The relevant metric boundaries were normalized to EQR values and used for the calculation of the composite index. RO: The boundary setting between classes was realized on the basis of statistical analysis, respectively: - The 75th percentile for the high status; - The 50th percentile for the good status; - The 25th percentile for the moderate status.
3. **All relevant parameters** indicative of the biological quality element are covered (Table 1 in the IC Guidance).	HU/BG: HMMI_lakes is the average EQR of 3 metrics: families no, SH-diversity index and BMWP $HMMI\_lake = \frac{EQR_{family} + EQR_{diversity} + EQR_{BMWP}}{3}$ RO: number of families, ET abundance, molluscs abundance, Orthocladiinae/Chironomidae abundance ratio, Shannon-Wiener diversity index, abundance of feeding types; combination rule: weighting of average parameters
4. Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the WFD	HU/BG: Yes, 5 national types, all belonging to common intercalibration type EC1 RO: Yes, 7 national types, all belonging to common intercalibration type EC1

Compliance criteria	Compliance checking conclusions
Annex II and approved by WG ECOSTAT	
5. The water body is assessed against **type-specific near-natural reference conditions**	HU/BG and RO: A combination of all approaches (RO and HU, e.g. near-natural reference sites, at least disturbed sites and statistical analyses) has been used to identify RC at national level.
6. Assessment results are expressed as **EQRs**	HU/BG: Yes RO: Yes
7. Sampling procedure allows for **representative** information about water body quality/ ecological status **in space and time**	HU: One or twice per year, March to October. Multihabitat sampling. 10 (low diversity habitat) or 20 (high diversity habitat (ex. more than 5 habitat). All available habitats in wadeable littoral waters are sampled. BG: Multi-habitat sampling. Surveillance monitoring is once every three years, which is not enough for intercalibration and detailed validation of methods with that one lake only. RO: Samples: 2 times/year. Multi-habitat procedure. At least 3 consecutive years for data acquisition.
8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	BG: Yes HU: Yes RO: Yes
9. Selected taxonomic level achieves adequate **confidence and precision** in classification	BG: species, general genus level HU: Species/species groups Genus level Family level Other level: Oligochaeta Family: Turbellaria, Chironomidae, Other Diptera Genus: Bivalvia (*Pisidium*), Odonata (juv), Heteroptera (juv), Trichoptera (juv.), Coleoptera Species, species groups: Bivalvia, Hirudinea, Mollusca (Gastropoda), Crustacea, Plecoptera, Ephemeroptera, Odonata, Megaloptera, Trichoptera, Heteroptera, Coleoptera RO: Species and at least genus level for all macroinvertebrates groups incl. Oligochaeta and Chironomidae

Both methods respond adequately to the pressures addressed (see descriptions of national methods in annexes 1 and 2)

3. Methods' intercalibration feasibility check

3.1. Typology

The intercalibration is **feasible for EC-1** lakes regarding typology (Table 3).

Table 3. EastCont lake typology

Common IC type	Type characteristics	MS sharing IC common type	Appropriate for IC types / subtypes
EC1	Lowland very shallow hard-water Altitude <200m Depth< 6m Conductivity 300-1000 (µS/cm) Alkalinity < 4 (meq/l HCO3)	BG HU RO	HU/BG method RO method

3.2. Pressures

Both assessment methods were designed to address similar pressures

- HU/BG Method: Organic and nutrient pollution, hydro-morphological pressures, recreational pressures, fish stocking
- RO Method: Nutrient loads, organic loads, general degradation (land use, fishing, banks morphology degradation etc.)

The Intercalibration is feasible in terms of **pressures** addressed, because both methods address the same pressures.

3.3. Assessment concept

Both methods follow typical lake assessment concepts:

- HU/BG Method: Eulittoral macroinvertebrates community, sampled by handnet, multihabitat sampling. Community characteristics are similar: species richness, species composition, diversity features, functional trophic groups;
- RO Method: Structural and functional macroinvertebrates characteristics are considered, for example, taxa composition, diversity, the presence/absence of some sensitive or ubiquitous animal groups, the prevalence of some groups, the functional groups etc.

All parameters considered focus on the community of the eulittoral zone of the lake.

4. Collection of IC dataset

All data were compiled in a common database (s. Annex 3 for database fields). All data are of the intercalibration types EC_1 (Table 4)

Table 4. Number of sites and samples for each country in the IC dataset

Member State	Biological data	Physico- chemical data	Other pressures
BG	1 lake / 3 samples	all samples	all samples
HU	20 lakes / 29 samples	all samples	all samples
RO	22 lakes, 230 samples	all samples	all samples

In order to cover the main pressures affecting the natural lakes in the lowland area, where the EC_1 lakes are located, data on nutrient and organic pollution, hydro-morphological pressures, the bank structure, recreational activities and aquaculture (fishing) were compiled. The values for these pressures range from 1 (no or very little human activity) to 5 (intensive human activity).
Also data on the land use regarding artificial lands, intensive agricultural lands, less intensive agricultural lands and natural or semi-natural lands were taken into account for the analysis. They were collected in %-values.
Within the GIG, it was agreed that the accepted taxonomic level was species level, where possible except for Oligochaeta and Chironomidae, which Hungary delivered on family level.

The following data acceptance criteria were used for the data quality control (Table 5):

Table 5. Data acceptance criteria

Data acceptance criteria	Data acceptance checking
Data requirements (obligatory and optional)	Compulsory physico-chemical, hydro-morphological and biological parameters as well as national type specification.
The sampling and analytical methodology	BG, RO, HU: littoral, 250-500 µm mesh-size handnet, 5-20 replicates, MHS technique, max. 1,5 m depth, standardized time (3 min.)
Level of taxonomic precision required and taxa lists with codes	As low as possible (species and genus level), family level for chironomids, higher groups for Oligochaeta
The minimum number of sites / samples per intercalibration type	N.A. (all together 43 lakes per IC type, minimum 1 site/WB, 1-2 samples/site)
Sufficient covering of all relevant quality classes per type	Only for HU and RO since BG had only one EC1 lake and used the HU method ➔ was treated like a HU lake
Other aspects where applicable	no

5. Benchmarking

In summary, we used :

- The full regression curve procedure ("continuous benchmarking"), but
- no common reference conditions
- no common alternative benchmark for intercalibration

Following are explanations and details to these benchmarking procedures and why the continuous benchmarking was chosen.

5.1. Reference conditions

The following criteria were used to identify reference sites (Table 6) in the attempt to derive reference conditions. According to these, **the intercalibration dataset did not contain a sufficient number of reference sites** to make a statistically reliable estimate.

Table 6. Reference criteria

Pressure type	**Criterion**
Diffuse source pollution	"Reference" threshold < 20% of intensive agriculture in the catchment area. "Rejection" threshold >50% of intensive agriculture in the catchment area (estimated from Corine data). Intensive agriculture between 20% and 50%: validation with physico-chemical parameters at the site scale.
Point source pollution	Not known point source discharge, or very localized impact with self purification. If point sources are present, a validation with chemical and biological parameters is necessary.
Water abstraction	Only very minor reductions in flow level changes, having no more than very minor effects on the quality elements.
Littoral vegetation modification	Only minor modification of the shoreline. Ratio of the natural littoral vegetation > 90%. Complete zonation of the macrophytes in the littoral zone.
Biological pressures	No biomanipulation. No invasive species, but alien species which are not at the invasive stage are tolerated.
Chemical pressures	TP: 130 µg l-1 TN: 1550 µg l-1 BOD: 2.5 mg l-1 If values are higher validation with chemical parameters is necessary.
Other pressures	No nearby intensive recreational use at the site scale: no regular bathing activities or motor boating. Occasional recreational uses (such as camping, swimming, boating, etc.) should lead to no or very minor impairment of the ecosystem.

5.2. Alternative benchmarking

The intercalibration dataset was checked for a comparable pressure window preferably near natural conditions. However we did not succeed in finding a sufficient number of comparable alternative benchmark sites to make a statistically reliable estimate, but an idea on the metrics and taxonomic communities of near natural lakes could be derived.
Therefore the alternative benchmark approach was not applied in favour of the continuous benchmarking approach (s. following chapter 6.3.).

The following **alternative benchmark sites** for each Member State were identified:

- RO: Lata and Tarova Lakes;
- BG: Potential Srebarna Lake, Biosphere Reserve (Annex 4);
- HU: Egyeki Holt Tisza, Lipóti Morotvató.

All lakes are shallow-lowland, below 200 m altitude, hard-water, within the Danube river catchment itself or main tributaries (Tisza) catchments.

The selection of the alternative benchmark was validated with biological data:
Diversities (Shannon-Wiener, number of families, number of taxa), absence or sporadic presence of *Asellus aquaticus*, absence or sporadic presence of invasive species within the Danube catchment were selected for this validation. Annex 5 presents additional pressures criteria affecting natural lakes used for alternative benchmark identification. Statistical analyses in Annex 6 and boxplots below. 1 represents the least impacted lakes (alternative benchmark) and 2 represents the impacted lakes.

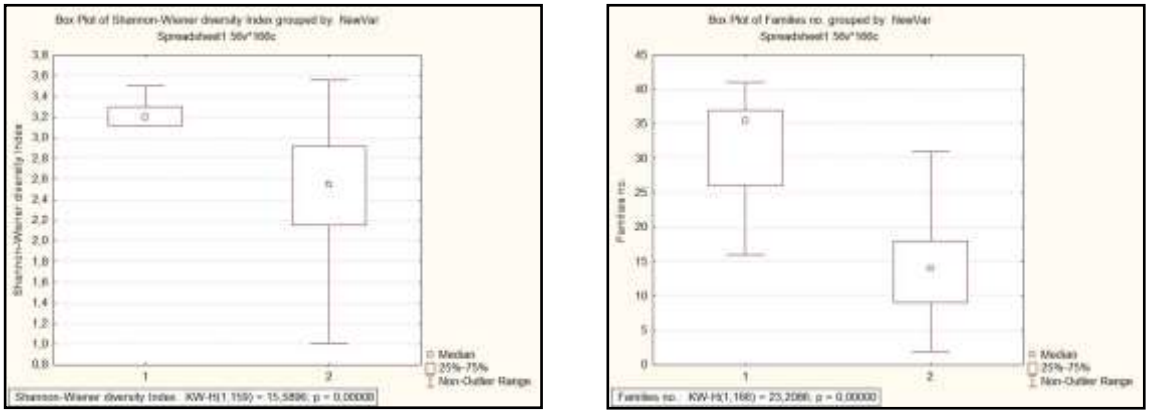

Fig 1. Comparison between the least impacted lakes (alternative benchmark) and the impacted lakes. 1 represents the least impacted lakes (alternative benchmark) and 2 represents the impacted lakes.

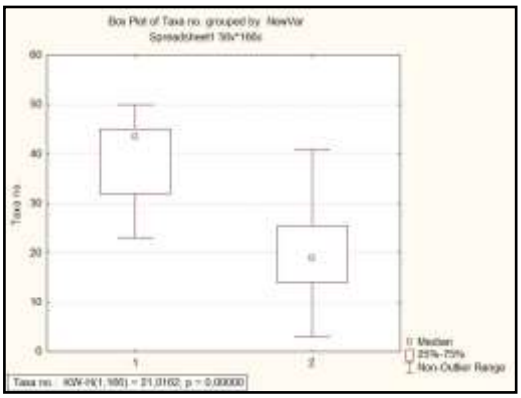

Fig 2. Comparison between the least impacted lakes (alternative benchmark) and the impacted lakes. 1 represents the least impacted lakes (alternative benchmark) and 2 represents the impacted

The **biological communities** at reference sites or at the alternative benchmark can be described as follows:

The biocoenosis typical for the **high status** of the stagnant water bodies are characterised by a high biodiversity of the present groups. The Chironomidae family is represented by species from the Chironominae group (*Tanytarsus sp., Einfeldia sp.*), Orthocladiinae (*Orthocladius sp., Cricotopus gr. silvestris*) and Tanypodinae (*Ablabesmyia longistyla, Tanypus punctipennis*), and the Oligochaeta are represented by species sensitive to various pressures (*Aelosoma tenebrarum, Criodrilus lacuum, Eiseniella tetraedra, Pristina longiseta, Stylaria lacustris*). The following species also form stable populations: *Dytiscus marginalis, Hydrophilus piceus*, *Noterus clavicornis* belonging to the Coleoptera, *Bithynia tentaculata, Lymnea stagnalis, Planorbarius corneus* belonging to the Gastropoda and *Coenanagrion puella, Lestes viridis, Libellula depressa* belonging to the Odonata. Due to the absence or presence of some minimal pressures, caused by fishing activities, agriculture and hydro-technical works, the populations of Heteroptera are represented by a high number of species (*Corixa punctata, Hydrometra stagnorum, Ilyocoris cimicoides, Ranatra linearis).*

5.3. Continuous benchmarking

Since sufficient reference or alternative benchmarks could not be found, **the whole dose response relationship was used as "continuous benchmark"**. Meanwhile this approach is well known in intercalibration. The adjustment is done by determining the standardisation offset for each type/country which adjusts it to the dose response regression for all types/countries together. This was done using Linear Mixed Models with the biological metrics as dependent variable, the combined pressure variable as covariates and the country as random factor. For this purpose the package 'lme4' of the 'R'-software was used. To obtain standardised metrics the offsets given by the model were subtracted from the metric values in most cases.

