European Commission

JRC TECHNICAL REPORTS

Intercalibration of the national classifications of ecological status for Eastern Continental lakes

Biological Quality Element: Macrophytes

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2018

Joint Research Centre

EUR 29336 EN

EU Science Hub
https://ec.europa.eu/jrc

JRC112692

EUR 29336 EN

PDF	ISBN 978-92-79-92968-7	ISSN 1831-9424	doi:10.2760/242521
Print	ISBN 978-92-79-92969-4	ISSN 1018-5593	doi:10.2760/941430

Luxembourg: Publications Office of the European Union, 2018

How to cite: PALL K, GECHEVA G, SOARU-MINEA A, LUKACS B, POIKANE S, Intercalibration of the national classifications of ecological status for Eastern Continental lakes: Biological quality Element: Macrophytes, EUR 29336 EN, Publications Office of the European Union, Luxembourg, 2018, ISBN 978-92-79-92968-7, doi:10.2760/242521, JRC112692

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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 macrophyte ecological assessment methods.

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

1 Introduction

The Eastern Continental Lake GIG was founded 2009 for Bulgaria, Hungary and Romania because it turned out that these countries have no common lake types with other neighbouring countries.

The report in hand deals with intercalibration of macrophyte-based methods for status assessment of lakes.

In the History of EC-GIG the number of member states changed. At the beginning there were three member states: Bulgaria, Romania and Hungary. At the end of 2012 Bulgaria officially postponed lake intercalibration. Hungary and Romania continued the IC process choosing Option 1 but didn’t succeed in the second round of intercalibration. In 2015 Bulgaria connected again the IC process and Romania developed an own method.

All methods address eutrophication and general degradation pressure and follow a similar assessment principle (considering species composition and abundance)

Therefore the intercalibration exercise could be continued 2016 with three countries and three methods, following Option 3.

The comparability analysis shows that the methods give a closely similar assessment (in agreement to comparability criteria defined in the IC Guidance), so only one boundary adjustment was needed (Hungary, “high-good” boundary).

The final results include EQRs of lake-macrophyte assessment-systems of Hungary, Romania and Bulgaria for the common type EC-1.

2 Description of national assessment methods

Three member states compared and harmonised their national lake macrophyte assessment systems. All member states have developed own assessment systems. They all are country specific adaptations of the German “Reference Index” method (SCHAUMBURG et al., 2004, 2007; STELZER et al. 2005).

Table 0.1: Overview of the national macrophyte assessment methods.

Member State	Method	Status
Hungary	**HU-RI**: Adapted Reference Index calculated according to a specific Hungarian list of indicator taxa (A – reference taxa, B – indifferent taxa and C – degradation indicators) respecting reference conditions in Hungarian lakes. The relatice share of A-, B- and C-taxa gives the assessment result. In contrast to the original method species classification is not performed depth specific. “Dominant stands”: used as an additional metric if selected species (e.g. *Ceratophyllum demersum*) reach huge (DAFOR: 4-5) plant quantities.	Finalized but not formally agreed national method (LUKÁCS et al., 2015)
Romania	**MIRO:** Adapted Reference Index calculated according to a specific Romanian list of indicator taxa (A – reference taxa, B – indifferent taxa and C – degradation indicators) respecting reference conditions in Romanian lakes. The relatice share of A-, B- and C-taxa gives the assessment result. In contrast to the original method species classification is not performed depth specific.	Finalized but not formally agreed national method (PALL et al., 2015)
Bulgaria	**RI-BG:** Adapted Reference Index calculated according to a specific Bulgarian list of indicator taxa (A – reference taxa, B – indifferent taxa and C – disturbance indicators respecting reference conditions in Bulgarian lakes. The relatice share of A-, B- and C-taxa gives the assessment result; species classification is performed depth specific.	Finalized and formally agreed national method (GECHEVA et al., 2013) (Regulation N-4 from 14.09.2012 towards characterising surface waters)

Detailed method descriptions are given in the ANNEX.

Methods and required BQE parameters

All methods include:

- Taxonomic composition and
- Abundance of macrophytes

Table 0.2: Overview of the metrics included in the national macrophyte assessment methods.

Member State	Full BQE method	Taxonomic composition	Abundance [a]	Combination rule of metrics
Hungary	Hydrophytes and selected amphi- and helophytes enter the assessment. Hydrophytes and amphiphytes are Algae, Mosses, Batrachoid, Myriophyllid, Nymphid, Plesutophyte, Potamid, Sagittarid and Stratiotid according to WIEGLEB (1991) life forms. Most helophytes are treated separately, as they indicate different pressures.	Relative share of type specific reference taxa, indifferent taxa and disturbance indicators.	Using DAFOR values (five-level scale, according to KOHLER, 1978). Additional metric: "dominant stands" of selected species.	Combined in one metric: Calculation of the Reference Index. Downgrading of the index value in case of "dominant stands" of selected species.
Romania	Hydrophytes, amphiphytes and selected helophytes out of Charophytes, Bryophytes, Pteridophytes and Spermatophytes enter the assessment. The relative share of reference species, indifferent species and disturbance indicators gives the assessment result.	Relative share of type specific reference taxa, indifferent taxa and disturbance indicators.	Using DAFOR values (five-level scale, according to KOHLER, 1978).	Combined in one metric: Calculation of the Reference Index. Only one metric, no combination
Bulgaria	Hydrophytes, amphiphytes and selected helophytes out of Charophytes, Bryophytes, Pteridophytes and Spermatophytes enter the assessment. The relative share of reference species, indifferent species and disturbance indicators gives the assessment result.	Relative share of type specific reference taxa, indifferent taxa and disturbance indicators.	Using DAFOR values five-level scale, according to KOHLER, 1978).	Combined in one metric: Calculation of the Reference Index. Only one metric, no combination.

Sampling and data processing

All countries use similar sampling strategies and data processing techniques (***Table 0.3***).

Table 0.3: Overview of national sampling procedures.

Member State	Sampling time and frequency	Surveyed Habitat	Data processing	Sampling devices	Identifica-tion level	How is abundance measured
Hungary, Romania, Bulgaria	Sampling is carried out once during the vegetation period (June to September)	The whole water column in the littoral zone, including the helophyte vegetation at the lake shore	All methods use belt transects located perpendicular to the shore. The number of transects depends on criteria given in CEN 15460 (RO) or on lake size according to Schaumburg et al. 2007, with country-specific inter-pretations and rules (BG and HU). The assessment of the lake results as average of the investigated transects.	Rake, grapnel or aqua-scope (*optional*)	Species level, except for Charophytes (genus level)	The species abundance is estimated as DAFOR-values (five-level scale, according to KOHLER, 1978).)

National reference conditions

The definition of reference criteria in all member states was performed on national level, using historical information (if available), expert judgement and information from other GIGs. All member states state that there are no real reference lakes within their datasets of EC-1-type lakes.

Accordingly, least disturbed sites were choosen as benchmark. ***Table 0.4*** gives the criteria for selecting benchmark lakes used in the different member states.