All in all we followed the same IC procedure used for the CB- and AL-GIGs in IC phase 2. Only the pressure variable differed:

A multivariate combined stressor index was used which was derived by a generalized linear model and took into account all available pressure information.

The equation was:

Combined stressor = 0.221-0.002*"Natural_norm"-0.002*"Landuse_norm" +0.044*Uses+0.395*"Total Phosphrous (mgP/L)"-0.014*"N-NH4 (mgN/L)" -0.124*"N-NO3 (mgN/L)"+0.035*"CBO5 (mgO2/L)"+0.007*"CCO-Cr (mgO2/L)" +0.673*"P-PO4 (mgP/L)"-0.319*"N-NO2 (mgN/L)"

where

"Natural_norm" = 5-[Natural and semi-natural areas (%)]/100*4

"Landuse_norm" = (4*[Artificial land use (%)]+2*[Intensive agriculture areas (%)] +[Low intensity agricultural areas (%)])/300*4+1

Uses= ([Hydromophological pressure]+[Recreational pressure]+[Fishing])/3

and

"Total Phosphorus (mgP/L)", "NH4 (mgN/L)", "N-NO3 (mgN/L)", "BOD (mgO2/L)",

"CCO-Cr (mgO2/L)", "P-PO4 (mgP/L)", "N-NO2 (mgN/L)"

are the measured values of the chemical parameters.

7. Design and application of the IC procedure

7.1. Choice of the appropriate intercalibration option.

There are differences in national lake types, species determination level (RO with, HU without chironomids), Sampled lake zones (RO included eulittoral – was changed in the last year), Sampling frequency (RO mostly 2x/year, HU mostly 1x), Month for first sample and also sampled area. All differences together would not allow option 1 or 3 intercalibration.

7.2. IC common metrics (When IC Options 2 or 3 are used)

The Common Multimetric Index (ICM) consists of three metrics:

- Shannon-Wiener index, representing diversity,
- BMWP, representing mainly tolerance,
- Percentage of dominating families, representing abundance and composition.

Metric were normalised using 10- and 90-%tiles of all metric values as anchors (Table below).

The normalised metric were averaged to obtain the intercalibration common metric (ICM).

Table 7. Metric normalization statistics

Metric	**Upper anchor** (= value close to reference condition)	**Lower anchor** (= approximate median value at bad status)
Shannon-Wiener index	3.0	1.46
BMWP	82.5	19.3
Percentage of dominating families	25.8	59.6

The correlations of the ICM with the national assessment EQRs are highly significant:

HU/BG R=0.89 P=0.0000

RO R=0.49 P=0.0000

The correlation for HU meets the R≥0.5 criterion. For RO the R-value is just below 0.5, but can be seen as sufficient as well, because the pressure gradient is shorter.

8. Boundary setting / comparison and harmonization in common IC type

The boundaries were set at national level, following the boundary setting protocols (see chapter 3 on compliance checking and annexes 1 and 2 for details within the detailed method descriptions). Therefore the average view was taken as common view of the boundaries.

8.1. Description of boundary setting procedure set for the common IC type

Not applicable, because boundaries were set on country level.

However, the table in the following chapter describes the differences between the status classes and compares it with the normative definitions of WFD Annex V. This was used to check the plausibility of the common view of the boundaries.

8.2. Description of IC type-specific biological communities representing the "borderline" conditions between good and moderate ecological status, considering possible bio-geographical differences.

The communities of macroinvertebrates typical for the **good status** of lakes make part of the Chironomidae, Bivalvia, Gastropoda, Heteroptera, Odonata si Oligochaeta families. A high specific biodiversity is noticed for each family present in these conditions of quality.

The Chironomidae family is well represented by species from the Chironominae, Orthocladinae and Tanypodinae subfamilies (*Dicrotendipes nervosus, Endochironomus dispar, Glyptotendipes barbipes, Lauterborniella agrayloides, Micropsectra praecox, Parachironomus arcuatus, Polypedilum nubeculosum, Cricotopus bicinctus, Clinotanypus nervosus, Procladius choreus*). The good conditions of quality allow for the development of a biocoenosis dominated by species of Oligochaeta (*Nais barbata, Nais communis, Limnodrilus udekemianus, Dero obtusa*), Bivalvia (*Anodonta cygnaea, Pisidium casertanum, Sphaerium corneum, Unio pictorum, Unio tumidus*), Gastropoda (*Physa acuta, Planorbis planorbis, Radix ovata, Viviparus acerosus*), Heteroptera (*Micronecta* sp., *Plea leachi, Notonecta viridis, Sigara lateralis*) and Odonata (*Coenagrion mercuriale, Ischnura elegans, Sympetrum striolatum*).

The community of macroinvertebrates specific for the **moderate** status is represented by species tolerant to various pressures, belonging to the Chironomidae group (*Chironomus plumosus, Cricotopus curtus, Cryptochironomus defectus, Procladius choreus*), Diptera (*Bezzia varicolor, Chaoborus albipes, Hemerodromia praecatoria, Psychoda cinerea*) and Oligochaeta with species that realize high densities (*Branchiura sowerbyi, Tubifex tubifex, Potamothrix hammoniensis*). Dominant populations within the biocoenosis are also the species *Theodoxus fluviatilis, Lithoglyphus naticoides, Valvata piscinalis* belonging to the gastropods.

The following graphs demonstrate the changes of relevant metrics within the status classes. They were used to derive the description below the graphs:

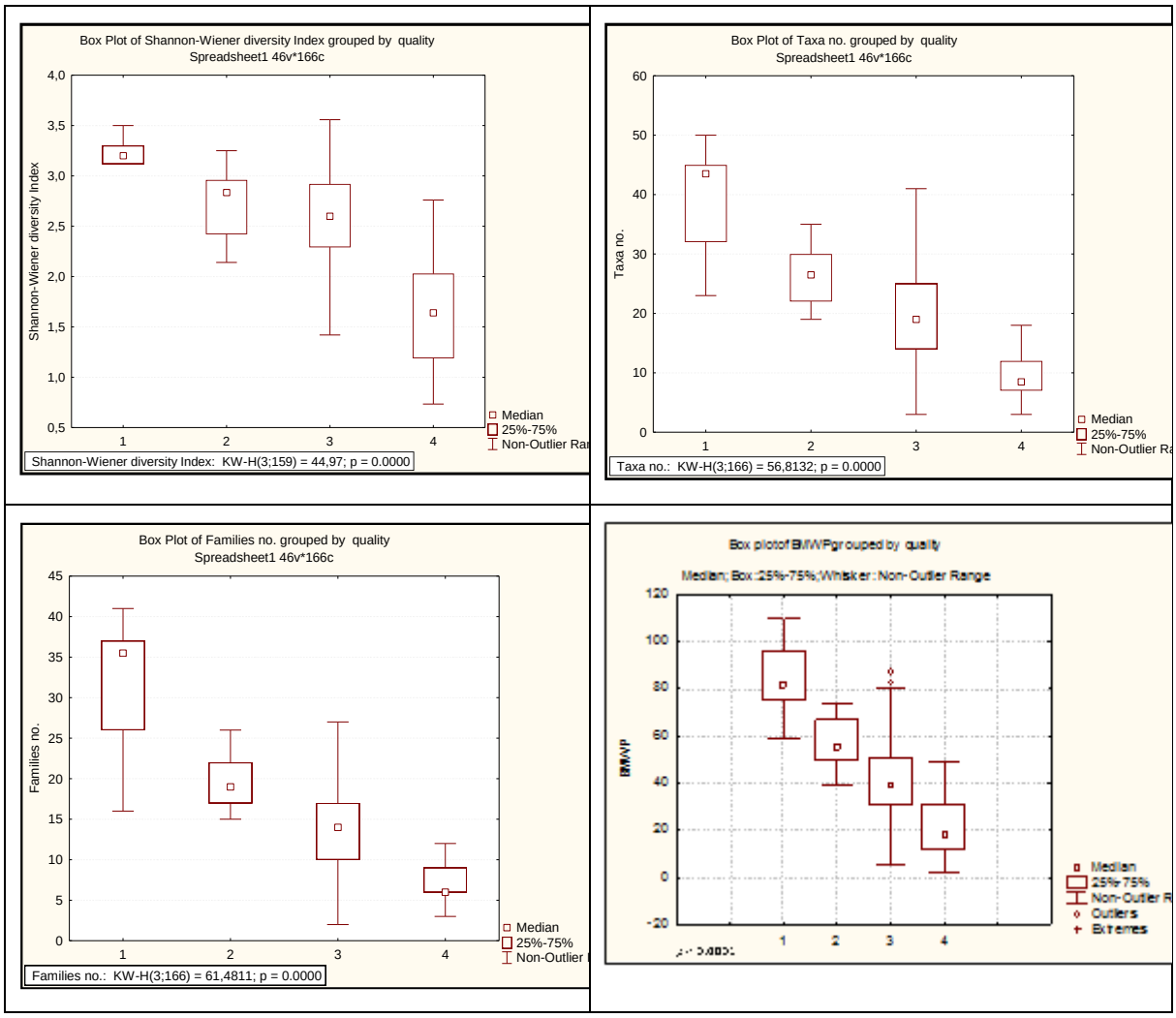

Figure 3. The changes of biological metrics within the status classes

Table 8. Description of biological conditions in the ecological status classes and comparison with the normative definitions in WFD Annex V for lake

Ecological status	Normative definition (WFD)	Interpretation
High EQR 0.8–1.0	The taxonomic composition corresponds totally or nearly totally to undisturbed conditions. The ratio of disturbance sensitive taxa shows no signs of alteration from undisturbed conditions. The level of diversity of invertebrate taxa shows no sign of alteration from undisturbed levels	Species diversity is high (Shannon-Wiener Diversity Index min. - 3,12). Balanced proportion of the macroinvertebrates taxa (no. of families – min. 26). High abundance of benthic fauna, but no dominant group. Presence of sensitive taxa (BMWP – min. 75). Presence of Odonata, Heteroptera, Trichoptera, Ephemeroptera, Gastropoda, Chironominae, Orthocladiinae, Tanypodinae, Naididae, Coleoptera taxa. The normalised value of the metric >0,8.
Good EQR = 0.6–0.8	There are slight changes in the composition and abundance of invertebrate taxa compared to the type-specific communities. The ratio of disturbance sensitive taxa shows slight sign of alteration from type-specific levels. The level of diversity of invertebrate taxa shows slight signs of alteration from type-specific levels	Slight decrease of all parameters. Shannon-Wiener Diversity Index is min. 2.53. No. of families is – min. 20. BMWP – min. 60. Some sensitive taxa could disappear due to anthropogenic impact. Taxa such as Chironomidae (Chironominae, Orthocladiinae, Tanypodinae), Bivalvia, Gastropoda, Heteroptera, Odonata and Oligochaeta (Naididae, Tubificidae) are presented in balanced populations. The normalised value of the metric >0.6.
Moderate EQR = 0.4–0.6	The composition and abundance of invertebrate taxa differ moderately from the type-specific communities. Major taxonomic groups of the type-specific community are absent. The ratio of disturbance sensitive to insensitive taxa, and the level of diversity are substantially lower than the type-specific level and significantly lower than for good status	Low diversity of benthic fauna (Shannon-Wiener Diversity Index is min. 2). Sensitive taxa could disappear. No. of families is min. 14. BMWP – min. 45. Chironomidae and Oligochaeta groups can be dominated. *Asellus aquaticus* (Isopoda) is characteristic to this status. Some species of Gastropoda and Bivalvia groups could be in large amounts of populations. The normalised value of the metric >0.4.

8.3. Boundary comparison and harmonization

Boundary comparison was carried out with the Intercalibration Excel Template Sheets (v1.24) for option 2.

Boundaries were compared using IC option 2 with a boundary translation against a common metric scale. Since the common metric was already standardised by continuous benchmarking, the offset was not established using benchmark sites, but was manually set to 0.

Boundary bias was >0.25 class equivalent in negative direction for both high/good (H/G) and good/moderate (G/M) boundaries for RO and in positive direction for HU for both eulittoral methods:

- RO_{HG} = -0.625, RO_{GM} = -0.474
- HU_{HG} = 0.484, HU_{GM} = 0.294.

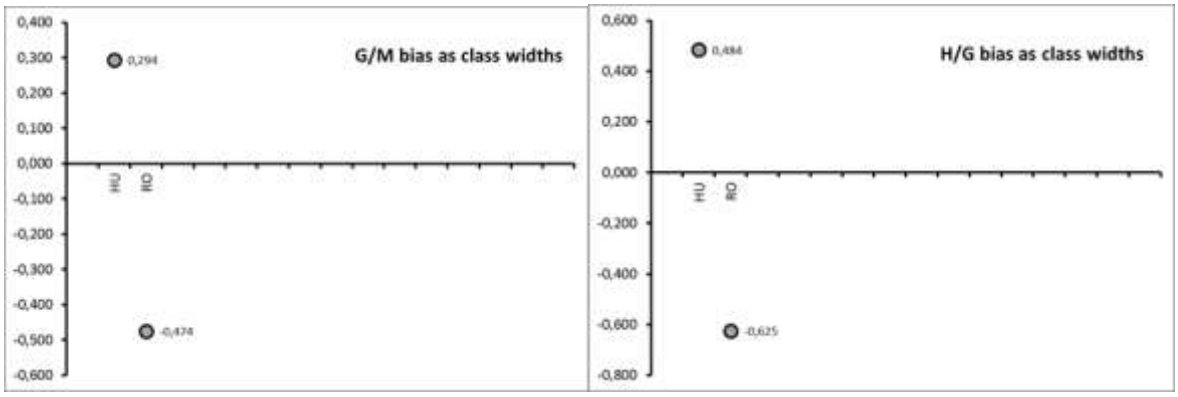

Figure 4. Boundary bias of comparison of national classification system

To account for the deviation of the acceptance band RO will have to raise the GM boundary as well as the HG boundary. For the HG boundary it is also necessary to raise the reference condition. RO agreed to do so.

For **Bulgaria** the only lake can be considered as a HU lake, which fits into the HU typology – assessed with the same method and same boundaries. So, the only question is, if the assessment fits into the HU lake assessments or not.

The graph below shows that the BG results fit in nicely:

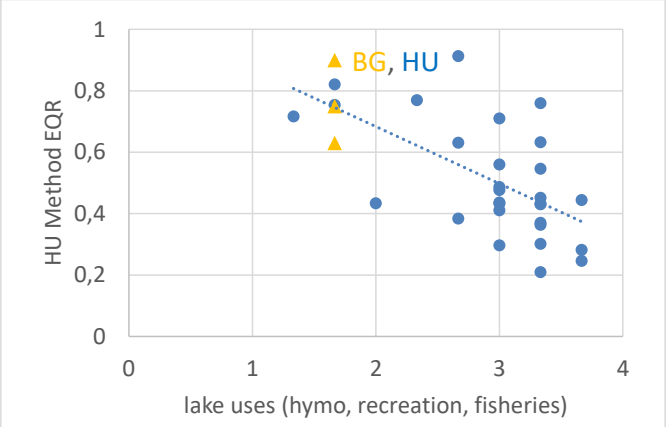

Figure 5. Relationship between pressure gradient and HU/RO assessment system

9. Conclusions

The following boundaries were intercalibrated:

Table 9. Initial boundaries of MS assessment systems

Member State	Classification	Ecological Quality Ratios	
	Method	High-good boundary	Good-moderate boundary
HU/BG	HMMI lakes	0.85	0.65
RO	ECO-NL-BENT	0.75	0.55

The following adapted boundaries resulted from the intercalibration exercise:

Table 10. Initial boundaries of MS assessment systems

Member State	Classification	Ecological Quality Ratios	
	Method	High-good boundary	Good-moderate boundary
HU/BG	HMMI lakes	0.85	0.65
RO	ECO-NL-BENT	0.93	0.60

The reference condition for RO has to be raised to 1.25.

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Annex 1: Romanian method description

ROMANIA - METHODOLOGICAL ASPECTS REGARDING THE ASSESSMENT OF THE ECOLOGICAL STATUS OF THE NATURAL LAKES BASED ON MACROINVERTEBRATES COMMUNITIES

Introduction

The macroinvertebrates are used for assessing the ecological status of the natural lakes, due to their numerous advantages. The described method is used for the monitoring activity.

The assessment method described below, based on the macroinvertebrates, is used exclusively for the natural lakes and complies with the requirements of the Water Framework Directive.

Sampling and analysis techniques

The aim of collecting data on the macroinvertebrate community is to facilitate the assessment of ecological status of lake water bodies.

The sampling method for the eulittoral zone of shallow lakes is generally handnet sampling that is specified by the international standard (SR EN ISO 10870:2012).

This method focuses on a multihabitat scheme designed to sample major habitats in proportional representation within a sampling area. Benthic macroinvertebrates are collected systematically from all available habitats by kicking the substrate with a handnet. A total of 5 - 20 subsamples are taken from all major habitats types based on expert judgment and diversity of habitats (covering 0,3 – 1,2 m^2 per sample). Sampling locations were at different distances from the bank but no more than 1 m depth.

The subsamples collected from the multiple habitats (from eulittoral zone) will be composited to obtain a single homogeneous sample.

The sampling period is from April to September/October, two times per year in each water body and section.

Sample treatment consists of:

1. The complete sample must be sieved through a coarse mesh of 500 µm.

2. The biological material is transferred to sample containers (1000 ml or larger).

3. Preserved either with formaldehyde to final concentration of the 4% or with ethanol having the final concentration of 70%.

4. Appropriate labelling of sampling containers or bags.

Macroinvertebrates samples collected by multihabitat method are processed in the laboratory under controlled conditions. Aspects of laboratory processing include subsampling, sorting, and identification of organisms.

As detailed as possible identification of the benthic invertebrates is recommended. Species level taxon list, whenever possible, can be used for calculation of the metrics/indices. The level of identification is: *Turbellaria, Oligochaeta, Hirudinea, Mollusca, Ephemeroptera, Heteroptera, Coleoptera, Trichoptera, Diptera* - genus, species level; *Crustacea, Megaloptera* - species, genus levels; *Odonata* – species level. Numbers are expressed as individual counts abundance per surface area (ind/m^2).

References condition/least disturbed conditions

The references were set out by identifying some natural, near natural or little impacted lakes, by statistical analysis of the existing data or by the expert's judgment.

In developing the national methodology, dates for 18 typologies of natural lakes, referring both to lakes from the mountain and plain area, have been processed. Some of these lakes were considered of reference, near natural or little impacted for those typologies and at national level. For example: Rosu Lake (ROLN17), Stiucilor Lake (ROLN16), Bucura Lake (ROLN18), Balta Lata Lake (ROLN01) or Tarova Lake (ROLN02) (Fig.1-4). In case of those typologies for which no references existed, alternative benchmarks or guide values for the reference status have been described (Fig. 5).

Fig, 1: Rosu Lake

Fig.2: Stiucilor Lake

Fig.3: Bucura Lake

Fig.4: Tarova Lake

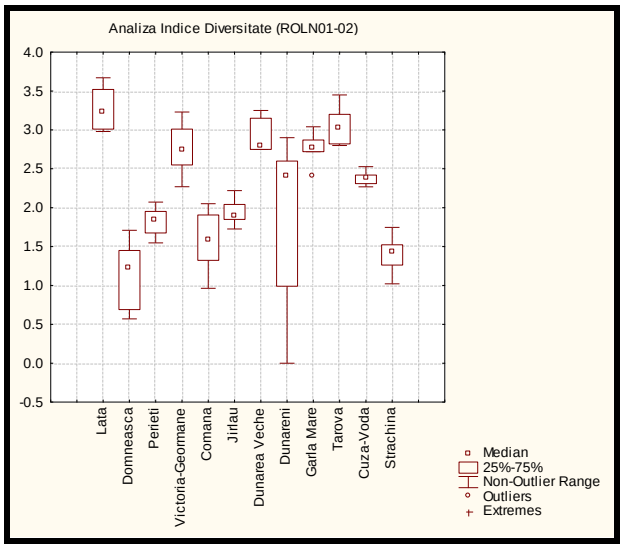


Fig. 5: Analysis model for the Diversity Index (ROLN01-02)

For setting out the alternative benchmark, the percentiles 90 for the least impacted sites were taken into account. Statistical analysis assessments are presented below, for 2 metrics: the diversity index and the number of families (Tables 1 and 2).

Tabel 1: Statistical analyses for Diversity Index

Type	Valid N	Mean	Confidence -90	Confidence +90	Median	Minimum	Maximum	Percentile 25%	Percentile 75%	Percentile 10%	**Percentile 90%**	Std-_dev
ROLN01+02	57	1.706070	1.901637	2.230503	1.986000	0.570000	3.670000	1.348000	2.307000	0.840000	**3.150000**	0.742257
ROLN03+04+05	80	1.451250	1.432842	1.737658	1.580000	0.190000	3.160000	0.750000	2.105000	0.450000	**2.780000**	0.819033
ROLN06	17	1.244706	1.193338	1.796074	1.480000	0.170000	3.270000	0.850000	1.607000	0.560000	**2.350000**	0.717176
ROLN10+11+12+13	29	1.699759	1.417520	1.981998	1.670000	0.332000	2.900000	0.634000	2.430000	0.451000	**2.880000**	0.893467
ROLN14T	14	1.240714	1.129138	1.690291	1.379500	0.533000	2.224000	0.754000	1.736000	0.456000	**2.125000**	0.592807
ROLN16	18	0.800069	0.846597	1.169540	1.081336	0.131692	1.546869	0.573012	1.082563	0.352335	**1.511359**	0.393805
ROLN17+18	23	1.252227	1.255921	1.708534	1.280000	0.212000	2.580000	0.968000	1.908000	0.608000	**2.440000**	0.632053

Tabel 2: Statistical analyses for no. of families

Type	Valid N	Mean	Confidence -90	Confidence +90	Median	Minimum	Maximum	Percentile 25%	Percentile 75%	Percentile 10%	**Percentile 90%**	Std-_dev
ROLN01+02	73	6.835616	7.165518	10.50571	5.000000	1.000000	41.00000	4.000000	10.00000	2.210000	**15.00000**	8.563511
ROLN03+04+05	84	6.059524	6.832010	9.28704	5.000000	1.000000	25.00000	4.000000	9.000000	2.000000	**12.00000**	6.763387
ROLN06	17	6.176471	3.975019	8.37792	5.000000	2.000000	24.00000	4.000000	8.00000	2.000000	**10.00000**	5.199892
ROLN10+11+12+13	31	6.270968	5.443883	8.29805	6.000000	1.000000	18.00000	3.000000	7.00000	2.000000	**11.00000**	4.681467
ROLN14T	14	4.285714	3.467434	5.10399	3.500000	2.000000	8.00000	3.000000	5.00000	2.000000	**6.00000**	1.728876
ROLN16	20	2.900000	2.326512	3.47349	3.000000	1.000000	6.00000	2.000000	3.50000	1.000000	**5.50000**	1.483240
ROLN17+18	24	5.000000	3.837413	6.16259	4.000000	1.000000	14.00000	3.000000	6.50000	2.000000	**11.00000**	3.323173

Each of the 6 proposed indexes is calculated (number of families, ET (Ephemeroptera-Trichoptera) abundance, the Shannon – Wiener diversity index, abundance of molluscs, Orthocladiinae/Chironomidae numerical ratio, functional groups), based on the species list from a monitoring section, in order to assess the ecological status based on the macroinvertebrates communities.

Pressures

The method description took into account the main pressures (organic pollution, nutrient pollution and general degradation) to which the communities of macroinvertebrates from the natural lakes respond.

The selection of the parameters used for assessing the ecological status of the natural lakes was made on the basis of the correlation between these parameters and the main pressures or stressing factors affecting the communities of macroinvertebrates. Correlations of the different metrics/variables (organic and nutrient pollution pressures) for the macroinvertebrates (with standardised data) with a single pressure index of those mentioned for the natural lakes for which data existed in the data base are provided (Fig. 6 and Fig. 7).