Table 0.4: Overview of the methodology used to select benchmark lakes

Member State	Key source to derive benchmark sites	Geographical scope	Number of benchmark sites	Location of benchmark	Time period
Hungary	Least disturbed sites concerning physical/chemical pressures (above all nutrient concentrations) and other anthropogenic pressures (shoreline degradation, recreation, fishing).	Ecoregion 11	3 (only one of them WFD-relevant)	Vajai-tározó (Tápió-szecsői-I-sz-halastó Vamo-spercsi-tarozo)	2010 (2005)
Romania	Least disturbed sites concerning physical/chemical pressures (above all nutrient concentrations). Out of this pool sites with no or very low pressure concerning shoreline degradation, shipping, water abstraction, recreation, fishing and fishfarming, intensive agricultural use in a 5km buffer around the lake.	Ecoregion 11 and 12	11	Bentul Latenilor Brat Dunarea Veche Gorggostel Lata Puiu Razim Rosu Snagov Somova Tarova Victoria Geormane	2006-2014
Bulgaria	No benchmark sites available in BG, the GIG benchmark sites were used.	Ecoregion 12	0		

For further details see Annex.

National boundary setting

Table 0.5 summarises the methodology used to derive class boundaries.

Table 0.5: Overview of the methodology used to derive national class boundaries

Member State	H/G boundary	G/M boundary	Other boundaries
Hungary	H/G boundary is the point at which A-species loss their overall dominance. For the boundary setting, the least disturbed sites as alternative benchmark sites were used. H/G boundary corresponds to the 75th percentile of HU benchmark lakes EQR's.	G/M boundary was determined according to the relative abundance distribution of 'A' and 'C' species along the EQR range: turning point among 'A' and 'C' species. This corresponds to the 25th percentile of the reference index in the HU benchmark lakes.	The bad status means a loss of macrophytes. Therefore the boundary for the bad status is 0. The M/P boundary was considered as the arithmetic mean of the G/M boundary and the P/B boundary. This is the point where C species start to become overall dominant.
Romania	H/G boundary is the point where A-species loss their dominance over B- and C-species. For the boundary setting, the least disturbed sites as alternative benchmark sites were used. H/G boundary was considered as the 75th percentile of the reference index in the RO benchmark lakes.	G/M boundary is the point where C-species start to overwight A-species. This corresponds to the 10th percentile of the reference index in the RO benchmark lakes.	M/P boundary is the point where C species start to become dominant over A- and B-species. This corresponds to the arithmetic mean of the good/moderate boundary and the poor/bad boundary. The bad status means no macrophytes. P/B boundary is the loss of macrophytes (EQR=0).
Bulgaria	The H/G boundary was assumed as the point at which sensitive species (Group A) were in quantity approximately more than 50% of the quantity of all groups. Because of lack of such sites in the dataset, one quarter (0.25) was added to the assessed highest EQR value from the dataset. This was checked with GIG benchmark sites.	Group B (indifferent taxa) was dominant and/or Group A and C were with equal quantities. The highest EQR value from the dataset was applied as G/M boundary. The G/M boundary was the point at which average value of sites, where loss of dominance of reference and sensitive species was observed.	The M/P boundary was set as the average where the community is dominated by disturbance indicators (Group C). It is characterised by disappearance of water lilies and dominance of elodeids. The P/B boundary is the point at which macrophyte species are extinct due to anthropogenic pressures.

For further details see Annex.

Pressures addressed

All methods address the pressures eutrophication and general degradation. Table 2.6 gives an overview on the pressure response relationships performed by the differernt national methods.

Table 0.6: Overview on pressure-response relationships of national methods

Member State	Pressure or combination of pressures	Pressure indicators / Strength of relationship
Hungary	Eutrophication and general degradation	TP: Spearman R=-0.66, r^2=0.44, p<0.001 Chla: Spearman R=-0.28, r^2=0.07, p=0.13 TN: Spearman R=-0.23, r^2=0.05 , p=0.13 Corine intensive agriculure area: Spearman R=-0.39, r^2=0.15, p=0.003
Romania	Eutrophication and general degradation	Cond.: r=-0.71, r^2=0.50, p<0.001 BOD_5.: r=-0.55, r^2=0.30, p<0.001 TP: r=-0.30, r^2=0.09, p<0.05 TN: r=-0.73, r^2=0.53, p>0.001 NO_3-N: r=-0.51, r^2=0.26, p<0.001 NH_4-N: r=-0.50, r^2=0.25, p>0.001 Combined Nutrient Pressure (rank sum of eutrophication-relevant physico-chemical and landuse parameters) r=0.76, r^2=0.58, p<0.001
Bulgaria	Eutrophication and general degradation	Chl a, TP, BOD_5, NO_3-N multiple regression: r=0.542, r^2=0.294, p=0.541

3 WFD compliance checking

General conclusion of the compliance checking:

- The GIG considers that all methods are compliant with respect to the criteria given in the IC guidance.
- All methods show significant correlations with eutrophication parameters.
- The GIG agrees in that macrophytes are indicative for the **BQE** as a whole for long-term changes and are responsive to the main anthropogenic pressures on lakes.

Table 0.1: Compliance check

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	All MS – Yes
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions (Boundary setting procedure)**	All MS – Yes For details see Table 2.5.
3. **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.	All MS consider taxonomic composition and abundance of macrophytes. These two parameters are combined in one metric, the Reference Index. Hungary uses an additional metric (mass stands) and has defined a comination rule.
4. Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the WFD Annex II and approved by WG ECOSTAT	All MS – Yes All systems are appropriate for the common type EC-1
5. The water body is assessed against **type-specific near-natural reference conditions**	Due to the lack of real reference sites the MS use benchmark sites. For Details see Table 2.4.
6. Assessment results are expressed as **EQRs**	All MS – Yes
7. Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	All MS – Yes**; In space**: With use of transects. MS have rules for the number of transects (BG and HU depending on the size of the lake; RO following CEN 15460); **In time**: All MS's assess once during the growing season (lake-years); For macrophytes this is the appropriate time scale, with at least one sample during the peak of the growing season (June-Aug).
8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	In all MSs parameters for abundance and species composition are covered.
9. Selected taxonomic level achieves adequate **confidence and precision** in classification	Species must be taxonomically identified to species level in all MSs (exception: Charophytes).

4 IC Feasibility checking

Typology

Intercalibration is feasible in terms of typology –all assessment methods are appropriate for the common lake type EC-1 (see ***Table 0.1***):

Table 0.1: Description of Eastern Continental GIG intercalibration lake types

Common IC type	Type characteristics	MS sharing IC common type
EC-1 - Lowland very shallow hard-water	Altitude <200m.s.l. Depth< 6m Conductivity 300-1000 (µS/cm Alkalinity 1-4 (meq/l HCO3)	HU – Yes RO – Yes BG – Yes
EC-2 - Lowland very shallow but very high alkalinity	Altitude <.200m.s.l. Depth< 6m Conductivity >1000 (µS/cm) Alkalinity >4 (meq/l HCO3)	HU – No RO – Yes BG – No
EC-3	Altitude 200-800m.s.l. Depth <6m Conductivity 200-1000(µS/cm) Alkalinity 1-4 (meq/l HCO3)	HU – No RO – Yes BG – No
EC-4	Altitude 200-800m.s.l. Depth>6m Conductivity 200-1000(µS/cm) Alkalinity 1-4 (meq/l HCO3)	HU – No RO – No BG – No
EC-5 - Reservoirs	Altitude 200-800m.s.l. Depth>6m Conductivity 200-1000(µS/cm) Alkalinity 1-4 (meq/l HCO3)	HU – No RO – No BG – Yes

Only the lake type EC-1 is a common type.