Boundary setting. Values of the metrics.

The method describes 5 ecological status. The results are expressed as EQR.

The boundary setting between classes was realized on the basis of statistical analysis, respectively:
- The 75th percentile for the high status;
- The 50th percentile for the good status;
- The 25th percentile for the moderate status;
- The 10th percentile for the poor status;
- What is below the 10th percentile for the bad status.

The discontinuities for boundary setting were used.

The tables 3 to 8 present the values for each of the indexes proposed for assessing the ecological status.

Table 3: Proposed values for the number of families

Type	High ecological status (min.)	Good ecological status (min.)	Moderate ecological status (min.)	Poor ecological status (min.)	Bad ecological status
ROLN01+02	10	7	4	2	>2
ROLN03+04+05	9	6	4	2	>2
ROLN06	8	6	4	2	>2
ROLN10+11+12+13	7	6	3	2	>2
ROLN14T	5	4	3	2	>2
ROLN16	4	3	2	1	>1
ROLN17+18	7	5	3	2	>2

Table 4: Proposed values for the Shannon - Wiener diversity index

Type	High ecological status (min.)	Good ecological status (min.)	Moderate ecological status (min.)	Poor ecological status (min.)	Bad ecological status
ROLN01+02	2,3	1,7	1,3	0,8	>0.8
ROLN03+04+05	2	1,4	0,7	0,4	>0.4
ROLN06	1,6	1,2	0,8	0,5	>0.5
ROLN10+11+12+13	2,4	1,6	0,6	0,4	>0.4
ROLN14T	1,7	1,2	0,7	0,4	>0.4
ROLN16	1	0,8	0,5	0,3	>0.3
ROLN17+18	1,9	1,25	0,9	0,6	>0.6

Table 5: Proposed values for the Orthocladiinae/Chironomidae (%)

Type	High ecological status (min.)	Good ecological status (min.)	Moderate ecological status (min.)	Poor ecological status (min.)	Bad ecological status
ROLN01+02	37	18	3	1	>1
ROLN03+04+05	45	29	17	5	>5
ROLN06	25	14	7	4	>4
ROLN10+11+12+13	27	17	8	4	>4
ROLN14T	14	12	9	3	>3
ROLN16	12	10	5	2	>2
ROLN17+18	36	23	12	2	>2

Table 6: Proposed values for the functional groups (%)

Type	High ecological status (min.)	Good ecological status (min.)	Moderate ecological status (min.)	Poor ecological status (min.)	Bad ecological status
ROLN01+02	36	25	10	1	>1
ROLN03+04+05	40	30	8	0	0
ROLN06	47	23	8	0	0
ROLN10+11+12+13	40	24	13	7	>7
ROLN14T	50	37	24	8	>8
ROLN16	51	35	18	8	>8
ROLN17+18	37	26	5	0	0

Table 7: Proposed values for the ET abundance (%)

Type	High ecological status (min.)	Good ecological status (min.)	Moderate ecological status (min.)	Poor ecological status (min.)	Bad ecological status
ROLN01+02	3	2	1	0	0
ROLN03+04+05	3	2	1	0	0
ROLN06	3	2	1	0	0
ROLN10+11+12+13	3	2	1	0	0
ROLN14T	3	2	1	0	0
ROLN16	3	2	1	0	0
ROLN17+18	8	3	2	0	0

Table 8: Proposed values for the abundance of molluscs (%)

Type	High ecological status (min.)	Good ecological status (min.)	Moderate ecological status (min.)	Poor ecological status (min.)	Bad ecological status
ROLN01+02	26	5	1	0	0
ROLN03+04+05	49	14	3	0	0
ROLN06	75	45	15	3	>3
ROLN10+11+12+13	34	12	4	1	>1
ROLN14T	1,5	1	0	0	0
ROLN16	67	45	10	1	>1
ROLN17+18	3	2	1	0	0

The multimetric index is calculated. For the selected indexes, a weighting of their importance was proposed for the communities of macroinvertebrates and for the assessment of the ecological status, as follows:

–Number of families (FAM)	15%	
– Shannon-Wiener diversity index (ID)		30%
–Orthocladiinae/Chironomidae numerical ratio (IOC)		20%
–Functional groups (IGF)	15%	
–ET abundance (IET)	10%	
–Abundance of molluscs (IMo)		10%

The value of the multimetric index, which has to be situated between 0 and 1, will indicate the ecological status. In order to establish the ecological status, it is recommended to divide the variation field of the multimetric index values in 5 parts, as follows:

	Value
–High ecological status	min. 0.75
–Good status	min. 0.55
–Moderate status	min. 0.30
–Poor status	min. 0.18
–Bad status	max. 0.18

When several sections exist, the multimetric index for each section is calculated, then the index is averaged and the ecological status of the lake/water body is established. Furthermore, if there are several seasonal results for a lake/water body, the annual average of the multimetric index is calculated and the ecological status is established.

Correlations among EQR and metrics are shown in the graphs below (fig. 10 - 14)

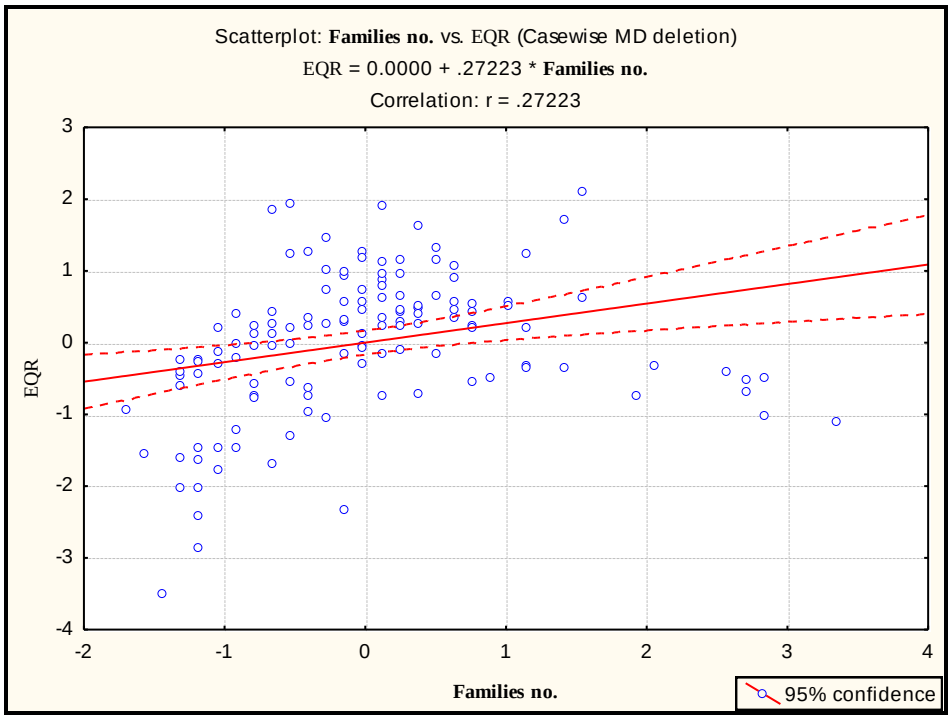


Fig.10

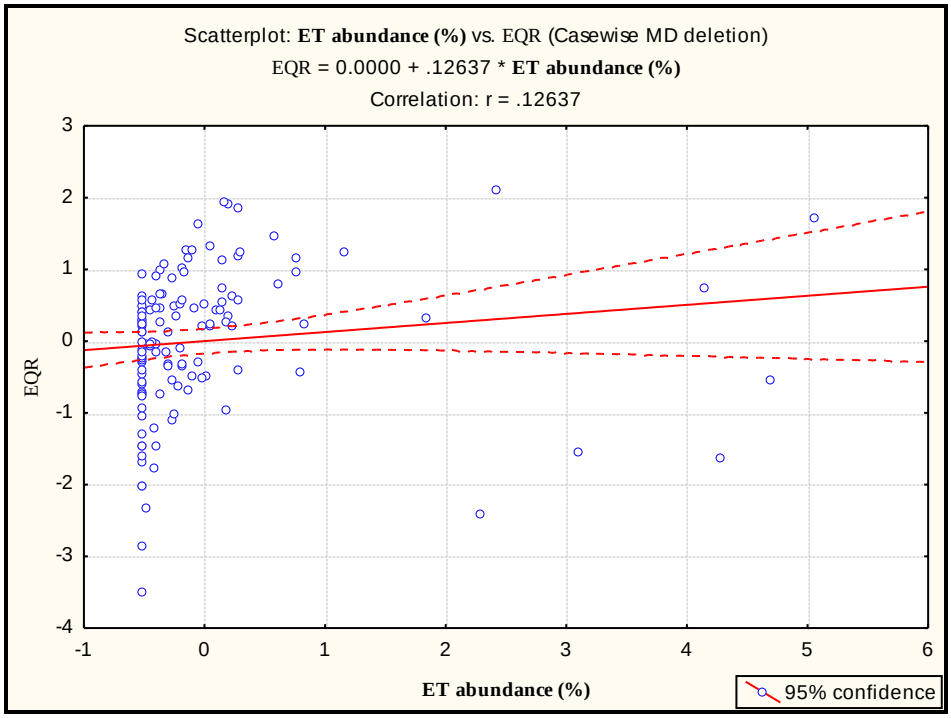


Fig.11

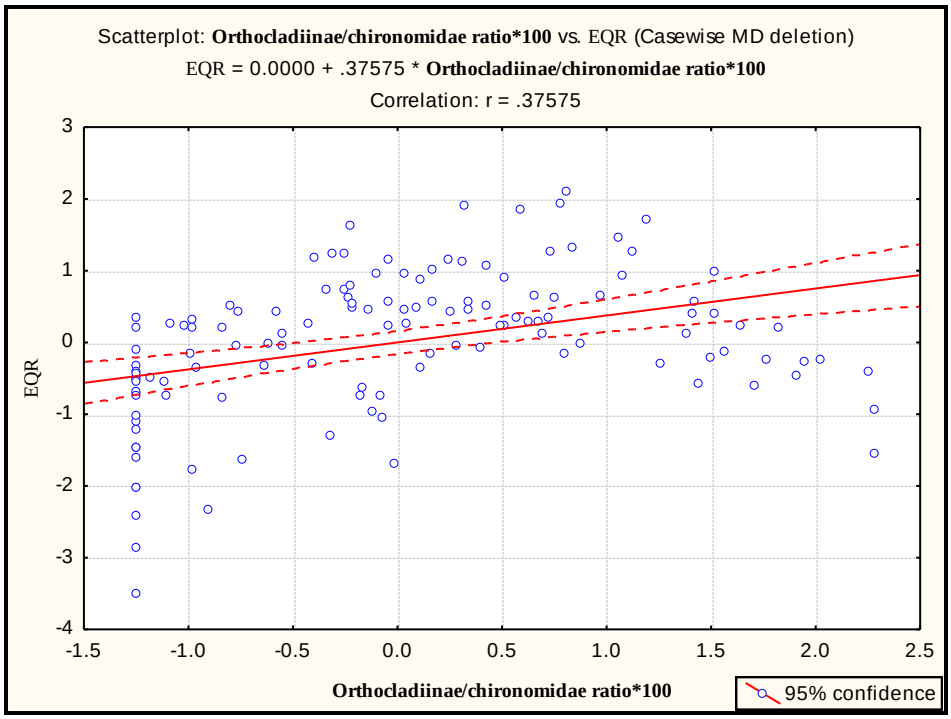


Fig.12

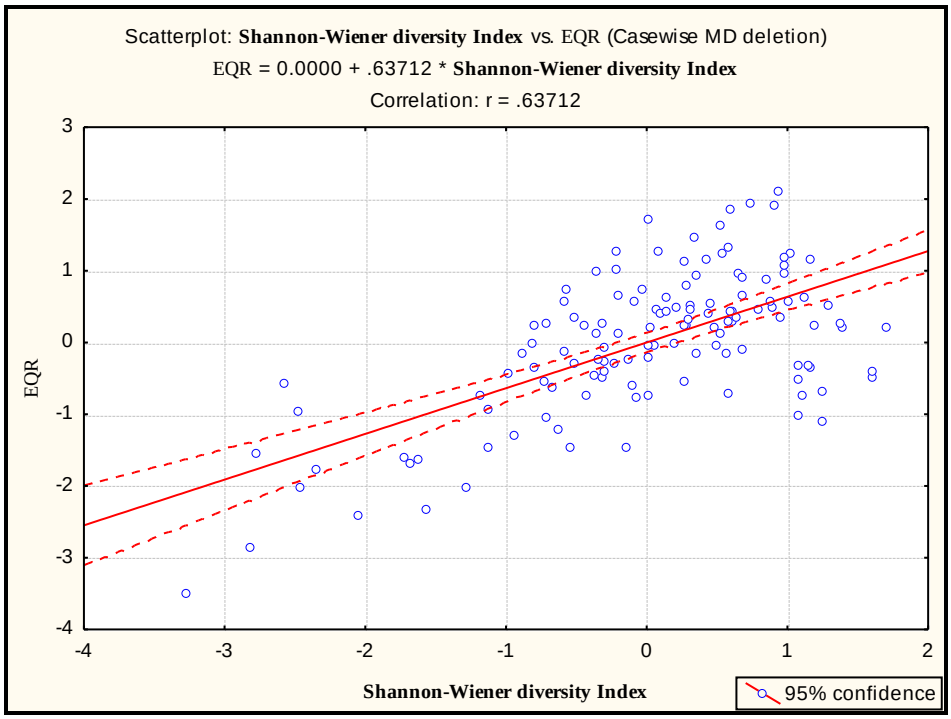


Fig.13

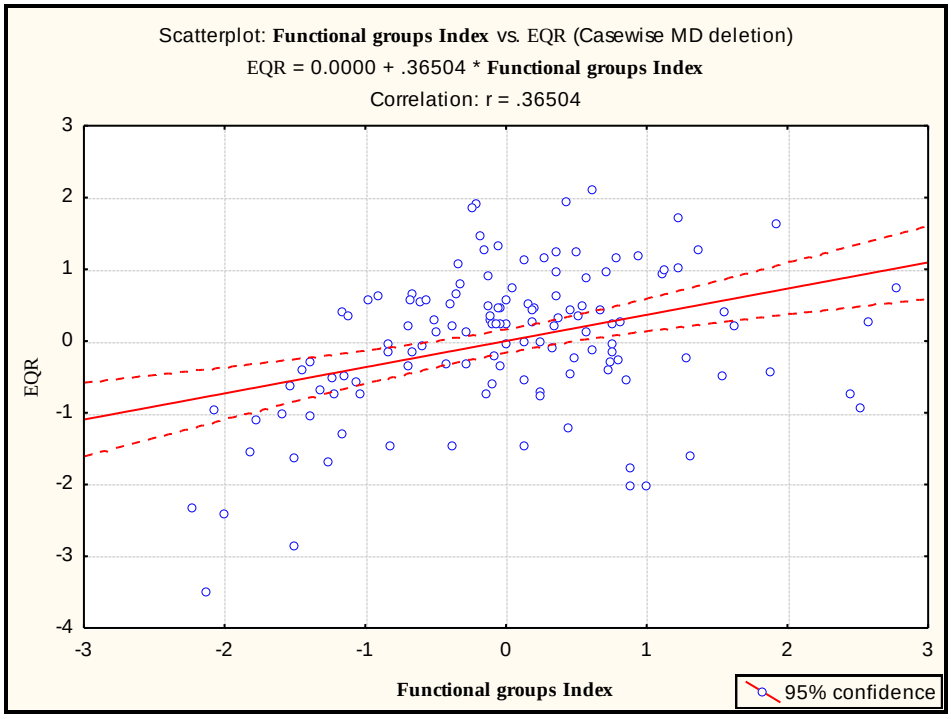


Fig.14

Asellus aquaticus is a species that also characterizes the moderate status in case of lakes. *Asellus aquaticus* is present in the lakes from different areas, but it does not appear in the typologies ROLN 14-18, from the hill and mountain areas. It was noticed that the higher numerical abundance of *Asellus* (15-46%, corresponding to densities of 39 – 148 ex/m^2) is correlated with

the decrease of the diversity index (1.29 – 1.83), which reflects the degradation of the lake ecological status. Taking into account the presence of this crustacean in the degraded lakes in the plain area, its abundance was considered in setting the boundaries for the Shannon-Wiener diversity index (Fig. 15 and 16). The value of the diversity index for the high status in case of lakes from the plain region is 2.4. The boundary of the entire range of values (1.6) is the value of the good status. Under this boundary, the status is moderated. All these values are also correlated with the main pressures (nutrient load, for example).

The characterization of the communities of invertebrates from the natural lakes for the high, good and moderate status was also realized. The main aspects are presented below:

The biocoenosis typical for the **high status** of the stagnant water bodies are characterized by a high biodiversity of the present groups. The Chironomidae family is represented by species from the Chironominae group (*Tanytarsus* sp., *Einfeldia* sp), Orthocladiinae (*Orthocladius saxicola, Cricotopus gr. silvestris*) and Tanypodinae (*Ablabesmyia longistyla, Tanypus punctipennis*), and the Oligochaeta are represented by species sensitive to various pressures (*Aelosoma tenebrarum, Criodrilus lacuum, Eiseniella tetraedra, Pristina longiseta, Stylaria lacustris*). The following species also form stable populations: *Dytiscus marginalis, Hydrophilus piceus, Noterus clavicornis* belonging to the Coleoptera, *Bithynia tentaculata, Lymnea stagnalis, Planorbarius corneus* belonging to the Gastropoda, *Coenanagrion puella, Lestes viridis, Libellula depressa* belonging to the Odonata.

Due to the absence or presence of some minimal pressures, caused by fishing activities, agriculture and hydro-technical works, the populations of Heteroptera are represented by a high number of species (*Corixa punctata, Hydrometra stagnorum, Ilyocoris cimicoides, Ranatra linearis).*

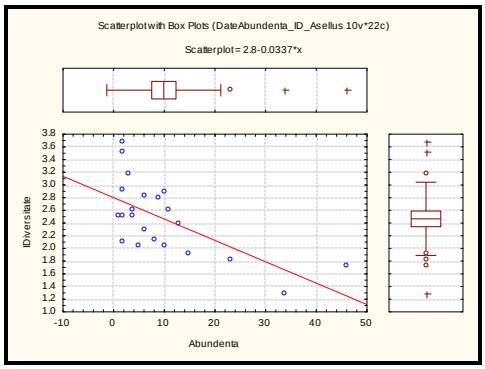


Fig.15: Correlations between the isopod Asellus and the diversity index

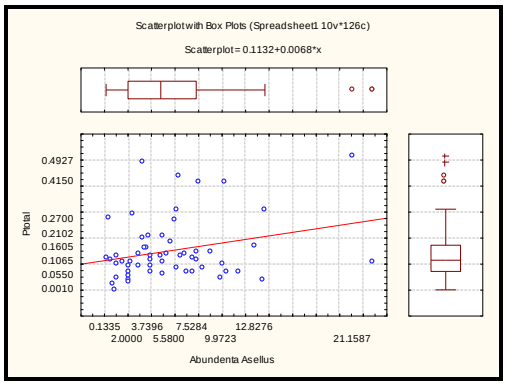


Fig.16: Correlations between the isopod Asellus and the total phosphorus concentration

The communities of macroinvertebrates typical for the **good status** of lakes make part of the Chironomidae, Bivalvia, Gastropoda, Heteroptera, Odonata and Oligochaeta families. A high specific biodiversity is noticed for each family present in these conditions of quality.

The Chironomidae family is well represented by species from the Chironominae, Orthocladiinae and Tanypodinae subfamilies (*Dicrotendipes nervosus, Endochironomus dispar, Glyptotendipes barbipes, Lauterborniella agrayloides, Micropsectra praecox, Parachironomus arcuatus, Polypedilum nubeculosum, Cricotopus bicinctus, Clinotanypus nervosus, Procladius choreus*). The good conditions of quality allow for the development of a biocenosis dominated by species of Oligochaeta (*Nais barbata, Nais communis, Limnodrilus udekemianus, Dero obtusa*), Bivalvia (*Anodonta cygnaea, Pisidium casertanum, Sphaerium corneum, Unio pictorum, Unio tumidus*), Gastropoda (*Physa acuta, Planorbis planorbis, Radix ovata, Viviparus acerosus*), Heteroptera (Micronecta sp., *Plea leachi, Notonecta viridis, Sigara lateralis*) and Odonata (*Coenagrion mercuriale, Ischnura elegans, Sympetrum striolatum*). In the lakes considered as having a good status are also presented species from the Trichoptera group (*Ecnomus tenellus, Limnephilus affinis, Limnephilus hirsutus*) and Ephemeroptera (*Caenis* sp and *Cloeon* sp.).

The community of macroinvertebrates specific for the **moderate** status is represented by species tolerant to various pressures, belonging to the Chironomidae group

(*Chironomus plumosus, Cricotopus curtus, Cryptochironomus defectus, Procladius choreus*), Diptera (*Bezzia varicolor, Chaoborus albipes, Hemerodromia praecatoria, Psychoda cinerea*) and Olighochaeta with species that realize high densities (*Branchiura sowerbyi, Tubifex tubifex, Pothamothrix hammoniensis*). Dominant populations within the biocenosis are also the species *Theodoxus fluviatilis, Lithoglyphus naticoides, Valvata piscinalis* belonging to the Gastropoda, as well as *Asellus aquaticus,* belonging to the Isopoda, that can have relatively high densities.

Pressure response of assessment results

A generalized linear model was built to predict the biological EQR-values from the abiotic pressure variables (water chemistry, land use, hydromorphology, recreational lake utilisation and fisheries). Using all intercalibration data and the Romanian assessment results for all sites the best subset was selected by the R2-values.

The resulting prediction equation for the Romanian EQR was:

"RO_EQR"=.717-.004*"Natural_norm"+.006*"Landuse_norm" -.0486*Uses-.186*"Total Phosphorus (mgP/L)"-.0713*"N-NH4 (mgN/L)"+.249 *"N-NO3 (mgN/L)"+.0617*" BOD (mgO2/L)"-.030*"CCO-Cr (mgO2/L)"-.761 *"P-PO4 (mgP/L)"+.007*"N-NO2 (mgN/L)"

	Test of SS Whole Model vs. SS Residual (Spreadsheet79)										
Dependnt Variable	Multiple R	Multiple R2	Adjusted R2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
RO_EQR	0.595380	0.354477	0.287235	0.750871	10	0.075087	1.367378	96	0.014244	5.271668	0.000004

In order to obtain a combined stressor variable ranging from 0 to 1, we subtracted the values from 1. The resulting R2 was 0.35 (R= -0.60) for all data and R2= 0.29 (R= -0.54) for RO sites (Fig. 17).

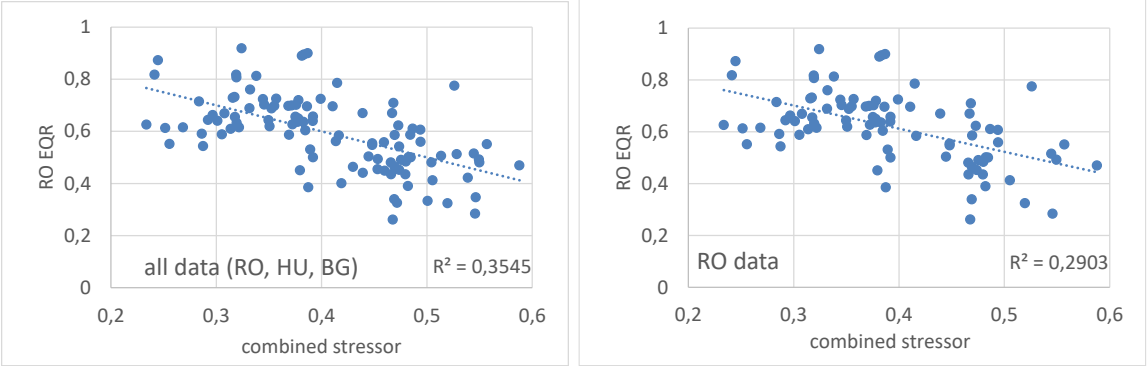


Fig.17: Correlations between RO assessment and combined stressor variable

Annex 2: Hungarian method description

HMMI lakes
Hungarian Multimetric Index for Lakes

1. General information

The macroinvertebrates are used for assessing the ecological status of the Hungarian lake types.

The assessment method described below, based on the macroinvertebrates, is used exclusively for the natural lakes and complies with the requirements of the Water Framework Directive.