Pressures addressed

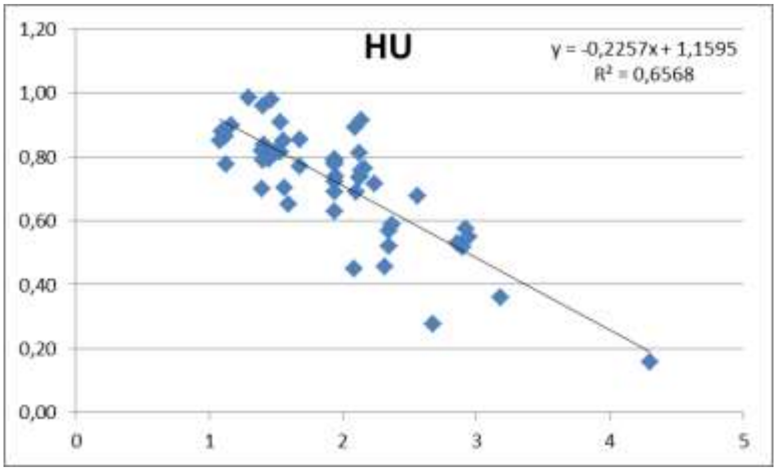
Intercalibration is fasible in terms of pressures addressed by the methods. The lake macrophyte assessment methods address mainly nutrient-enrichment (eutrophication) and general degradation (see Table 0.2).

Table 0.2: Pressures addressed by the assessment methods in EC 1 lakes using Pearsons R. In the Table we summarized the correlation for the whole GIG (HU+RO+BG) dataset

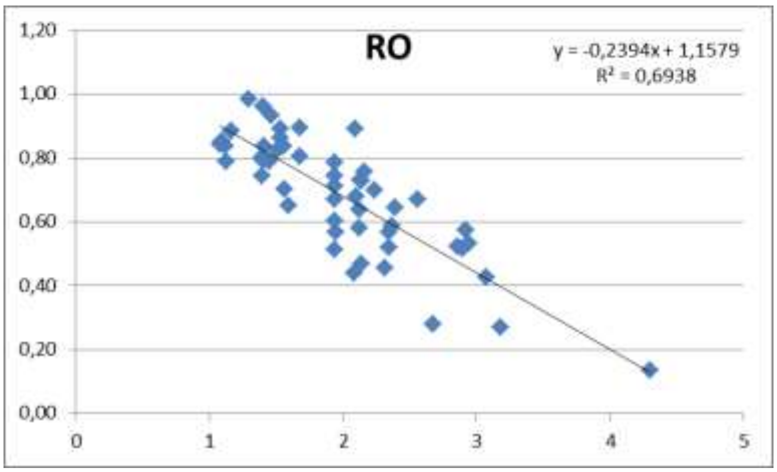
Relationship with pressure		Pressure indicators / Strength of relationship (Pearsons R)		
		HU	**RO**	**BG**
Nutrient-enrichment	Log TP	**-0.52***	**-0.46***	**-0.39***
	Log TN	**-0.49***	**-0.58***	**-0.58***
	Conductivity	**-0.69***	**-0.77***	**-0.64***
	Log BOD5	**-0.43***	**-0.53***	**-0.47***
	Intensive agricultural areas (%) (Corine)	-0.35*	-0.28*	**-0.39***
Combined nutrient pressure	Sum of normalised values of the above given parameters	**-0.81***	**-0.83***	**-0.80***

*) **p <0.001**, **p<0.01**, p<0.05

HU



RO



BG

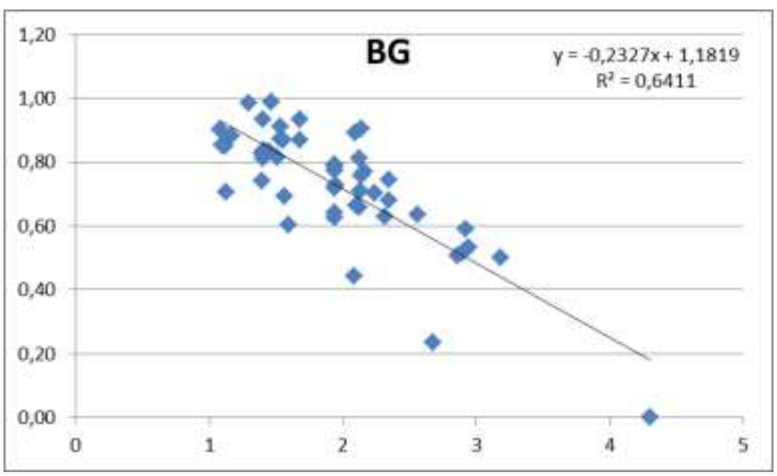


Figure 0.1: The response of MS methods in relation to the combined Nurtrient Stressor (Sum normalised values of the parameters Log-TP, Log-TN, Conductivity, Log-BOD5, Corine intensive agriculture). The figures based on the whole GIG (HU+RO+BG) dataset.

Assessment concept

All member states have developed own assessment systems. They all follow a very similar assessment concept.

Table 0.3: Assessment Concepts

Member State	HU	RO	BG
Assessment concept	Hydrophytes, amphiphytes and selected helophytes out of the taxonomical groups *charophyta*, *bryophyta*, *pteridophyta* and *spermatophyta*, enter the assessment. The entire littoral zone with all habitats as well as the reed belt has to be investigated along selected transects. The relative share of reference taxa, indifferent taxa and degradation indicators gives the assessment result. Additional criterion: Vegetation limit. Additional criterion: Very low abundance. Additional criterion: Monodominant stands (DAFOR = 4-5) of species.	Hydrophytes, amphiphytes and selected helophytes out of the taxonomical groups *charophyta*, *bryophyta*, *pteridophyta* and *spermatophyta*, enter the assessment. The entire littoral zone with all habitats as well as the reed belt has to be investigated along selected transects. The relative share of reference taxa, indifferent taxa and degradation indicators gives the assessment result. Additional criterion: Vegetation limit. Additional criterion: Very low abundance.	Hydrophytes, amphiphytes and selected helophytes out of the taxonomical groups *charophyta*, *bryophyta*, *pteridophyta* and *spermatophyta*, enter the assessment. The entire littoral zone with all habitats as well as the reed belt has to be investigated along selected transects. The relative share of reference taxa, indifferent taxa and degradation indicators gives the assessment result. Additional criterion: Vegetation limit. Additional criterion: Very low abundance.

Conclusion of the intercalibration feasibility

The GIG concluded that intercalibration is feasible in terms of typology, pressures addressed and assessment concepts.