The determination of the 'ecological status' required for the European Water Framework Directive (WFD) is based on characterizing reference conditions for water bodies. The WFD classification scheme for water quality includes five status classes: high, good, moderate, poor and bad. 'High status' is defined as the biological, chemical and morphological conditions associated with no or very low human pressure. This is also called the 'reference condition' as it is the best status achievable - the benchmark. These reference conditions are type-specific, so they are different for different types of rivers, lakes or coastal waters so as to take into account the broad diversity of ecological regions in Europe. Assessment of quality is based on the extent of deviation from these reference conditions, following the definitions in the Directive. 'Good status' means 'slight' deviation, 'moderate status' means 'moderate' deviation, and so on. The definition of ecological status takes into account specific aspects of the biological quality elements

Hence the reference conditions are hard to find in our country the WFD allows the use of so called benchmark sites which includes the sites with the best available conditions. According to the normative definitions of the WFD to describe the biological elements the following attributes have to be considered: composition, abundance, the ratio of disturbance sensitive taxa to insensitive taxa and the diversity, the numerical equivalent of these attributes called biological metrics. Aggregation of these metrics simplifies management and decision making (KARR et al. 1986). Thus a multimetric approach with qualitative and quantitative data should be used to reflect different environmental conditions and aspects of the community the multimetric assessment (KLEMM et al. 2002). Multimetric Indices are frequently used in routine water management. (HUGHES et al. 1998; BARBOUR et al. 1999; KARR & CHU 1999)

1.01 GIG: Eastern Continental
Relevant intercalibration types: EC-1 Lake types

1.02 Category: Lakes

1.03 BQE: Benthic Invertebrates

1.04 Country: Hungary

1.05 Specification: none

1.06 Method name: Hungarian Multimetric Macroinvertebrate Index for Lakes (HMMI_lakes)

1.07 Original name:
Magyar Makroszkopikus Vízi gerinctelen Multimetrikus Index Tavak

1.08 Status: Method is/will be used in second RBMP

1.09 Detected pressure(s):
Habitat destruction, Pollution by organic matter, Riparian habitat alteration

Specification of pressure-impact-relationship: multiple regression analysis
Pressure-impact-relationship:
Yes, with qualitative data .

1.10 Internet reference:
http://tiszaki.atomki.hu/Joomla/index.php/hu/interkalibracio

1.11 Pertinent literature of mandatory character:
None

1.12 Scientific literature:

G. Várbíró - Cs. Deák - G. Borics - E. Krasznai: Current issues in ecological water qualification: Developing multimetric macroinvertebrate index on lowland, small and medium sized watercourses - a case study Acta Biologica Debrecina Supplementum Oecologica Hungarica 21., 254 pp.

Várbíró, G. - Fekete, O. - Ortmann-Ajkai, A. - Ficsor, M. - Cser, B. - Kovács, K. - Kiss, G. - Czirok, A. - Horvai, V. - Deák, Cs.: Developing a multimetric macroinvertebrate index on mountainous, small and medium sized water bodies

Acta Biologica Debrecina, Supplementum Oecologica Hungarica 26., 220 pp.

Hering, D., O. Moog, L. Sandin & P.F.M. Verdonschot, 2004. Overview and application of the AQEM assessment system. Hydrobiologia 516: 1-20.

Moog, O., 1995. Fauna Aquatica Austriaca. Wassewirtschaftskataster, Bundesministerium für Land- und Fortwirtschaft, Wien.

1.13 Method developed by: Dr. Gabor Varbiro
Email of developer: varbirog@gmail.com
Institute of developer: Balaton Limnological Research Institute of Hungarian Academy of Science

1.14 Method reported by: Dr. Gabor Varbiro
Email of person reporting the method: varbirog@gmail.com
Email of institute reporting the method: Balaton Limnological Research Institute of Hungarian Academy of Science

1.15 Comments: none

2. Data acquisition

Field sampling/surveying

2.01 Sampling/Survey guidelines:

The method is a simplification of the AQEM Consortium, 2002. Manual for the application of the AQEM using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive.

2.02 Short description:

Multi-habitat sampling from major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 10-20sampling units taken from all habitat types at the sampling site with a share of at least 5 % coverage. A sampling unit is a equals the frame-size of the net (0.25 x 0.25 m). Sediments must be disturbed to a depth of 15-20 cm (where possible) depending on substrate compactness.
In case of lakes the littoral zone wadeable zone is sampled.

2.03 Method to select the sampling/survey site or area: Expert knowledge applying the AQEM compliant multi-habitat selection (avoid bridges, pollution impact, shade, any hydraulic structures like submerged weirs, etc.)

2.04 Sampling/survey device: Hand net

2.05 Specification: Hand net: frame 25 x25 cm, length of net 1 m.

2.06 Sampled/surveyed habitat:
Specification of sampled habitat:
All available habitats in the wadeable zones;

Sampled habitat: All available habitats per site (Multi-habitat)

2.07 Sampled/surveyed zones in areas with tidal influence: not relevant

2.08 Sampling/survey month(s): May to October

2.09 Number of sampling/survey occasions (in time) to classify site or area: 2 occasions

2.10 Number of spatial replicates per sampling/survey occasion to classify site or area: 10-20 (10 in case of small diverse habitats-less than 3 type)

2.11 Total sampled/surveyed area or volume or total sampling duration to classify site or area:
Benthic invertebrates: 20 x 0.0625= 1.25 m^2

10 x 0.0625= 6.125 m^2

Sample processing

2.12 Minimum size of organisms sampled and processed: 1000 µm (mesh-size of hand net)

2.13 Sample treatment:

Samples sorted and identified in the laboratory.

2.14 Level of taxonomical identification:
Level: Family, Genus, Other, Species/species groups

Specification of level of determination:
Species, species groups:, , Hirudinea, Mollusca (Gastropoda), Crustacea, Plecoptera, Coleoptera, Ephemeroptera, Odonata, Megaloptera, Trichoptera
family: Oligochaeta, Trichoptera, Chironomidae, other Diptera

2.15 Record of abundance:
Determination of abundance: Individual counts
Abundance is related to: Area
Unit of the record of abundance: Number of individuals per one m^2

3. Data evaluation

Evaluation

3.01 List of biological metrics:

$$HMMI\_lake = \frac{EQR_{family} + EQR_{diversity} + EQR_{BMWP}}{3}$$

Number of families, Shannon-Wiener Diversity Index, BMWP

3.02 Does the metric selection differ between types of water bodies: Not relevant

3.03 Combination rule for multi-metrics: Average metric scores

3.04 From which biological data are the metrics calculated:
List of biological metrics: Aggregated data from multiple spatial replicates

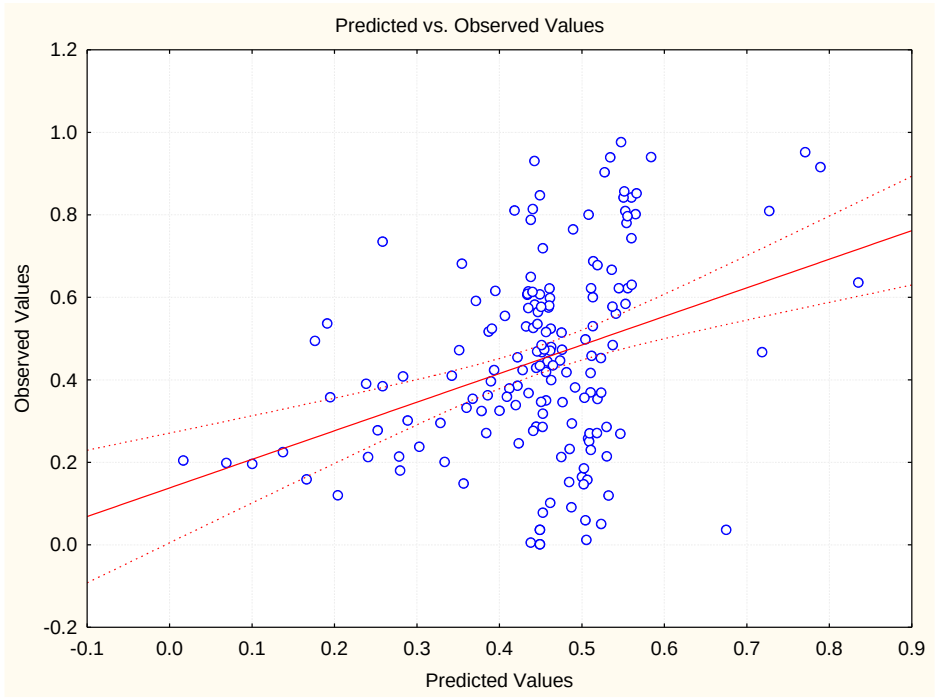
Pressure Impact relation:

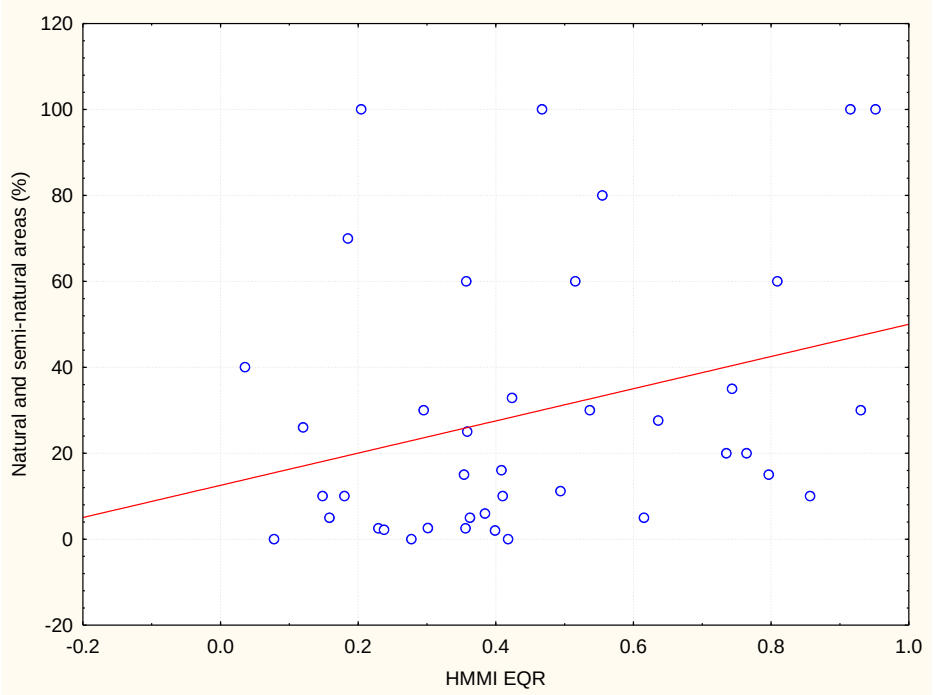
The relation was tested by correlation analyses then the chosen biological metric by the boundary setting procedure were transform dint a normalized EQR.