5 IC dataset collected

Table 0.1 gives an overview of the dataset collected within the EC-GIG and lists the provided data per member state, ***Table 0.2*** shows the data acceptance criteria.

Table 0.1: Overview of the number of lakes and surveys.

Member State	Biological, physico-chemical and pressure data	
	No. of lakes	No. of surveys
Hungary	15	15
Romania	22	32
Bulgaria	2	5
TOTAL	**39**	**52**

Table 0.2: List of the data acceptance criteria used for the data quality control and the data acceptance checking

Data acceptance criteria	Data acceptance checking
Data requirements (obligatory for ALL MS)	• Species list with species-specific abundances in a five-level scale for each lake / survey • Genus level was accepted in case this didn't hamper assessment by other methods (this was the case with Charophytes) • Assessment results were only accepted in case of at least 3 indicator species with an abundance of at least 3 (=plant quantity 27) • Supporting physico-chemical and pressure data for each lake / survey
Sampling and analytical methodology (ALL MS)	All data could be accepted as • Member states used the same ordinal scale (five-level descriptor: DAFOR- or KOHLER-scale) for abundance of macrophytes species. • Member states used the same accuracy for the chemical physical analyses • Member states used the same scale for other pressure data
Level of taxonomic precision required and taxalists with codes (ALL MS)	Species level (exept for Charophytes). Unique taxa codes were used.
The minimum number of sites / samples per intercalibration type	At minimum 5 surveys from 2 lakes were provided (in BG no more lakes and surveys of the common lake-type were available).
Sufficient covering of all relevant quality classes per type	The full quality spectrum is covered.

Table 0.3 and **Table 0.4** show the ranges of physico-chemical and pressure data and give an overview of the whole dataset, respevtively.

Table 0.3: Ranges of physico-chemical and pressure data

Abiotic data and pressure information	*HU*	*RO*	*BG*	*TOTAL*
LAKE INFORMATION				
Lake area [km²]	*0.4-2.6*	*0.4-25.0*	*0.4-6.3*	0.4 – 25.0
Altitude [m.s.l].	*<200*	*1-82*	*1-10*	1-<200
Average depth [m]	*0.9-3.8*	*1.0-3.0*	*1.0-2.0*	0.9 – 3.8
Conductivity [uS/cm]	*467-1113*	*350-1142*	*281-478*	281 – 1142
Alcalinity [mmol/l]	*3.03-4.40*	*2.42-4.50*	*1.79-4.47*	1.79 – 4.50
Physico-chemical data				
Water temperature [°C]	*12-25*	*17-24*	*20-25*	12 – 25
pH	*7.52-8.73*	*7.16-8.69*	*7.66-8.12*	7.16 – 8.72
Dissolved oxygen [mg{l]	*3.10-10.03*	*3.96-10.74*	*3.38-10.10*	3.10 – 10.74
Oxygen saturation [%]	*33-126*	*35-121*	*34-99*	33 – 126
BOD5 [mgO2/l]	*1.20-7.70*	*1.97-46.16*	*1.03-4.35*	1.03 – 46.16
TP [ug/l]	*60-450*	*28-339*	*20-130*	20 – 450
PO4-P [ug/l]	*13-375*	*9-277*	*10-30*	9 – 375
TN [ug/l]	*469-2470*	*605-6772*	*470-1510*	469 – 6772
NO3-N [mg/l]	*45-692*	*37-1892*	*40-100*	37 – 1892
NH4-N [mg/l]	*19-156*	*39-577*	*42-207*	19 – 577
Chl a [mg/l]	*5.1-144.8*	*no data*	*23.3-46.0*	5.1 – 46.0
Pressure informantion				
Shoreline degradation (4 classes: 0-3)	*1-3*	*0-3*	*0*	0 – 3
Recreation (4 classes: 0-3)	*0-3*	*0-1*	*0-3*	0 – 3
Fishing pressure (4 classes: 0-3)	*1-3*	*0-2*	*0-1*	0 – 3
Eutrophication, diffuse (4 classes: 0-3)	*0-3*	*0-3*	*1-2*	0 – 3
Corine artificial surfaces [%]	*0-36*	*0-17*	*8-23*	0 – 36
Corine intensive agriculture [%]	*38-90*	*0-92*	*0-44*	0 – 92
Corine low intensive agriculture [%]	*0-45*	*0-67*	*0-11*	0 – 67
Corine natural lands [%]	*0-60*	*0-100*	*37-77*	0 – 100

Table 0.4: Overview of the EC-GIG dataset

Size of common dataset	52 surveys out of 39 lakes
Number of member states	3
Repackage/disaggregation of samples	No, use of survey data
Gradient of ecological quality	High to bad
Coverage per ecological quality class	High 7%; Good 32%; Moderate 49% Poor 11%; Bad 1%

6 Common benchmarking

Due to the lack of reference sites and a sufficient number of near natural benchmark sites, we applied "continuous benchmarking" (BIRK et al., 2013, 2016) in this exercise. The principle of "continuous benchmarking" is to adjust all national regression lines (national metric versus pressure gradient) to a common regression line for all data together. Benchmark standardization serves to homogenize the EQR results of common datasets where needed, minimising typological and methodological differences between the Member States which may otherwise influence the comparability of their classifications.

In order to model the standard and to receive the correction values directly, we used a Linear Mixed Model (LMM in R). Using the package lme4 in R the model can be specified as "fit.mm2 <- lmer(Metric ~ Pressure + (1|country),data=data)" with "Metric" being the metric variable, "Pressure" the pressure variable and "country" the groups for standardisation (country). The following R-script was used:

```
#Load packages
library(lattice)# for scatterplot
library(lme4)# for mixed model
rm(list= ls()) # clear data
setwd("your_path") #set working directory
data<- read.csv(file = "LR_BF_EQR_standardisation.csv",header = TRUE) #Load Data
names(data) # view variables
dim(data) # view number of columns and rows
```

The following offsets resulted:

Table 0.5: List of country-specific offsets

MS / MS-EQR	BG-EQR	HU-EQR	RO-EQR
BG	-0,089819092	-0,05274543	-0,04646276
HU	0,080502697	0,04945146	0,06283233
RO	0,009316395	0,00329397	-0,01636957

All EQR-values were standardised by subtracting the offset from the national EQR-value.

7 Comparison of methods and boundaries

IC Option and Common Metrics

IC Option 3 was used: due to similar sampling procedure, similar data structure all national assessment methods can reasonably be applied to the data of other countries. For comparison of the MS assessments, a pseudo-common metric (PCM) – the average of the benchmark standardised EQR-values of, in each case, all other member states – was used.

Results of the regression comparison

All methods have significant correlations to the pseudo-common metric (***Figure 0.1*** to ***Figure 0.3***). The correlation coefficients (r) and the probability (p) for the correlation of each method with the common metric are given in ***Table 0.1***.

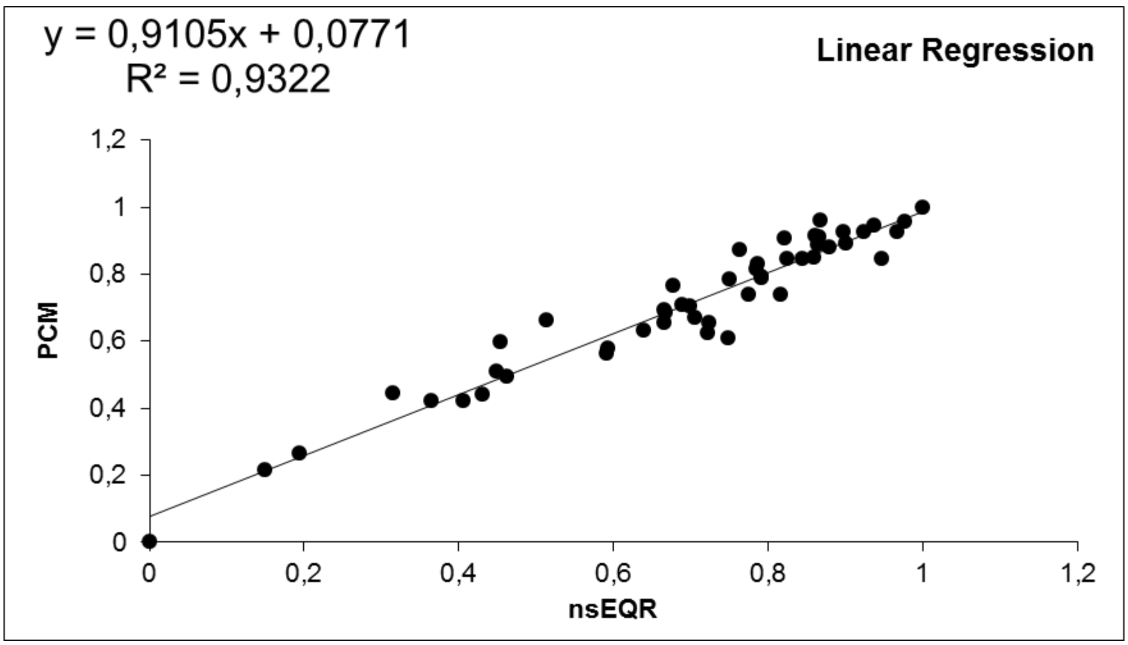


Figure 0.1: Linear regression of the Hungarian assessment results (bsEQR-HU) to the PCM.

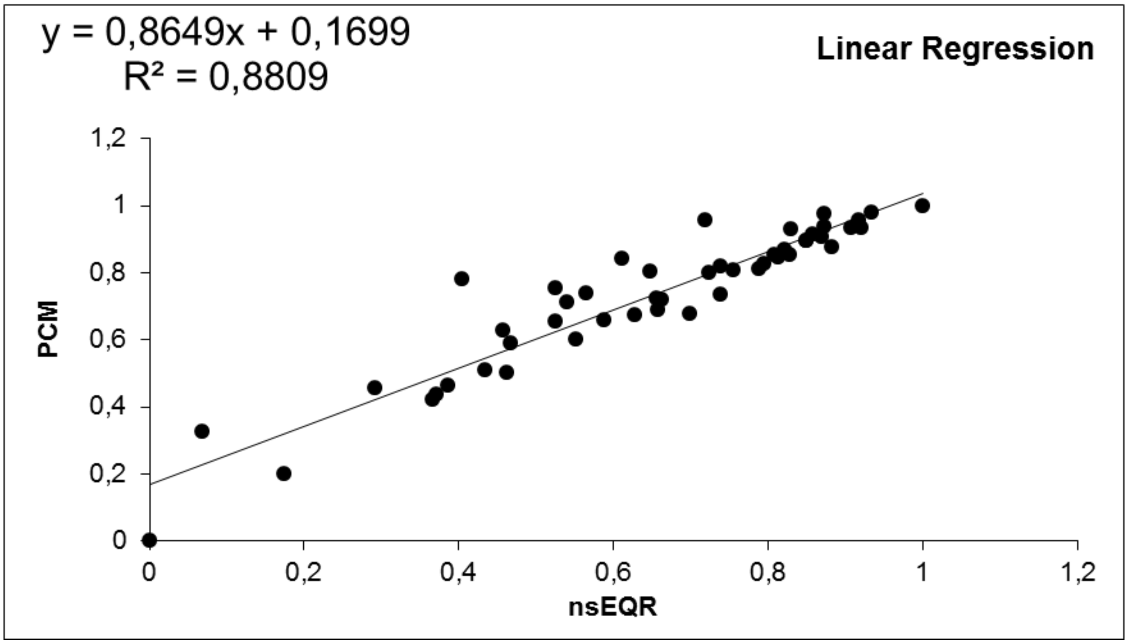


Figure 0.2: Linear regression of the Romanian assessment results (bsEQR-RO) to the PCM

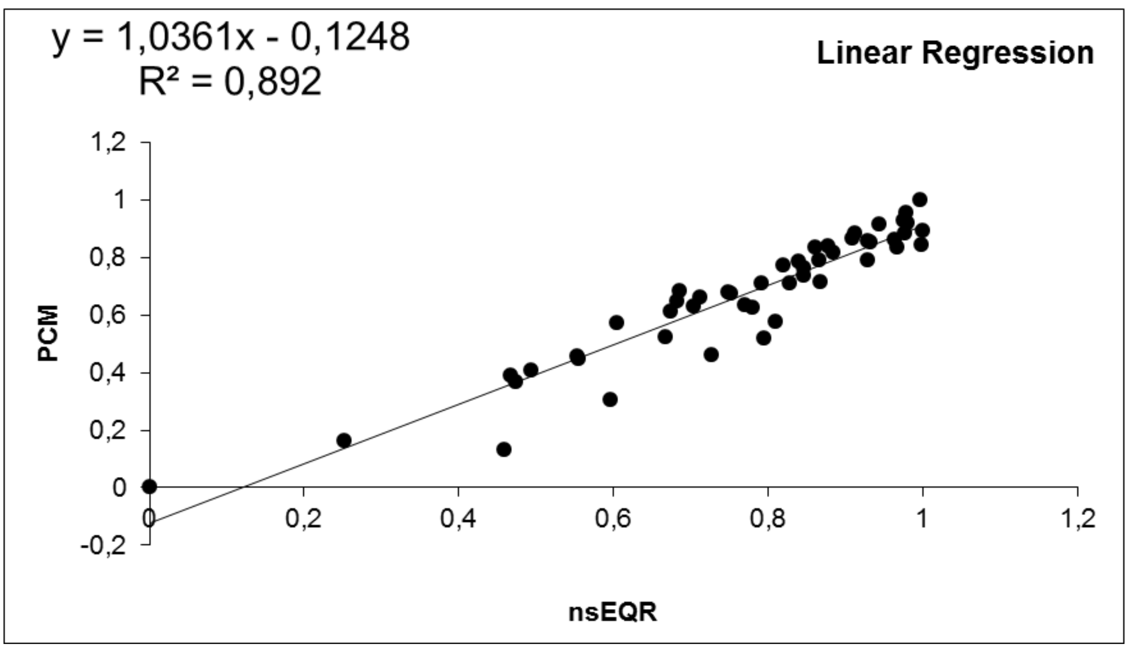


Figure 0.3: Linear regression of the Bulgarian assessment results (bsEQR_BG) to the PCM

Table 0.1: The correlation coefficients (r) and the probability (p) for the correlation of each method with the common metric (PCM)

Member State/Method	r	r^2	p	slope
Hungary	0.966	0.932	< 0.001	0.910
Romania	0.939	0.881	< 0.001	0.865
Bulgaria	0.944	0.892	< 0.001	1.036

The outcomes of the regression complied with the following characteristics according to the IC Guidance:

- All relationships were highly significant p<=0.001,
- Assumptions of normally distributed error and variance (homoscedasticity) of model residuals are met,
- Common metric must represent all methods (r^2>0.5),
- Observed minimum r^2 was > half of the observed maximum r^2,
- Slope of the regression should lie between 0.5 and 1.5.