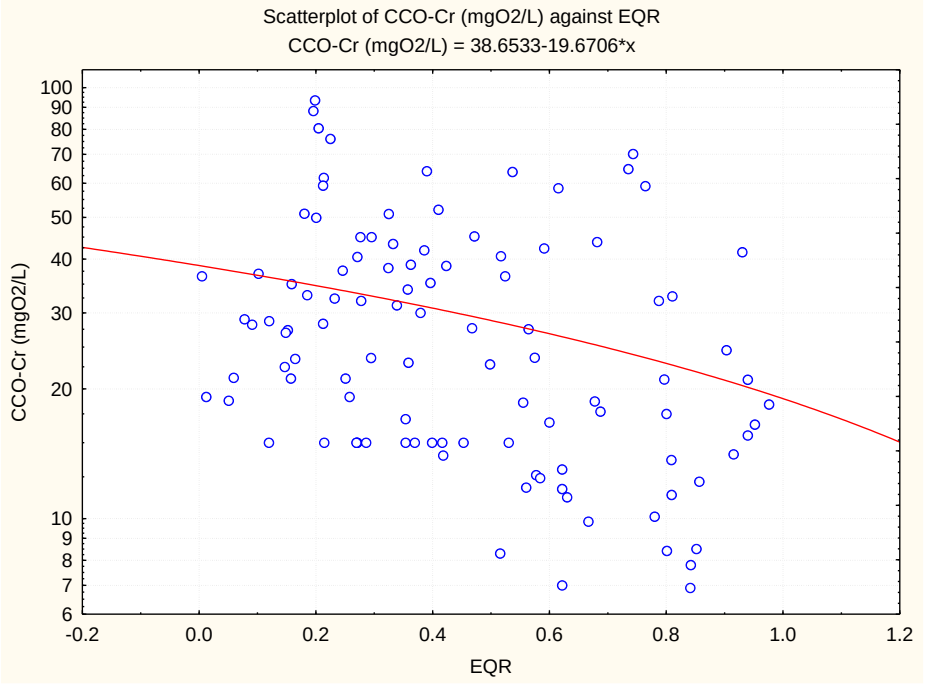
The EQR were averaged and the pressure impact was tested by multiple linear correlation. The results show significant correlation. (Multiple R = 0.7160, p<0,001)

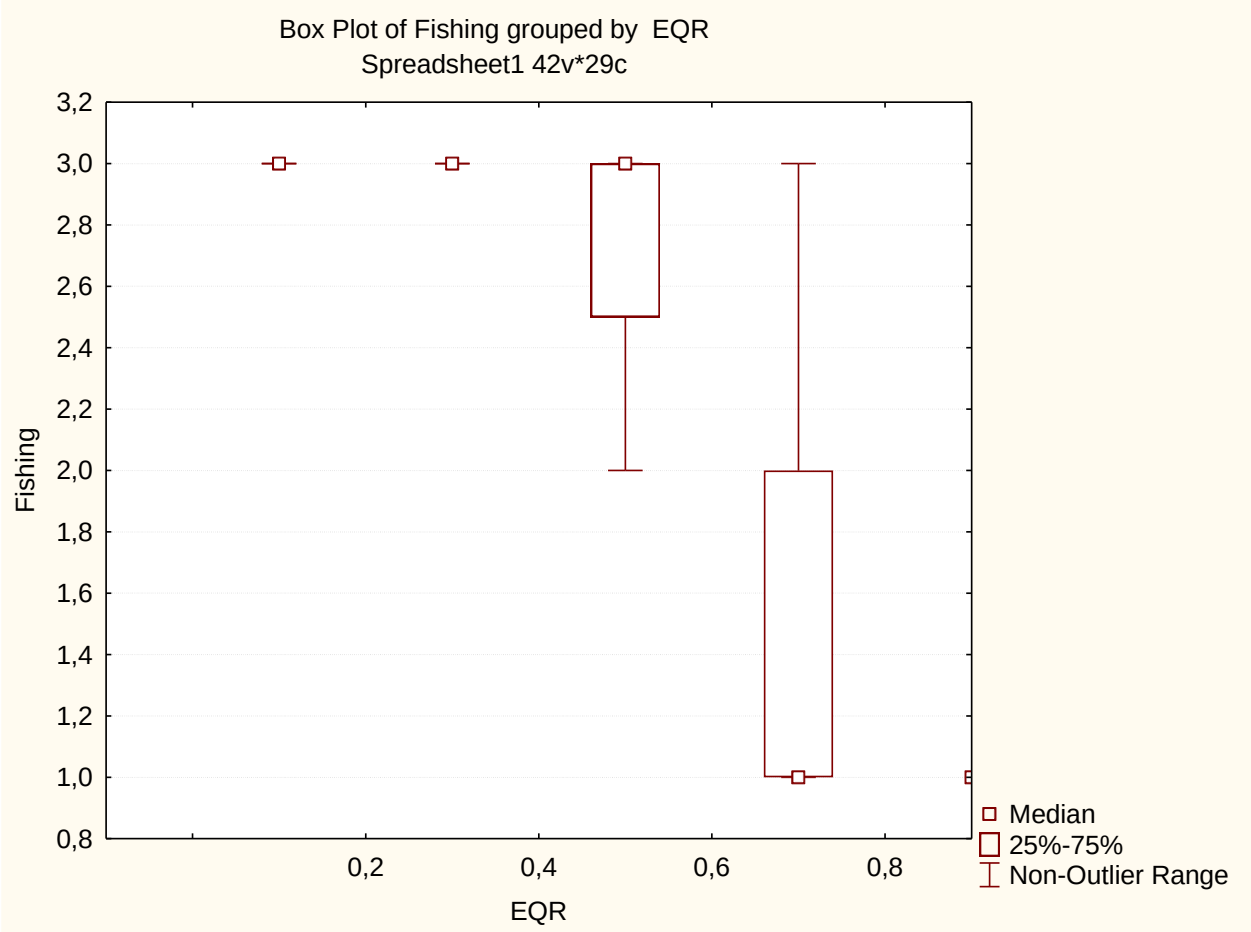
Multiple Regression Results			
Dependent: EQR	Multiple R =	.71605573	F = 2.630687
	R2=	.51273580	df = 8,20
Min. pairw. N: 29	adjusted R2=	.31783013	p = .037906

Standard error of estimate: .195787362
Intercept: 5.066410976 Std.Error: 1.640965 t(20) = 3.0875 p = .0058
CCO-Cr (mgO2/ beta=-.49 Artificial la beta=-2.8
Intensive agr beta=-4.0 Low intensity beta=-4.1 Natural and s beta=-5.3
Recreational beta=-.69 Fishing beta=-.24
(significant betas are highlighted)




120
100
80
60
40
20
0
-20
Natural and semi-natural areas (%)
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
HMMI EQR


Scatterplot of CCO-Cr (mgO2/L) against EQR
CCO-Cr (mgO2/L) = 38.6533-19.6706*x
100
90
80
70
60
50
40
30
20
10
9
8
7
6
CCO-Cr (mgO2/L)
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
EQR



Reference conditions

3.05 Scope of reference conditions: Surface water type-specific

3.06 Key source(s) to derive reference conditions:
Scope of reference conditions:
Benchmark sites from the IC database

3.07 Reference site characterisation:

Reference criteria:

absence of major point sources in catchment,

complete zonation of the macrophytes in the littoral zone,

no (or insignificant) artificial modifications of the shore line,

no mass recreation (camping, swimming, rowing,

low/moderate fishing (Fish stock <50kg/ha).

combined stressor value* < 1.5 This means that:

Fishing is low (Fish stock ~ < 50kg/ha)

Vegetation period mean TP < 115 $\mu g l^{-1}$

Vegetation period mean TN < 1550 μgl^{-1}

Vegetation period mean COD < 32 mgl^{-1}

3.08 Reference community description:

The biocenosis typical for the **high status** of the stagnant water bodies are characterised by a high biodiversity of the present groups.

The following species form stable populations: *Dytiscus marginalis, Hydrophilus piceus*, *Noterus clavicornis* belonging to the Coleoptera, *Bithynia tentaculata, Lymnea stagnalis, Planorbarius corneus* belonging to the Gastropod a and *Coenanagrion puella, Lestes viridis, Libellula depressa* belonging to the Odonata. Due to the absence or presence of some minimal pressures, caused by fishing activities, agriculture and hydro-technical works, the populations of Heteroptera are represented by a number of species (*Corixa punctata, Hydrometra stagnorum, Ilyocoris cimicoides, Ranatra linearis)*

3.09 Results expressed as EQR: Yes

Boundary setting

3.10 Setting of ecological status boundaries:

Division of the EQR gradient
High-good boundary derived from metric variability at benchmark sites.

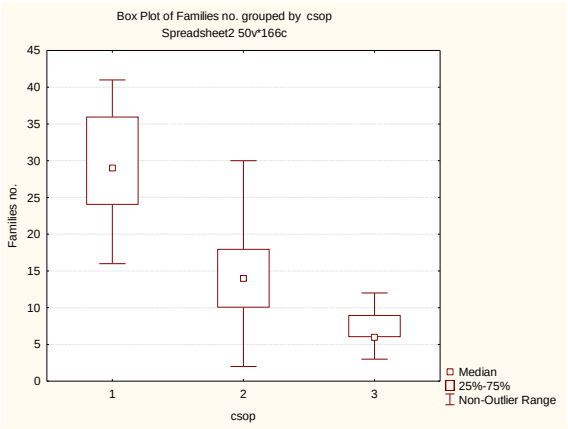
3.11 Boundary setting procedure:

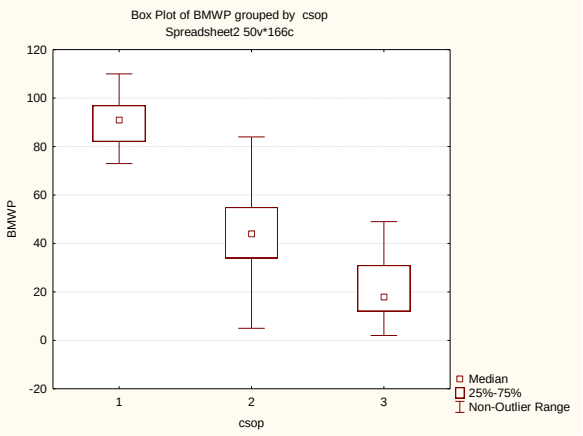
The IC database was divided into the following quality groups:

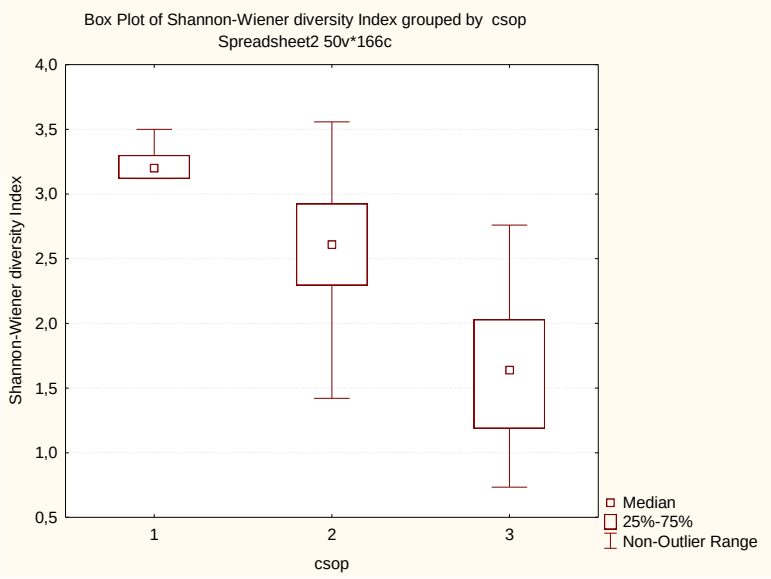
Quality groups: 1 Benchmark Tarova, Lata, Egyeki Holt Tisza

3 others

4 worst sites (Snagov)


Box Plot of Families no. grouped by csop
Spreadsheet2 50v*166c
Families no.
45
40
35
30
25
20
15
10
5
0
1
2
3
csop
Median
25%-75%
Non-Outlier Range


Box Plot of BMWP grouped by csop
Spreadsheet2 50v*166c
BMWP
120
100
80
60
40
20
0
-20
1
2
3
csop
Median
25%-75%
Non-Outlier Range


Box Plot of Shannon-Wiener diversity Index grouped by csop
Spreadsheet2 50v*166c
Shannon-Wiener diversity Index
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
1
2
3
csop
Median
25%-75%
Non-Outlier Range

The chosen metric were statistically described and the following quartiles were used for the EQR boundaries.

	HG	GM	MP	B	equation
Families no.	24	18	10	6	y = 0,0318x + 0,039
Shannon-Wiener diversity Index	3,12	2,92	2,29	1,18	y = 0,2814x - 0,1698
mz_bmwp_hu_i	82	55	34	12	y= 0,0086x + 0,1052

Comparison with WFD Annex V, normative definitions for each QE/ metrics and type

Ecological status	Normative definition (WFD)	Interpretation
High EQR 0.8–1.0	The taxonomic composition corresponds totally or nearly totally to undisturbed conditions. The ratio of disturbance sensitive taxa shows no signs of alteration from undisturbed conditions. The level of diversity of invertebrate taxa shows no sign of alteration from undisturbed levels	Species diversity is high (Shannon-Wiener Diversity Index min. – 3.12). Balanced proportion of the macroinvertebrates taxa (no. of families – min. 24). High abundance of benthic fauna, but no dominant group. Presence of sensitive taxa (BMWP – min. 82). Presence of Odonata, Heteroptera, Trichoptera, Ephemeroptera, Gasteropoda, Chironominae, Orthocladiinae, Tanypodinae, Naididae, Coleoptera taxa. The normalised value of the metric >0,8.
Good EQR = 0.6–0.8	There are slight changes in the composition and abundance of invertebrate taxa compared to the type-specific communities. The ratio of disturbance sensitive taxa shows slight sign of alteration from type-specific levels. The level of diversity of invertebrate taxa shows slight signs of alteration from type-specific levels	Slight decrease of all parameters. Shannon-Wiener Diversity Index is min. 2.92. No. of families is – min. 18. BMWP – min. 55. Some sensitive taxa could disappear due to anthropogenic impact. Taxa such as Chironomidae, Bivalvia, Gastropoda Heteroptera, Odonata are presented in balanced populations. The normalised value of the metric >0.6.
Moderate EQR = 0.4–0.6	The composition and abundance of invertebrate taxa differ moderately from the type-specific communities. Major taxonomic groups of the type-specific community are absent. The ratio of disturbance sensitive to insensitive taxa, and the level of diversity are substantially lower than the type-specific	Low diversity of benthic fauna (Shannon-Wiener Diversity Index is min. 2.29). Sensitive taxa could disappear. No. of families is min. 10. BMWP – min. 34. Chironomidae and Oligochaeta groups can be dominate. *Asellus aquaticus* (Isopoda) is characteristic to this status. Some species of

	level and significantly lower than for good status	Gastropoda and Bivalvia groups could be in large amounts of populations. The normalised value of the metric >0.4.