No method had to be exclude due to its low correlation with the PCM.

Evaluation of comparability criteria

Table 0.2 shows the results of the evaluations of comparability criteria according to the IC Guidance Annex V requirements.

Table 0.2: Comparison of national boundaries of EC-GIG macrophyte assessment systems before boundary adjustment, using comparability criteria

Member State	H/G boundary bias	G/M boundary bias	Class agreement
Requirement	**>-0.250**	**>-0.250**	**<1.000**
Hungary	**-0.360**	-0.193	0.408
Romania	0.188	0.196	0.327
Bulgaria	0.055	-0.086	0.327
Average	**0.201 ***	**0.158 ***	**0.354**

*calculated from absolute values of boundary bias

The following outcome can be described:

- The comparability criteria were met by Bulgaria and Romania for all boundaries
- For Hungary the comparability criteria were only met for the G/M boundary, the bias of the H/G boundary was above the allowed range.

Boundary adjustment

Only the Hungarian H/G boundary had to be adjusted. Table 7.3. shows the results of the evaluations of comprability criteria according to the IC Guidance Annex V requirements after boundary adjustment.

Table 0.3: Comparison of national boundaries of EC-GIG macrophyte assessment systems after boundary adjustment, using comparability criteria

Member State	H/G boundary bias	G/M boundary bias	Class agreement
Requirement	**>-0.250**	**>-0.250**	**<1.000**
Hungary	**-0.244**	-0.175	0.388
Romania	0.188	0.196	0.316
Bulgaria	0.055	-0.086	0.316
Average	**0.201 ***	**0.158 ***	**0.354**

*calculated from absolute values of boundary bias

8 Final results to be included in the IC-decision

Table 8.1. gives the final class boundaries to be included in the IC decision.

Table 0.4: Class boundaries of EC-GIG macrophyte assessment systems to be included in the IC-decision

Member State	Classification Method	Ecological Quality Ratios High-good boundary	Good-moderate boundary
Hungary	HU-RI	0.890	0.670
Romania	MIRO	0.860	0.660
Bulgaria	RI-BG	0.830	0.580

9 Description of biological communities representing the "borderline" conditions between good and moderate ecological status

Following biological quality element changes were detected along eutrophication gradient:

- Decrease of reference species;
- Increase of tolerant species. Decrease of sensitive species;
- Increase of disturbance indicating species;

The major changes in the abundance of different functional groups are consistent with an overall increase in lake use (Figure 0.4 and Figure 0.5).

Figure 0.4 indicates the relative abundances of all species (hydrophytes, amphiphytes and helophytes) along the EQR gradient. Growth-forms like Vallisnerid (e.g. *Butomus umbellatus*, *Sparganium erectum*), Peplid and Sagittarid appeared in remarkable amount in moderate ecological status. Growth-forms, known as disturbance indicators, like some algae, Vallisnerid (Vallisneria spiralis) and Pleustophyte (e.g. Lemna gibba) speceis decreased its abundance in the P/M boundary. On the other hand, some pleustophyte species (e.g. Lemna minor, L. trisulca) increased its abundance in the H/G boundary. Mosses, Myriophylloid and Nymphaeid species appeared in higher relative abundance in the higher EQR classes (high and good).

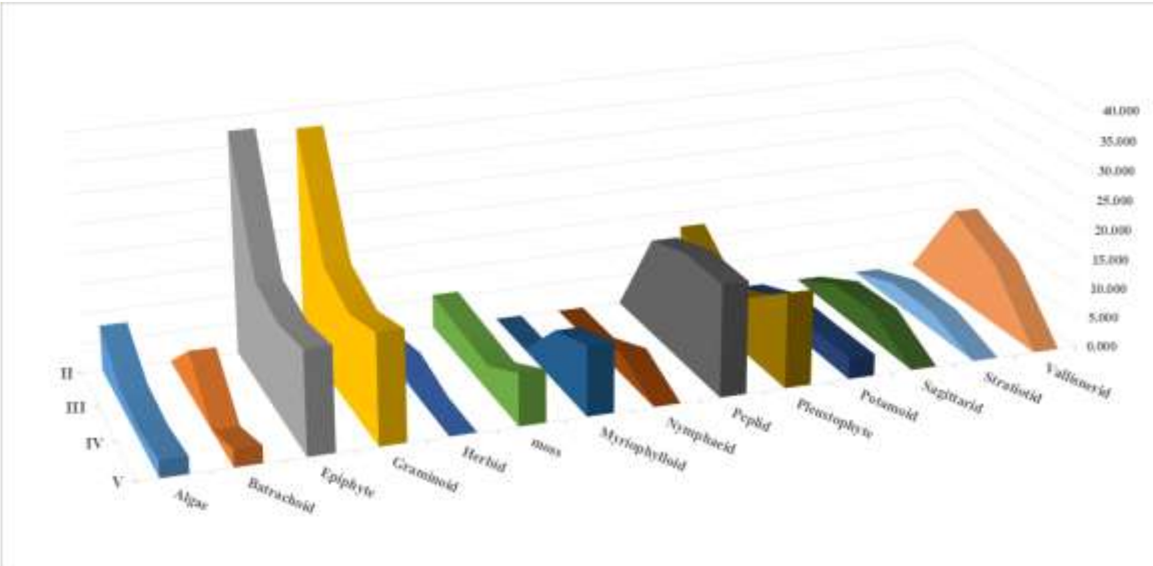


Figure 0.4: Graphical representation of modelled changes in plant growth forms (WIEGLEB 1991) across EQR classis in EC-1 lakes

Figure 0.5 indicates the relative abundance of hydrophyte species along the EQR gradient. We found a tendencious increase in the diversity of growth-forms along the ecological quality gradient (***Figure 0.6***); and found reliable increase of relative abundaces between high and good status in case of group 3 (e.g. *Butomus umbellatus, Nuphar lutea, Nymphaea alba, Sagittaria sagittifolia*), group 11 (*Mentha spp, Myosotis spp, Veronica spp*.), group 15 (*Potamogeton lucens, P. perfoliatus*), and group 16 (*Myriophyllum* spp). We found remarkable decrease of relative abundance in the H/G boundary the gradient in case of group 14 (Utricularia spp.), group 13 (e.g. *Ceratophyllum* spp.), 12 (e.g. *Najas marina, Potamogeton trichoides*) and 2 (*Glyceria fluitans*). Species known as nutrient tolerators e.g. group 6 (*Nymphoides peltata, Persicaria amphibia*) decreased its relative abundances in the G/M boundary, while group 18 (Lemna spp, Azolla filiculoides) remarkably decreased its relative abundances in the P/M boundary.

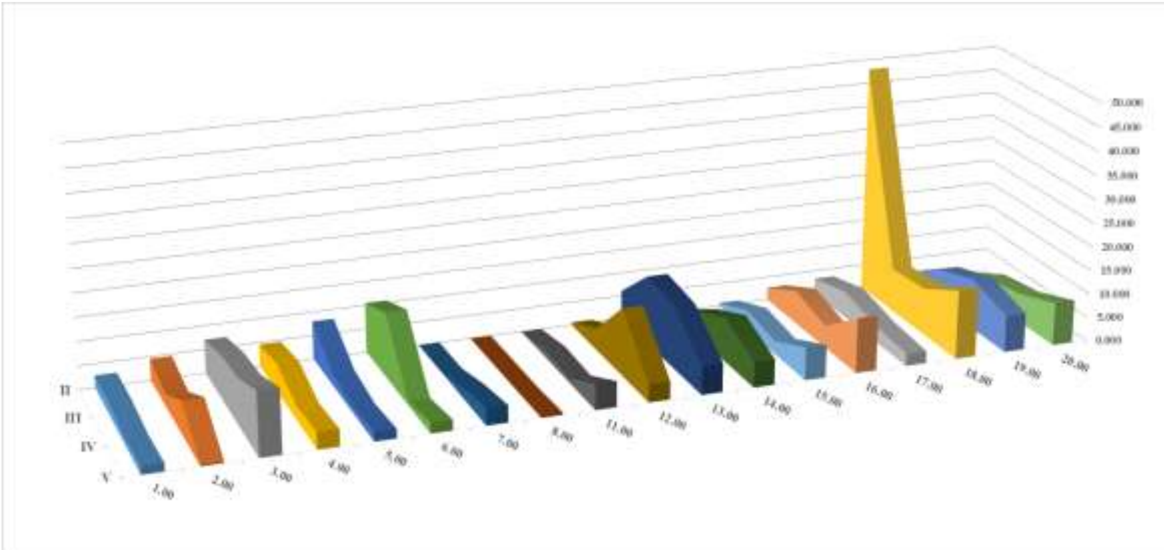


Figure 0.5: Graphical representation of modelled changes in plant growth forms (WILLBY et al. 2000) across EQR classis in EC-1 lakes. Numbers represent growth-forms obtained by trait attributes in Table 3. at WILLBY et al. 2000

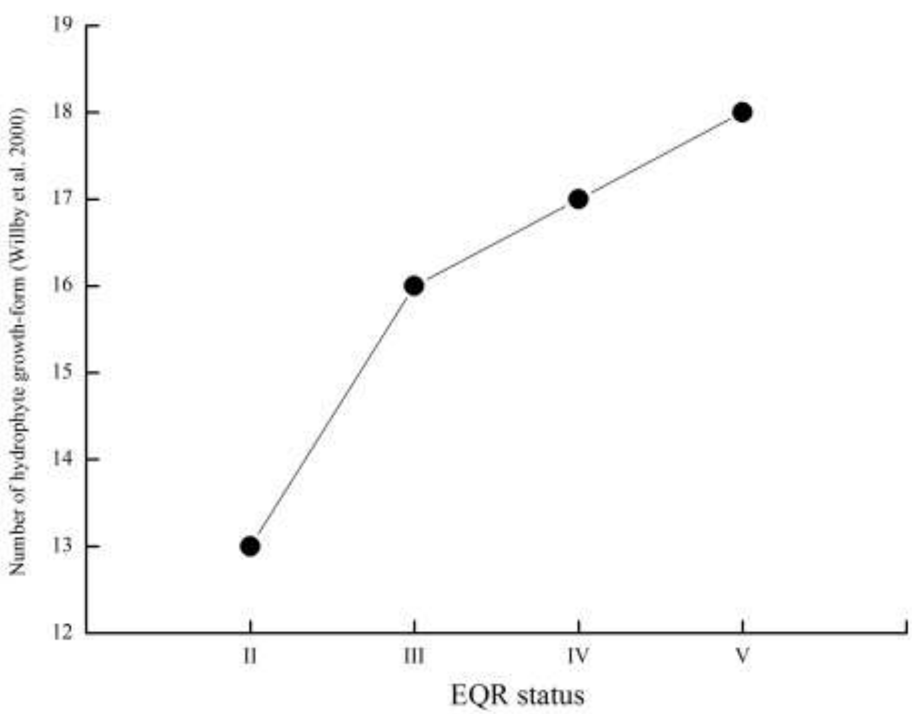


Figure 0.6: The relationship between number of hydrophyte growth-forms and EQR status

Figure 0.7 shows the changes of abundance and number of species along the degradation gradient.

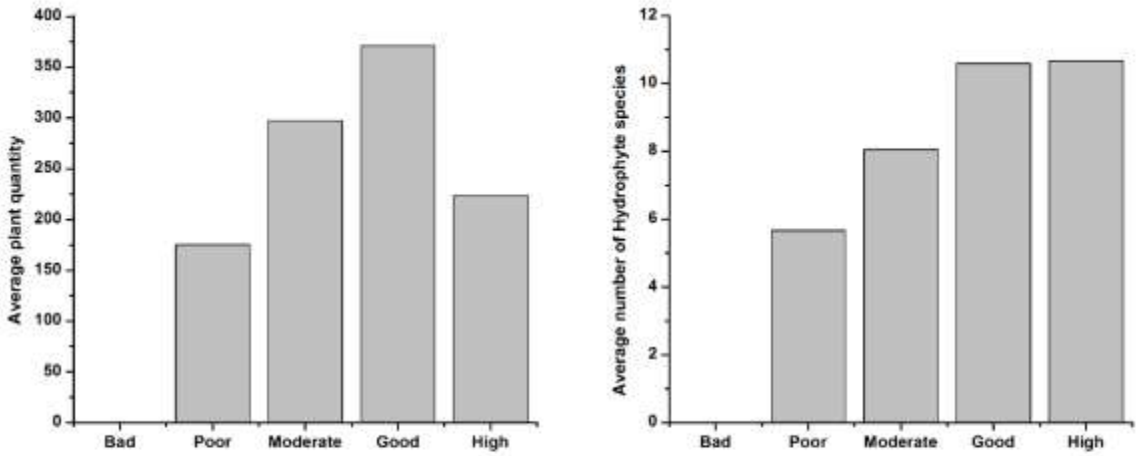


Figure 0.7: Average abundance (expressed as "plant quantity" = cubed Kohler-values) of hydrophtes in the different quality classes in EC-1 lakes and average number of hydrophte species in the different quality classes in EC-1 lakes.

Figure 0.8 to Figure 0.10 show the relative shares of "A", "B" and "C"-species in the different quality classes for the different countries.