Annex 3: Example for type-specific passport information on reference conditions/alternative benchmark conditions (BG lake type equivalent to EC1)

INFORMATION SHEET (PASSPORT) OF LAKE TYPE	
Lake type:	**L5: Riparian lakes and marshes (LC1)**
General description and geographic area in BG:	Mesotrophic to eutrophic conditions. Polymictic lakes. Highly heterogenic group. This type includes riparian lakes and marshes in flood-plain areas. Some heavily modified WBs and even artificial ones could be belonged to this group, e.g. semi-natural fish ponds, etc.
Hydro-morphological characteristics:	**Ecoregion/ Sub-ecoregion:** 12-1,2 **Altitude:** <80 m (variable) **Mean depth:** < 3 m (rarely more – for some artificial excavation „lakes") **Size/ area:** <5 km^2, very small to medium size **Depth (max.)**: <10 m **Retention time:** not relevant **Mixing characteristics:** polymictic **Salinity:** <0.5‰ (freshwater)

Geology and hydrogeology	Mixed: quaternary and neogenic alluvial sediments, rarely calcareous (Srebarna Lake – Srebarna Biosphere Reserve)
Picture:	
Physico-chemical conditions (25-75 percentiles):	**pH:** 7.7-8.4 **DO [mg/l]:** 6.05-9.5 **Conductivity [µS/cm]:** 280-430 **Temperature [^{0}C]:** 10.45-23.2 **COD - Cr [mgO_2/l]:** 2.28-4.70 **BOD5 [mgO_2/l]:** 1.2-3.0 **TN [mg/l]:** 0.39-0.61 **TP [mg/l**]: 0.076-0.29 **PO4-P[mg/l]:** 0.015-0.066 **N Kjeldahl [mg/l]:** 0.3-0.7 **NO3-N [mg/l]:** 0.06-0.22 **NO2-N [mg/l]:** 0.002-0.01 **NH4-N [mg/l]:** 0.04-0.14 Additional physico-chemical monitoring is needed for this lake type in BG.

Type-specific biological conditions	**Phytoplankton**	**Macrophytes**
	Cyanobacteria: *Anabaena scheremetievi*; *Anabaena spiroides; Aphanizomenon fos-aquae; Aphanizomenon elenkinii; Aphanocapsa incerta; Merismopedia glauca; Microcystis flos-aquae; Microcystis aeruginosa; Oscillatoria agardhii, Snowella lacustris* ***Chlorophyta:*** *Actinastrum hantzschii; Ankistrodesmus bibraianus; Ankistrodesmus gracilis; Coaelastrum microporum; Crucigenia tetrapedia; Dicellula planctonica; Eudorina elegans; Kirchneriella obesa; Lagerheimia genevensis; Lagerheimia wratislaviensis; Micractinium pusillum; Pandorina morum; Pediastrum boryanum;*	Important indicator species are the following submerged and emergent macrophytes: *Azolla filiculoides* *Ceratophyllum demersum* *Hydrocharis morsus-ranae* *Myriophyllum verticillatum* *Nuphar lutea* *Spirodela polyrrhiza* *Stratiotes aloides* *Trapa natans* Most of natural lakes are typical "macrophyte" type, overgrowth by various macrophytes.

	Pediastrum duplex; Pediastrum simplex; Phacotus lenticularis; Pteromonas aculeolata; Scenedesmus pectinatus; Scenedesmus opoliensis; Scenedesmus obliquus; Tetraedron caudatum; Tetrastrum staurogeniaeforme; Westella botryoides *Sphaerocystis planctonica;* ***Zygnemaphyta:*** *Closterium acutum* ***Chrysophyta:*** *Dinobryon divergens, Synura uvella* ***Bacillariophyta:*** *Attheya zachariasi; Aulacoseira granulata, Cocconeis placentula; Cocconeis pediculus; Cyclotella kuetzingiana; Cyclotella meneghiniana; Cymatopleura elliptica; Diatoma vulgare; Gomphonema acuminatum; Melosira varians; Rhoicosphenia curvata; Fragilaria ulna* ***Euglenophyta:*** *Euglena acus, Euglena ehrenbergii; Euglena spirogyra; Euglena texta; Lepocinclis fusiformis; Monomorphina pyrum; Phacus caudatus; Phacus longicauda; Phacus pleuronectes; Trachelomomas armata;*	Specific quantitative metrics are naturally variable. **Reference conditions**: Reference Index (RI) (Schaumburg et al., 2006) - **52 ÷ 100**, EQR (RI)- **0.76 ÷ 1.00** **MEP:** Reference Index (РИ) (Schaumburg et al., 2006) - **52 ÷ 100**, EQR (RI)- **0.76 ÷ 1.00**	

	Trachelomonas oblonga; Trachelomonas volvocina ***Dinoflagellata:*** *Ceratium furcoides* ***Cryptophyta:*** *Chroomonas caudata; Cryptomonas curvata* Chlorophyll-A (µg/l): <6 Transparency (m): 1 ÷ 4 m Algae Group Index (AGI) / Catalan Index: < 1,4 Total biovolume (mm^3/l): < 2,5 % Cyanobacteria: <10 Number of species (%) – general description: Dominated Chlorophyta, Bacillariophyta and Cyanobacteria (Cyanoprocaryota). In some cases Chrysophyta and Zygnemophyta are presented with a great number of species as well. Toxic species are present but never in "bloom" concentrations. There is usually not a distinct domination of a certain algae group. Phytoplankton groups are generally diverse. Biovolume (%): Often the highest percent - Chlorophyta,

Dinophlagellata (*Ceratium spp.*) and Bacillariophyta.

Typically there is no blooming species or it could be Dinoflagellata (*Ceratium*) and Chlorophyta (*Coelastrum*) but never Cyanobacteria.

Reference conditions: Algae Group Index (AGI) - **< 1.40**; Chlorophyll-A (µg/l) **< 6;** Total biovolume (mm^3/l) - **< 2.5**; SD **(**m) - **1 ÷ 4;** % Cyano bacteria – **< 10%;** Presence of slight „blooms"-**Yes.**

MEP: Equal to reference conditions.

Benthic macroinvertebrates	**Fish fauna**
There is not enough data for this variable lake type. Benthic macroinvertebrate communities are dominated by Diptera larvae: Chironomidae (Chironomus genus is only sporadic) and *Chaoborus* (data from Srebarna Lake). *Tubificidae* (<20 ind./m^2) Other *Oligochaeta* *Asellus aquaticus* (<20 ind./m^2)	**Reference conditions:** Typical Danube fish species are presented: *Leucaspius delineatus*, *Cyprinus carpio*, *Misgurnus fossilis*, *Pungitius platygaster*, *Umbra krameri*, *Scardinius erythrophthalmus, Esox lucius, Silurus glanis, Alburnus alburnus, Tinca tinca, Carassius carassius*. Very high species diversity: > 15 fish species; Very high biomass/abundance;

	Chaoborus Presence of organic silt and high seasonal fluctuations in water level. Low taxonomic diversity (≤6 taxa – genus level) and low abundance (<40 ind./m^2). *Total number of taxa (genus level): ≤6* *Total abundance: <40 ind./m^2* *%Oligochaeta: ≤10* Heterogenic type with a big natural variability. Additional investigative monitoring is needed. **Reference conditions:** Total number of taxa: **>5**; Abundance: **≤60 ind./m^2**; % Oligochaeta **≤20**; EQR - **0.80 ÷ 1.00.** **MEP:** Total number of taxa: **<6**; Abundance: **<40 ind/m^2**; % Oligochaeta: **≤20**; EQR - **≥ 0.8.**	Successful breeding of above-listed indicator species represented by multi-age populations. Successful spawning local migrations; Hydraulic connectivity (temporary or permanent) with the main river; Complicated trophic structure represented all trophic levels – from plankton-filtrators to predators. Invasive alien fish species (e.g. (*Lepomis, Pseudorasbora, Gambusia, Carassius gibelio*, *Percottus*, etc.) <20% **MEP:** In fact similar to reference conditions allowing by 20% the reduction of species composition and total biomass/abundance. Invasive alien fish species: <40%

Examples and reference sites	Natural riparian lakes and various wetlands, many of extensive riparian fish ponds, old isolated river meanders, some artificial lakes (sand & gravel excavation), etc. Examples: Srebarna Lake, Persina lakes, Malak Preslavetz Lake, Garvan marsh, Pojarevo marsh, Kalimok-Brushlen wetlands, artificial lakes round Sofia, etc. This lake type is rare in the Black Sea sub-ecoregion (ER12-2). Some examples are: fish-ponds "Krusha" (Kamchia RB) and Velyov Vir Lake (Ropotamo RB). **Potential reference sites:** Srebarna Lake (Srebarna Biosphere Reserve) – near to reference conditions. Velyov Vir Lake (Ropotamo Nature Reserve) – reference conditions.
Remarks:	High species diversity, stable and balanced phytoplankton communities. Possible occasional slight algae blooms but never toxic species. Heterogenic type with a big natural variability concerning water macrophytes vegetation. The conditions range from lacks of macrophytes to overgrowth. Many of these natural lakes have a periodic connection with the main river (e.g. the Danube). Most of such connections have been destroyed after river regulations and dyke construction affecting negatively the lake water regime. Some of such lakes have been transferred into fish ponds and fish farms. The most visible indicators for the degradation of lake ecosystem are invasive fish species, which dominate**,** such as *Carassius gibelio*, *Lepomis gibbosus*, *Rutilus rutilus*, etc.

Annex 4: Pressures criteria for natural lakes

Banks

Banks configuration	**Description**	**% alterations (max.)**	**Score**
Natural	natural banks, aquatic macrophytes vegetation, high diversity of habitats, food, nest, spawning places for organisms	0-5	1
Near natural (little modified)	natural banks, in general, but also some buildings (wood pontoon), aquatic macrophytes vegetation, good diversity of habitats, feed, nest, spawning places for organisms	6-15	2
Partly modified	natural banks, in general, but also parts hardened/consolidated with stone tiles, some buildings (wood pontoon), relatively abundant aquatic macrophytes vegetation, moderate diversity of habitats, food, nest, spawning places for organisms	15-30	3
Modified	natural banks, but large parts hardened/consolidated with stone tiles, some buildings (wood and concrete pontoon), low development of the aquatic macrophytes	31-50	4

	vegetation, low diversity of habitats, food, nest, spawning places for organisms		
Much modified	large concrete banks parts alternate with natural banks, some buildings (wood and concrete pontoon), low development or absence of the aquatic macrophytes vegetation, very low diversity of habitats, food, nest, spawning places for organisms, major anthropogenic impacts: localities, industry etc.	51-70	5

Recreational activities

Recreational activities	**Description**	**% (max.)**	**Score**
Absent	very little or absent recreational activities; natural status	5	1
Few	few recreational activities: ecological tourism, angling etc.	10	2
Moderate	moderate recreational activities: tourism, non-permanent localities, boats (rowing boats, kayak-canoe etc.), moderate angling	20	3
Many	intensive recreational activities: tourism, boats (including motorboats), buildings on	40	4

	bank (houses inhabited few months yearly, pontoons etc.), beach, intensive recreational fishing		
Very many	very many recreational activities: boats (motorboats including), beach, spa, tourism, buildings on bank (houses inhabited all over the year, pontoons etc.), commercial fishing	70	5

Fish culture

Fish culture/Fishing	**Description**	**Score**
Natural regime	no fish populated	1
Moderate fish populated	sporadically and moderately populated with valuable species for angling	2
Industrial fish populated	permanently/yearly and abundantly populated with different species economically important for commercial fishing	3

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