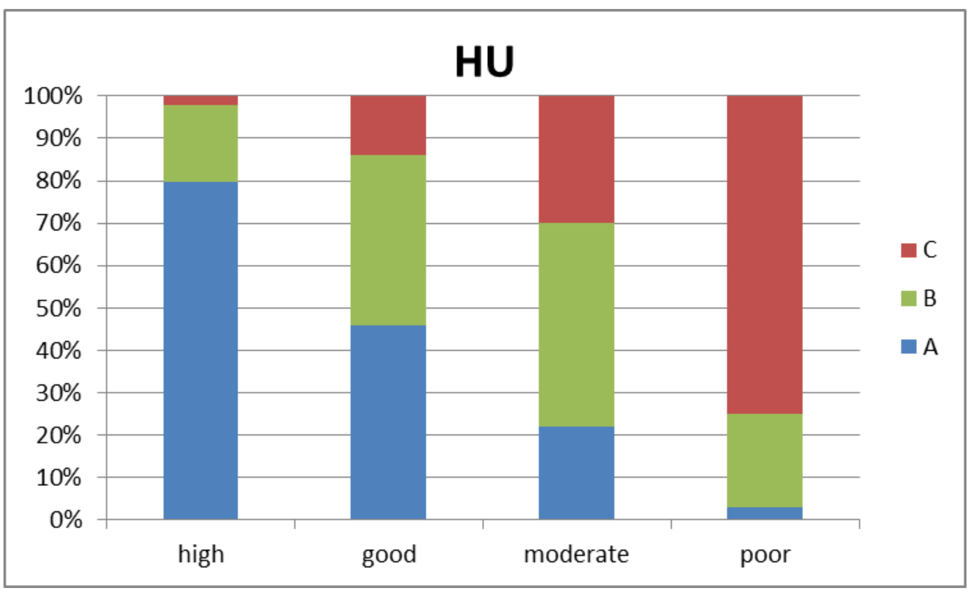


Figure 0.8: Relative share of A, B and C-species in the quality-classes – HU system

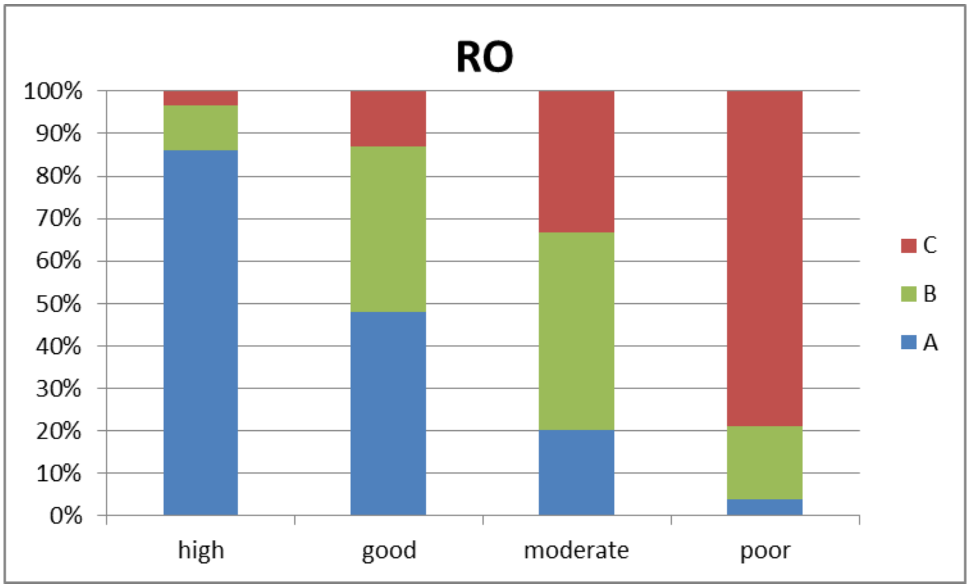


Figure 0.9: Relative share of A, B and C-species in the quality-classes – RO system

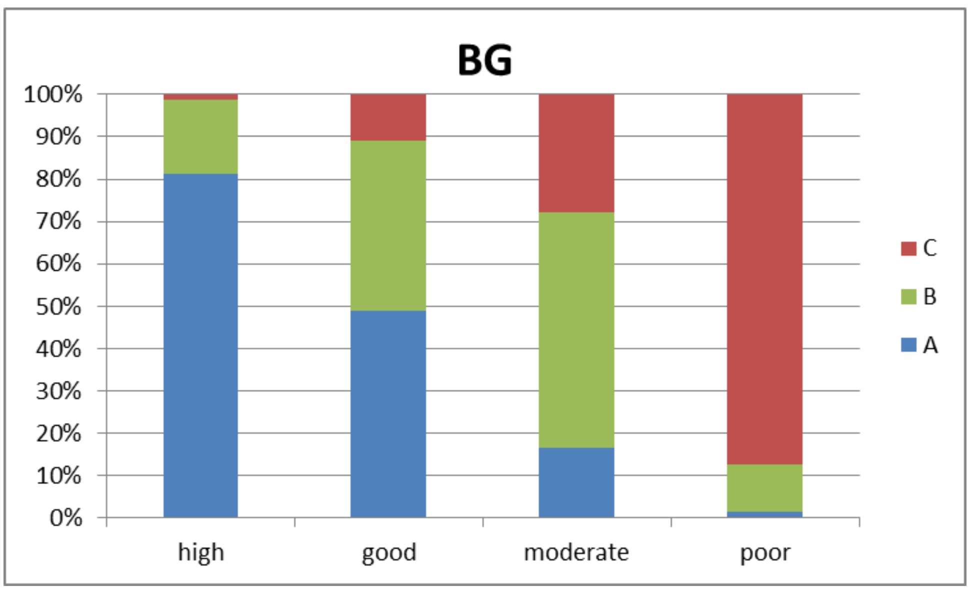


Figure 0.10: Relative share of A, B and C-species in the quality-classes – BG system.

10 Conclusion

Three countries participated in the intercalibration exercise and harmonised their assessment systems. Results are presented in Table 20 and included in the EC Decision on intercalibration (EC 2018).

Country	**National classification systems intercalibrated**	**Ecological Quality Ratios**	
		High-good boundary	***Good-moderate boundary***
Bulgaria	RI-BG - Adapted Reference Index	0.83	0.58
Hungary	HU-RI - Adapted Reference Index	0.89	0.67
Romania	MIRO - Macrophyte Index for Romanian Lakes (Adapted Reference Index)	0.86	0.66

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Annexes

A Hungarian macrophyte-based assessment system in EC-1 type lakes.

Status: national input for intercalibration, accepted national method, slight adjustments are still possible

1. Purpose

The macrophyte field survey procedure is part of a biological method to facilitate the establishment of good ecological status and general monitoring of ecological status in Europe with the Water Framework Directive (2000/60/EC).

The method can be use to give a qualitative assessment of whether a site be impacted by anthropogenic effects.

2. Biota sampled

The macrophyte field survey procedure is based on the presence and abundance of species of aquatic macrophyte. A macrophyte is defined as '*any plant observable with naked eye and nearly always identifiable when observed*' (HOLMES & WHITTON, 1977). It includes all higher aquatic plants, vascular cryptogams and bryophytes, together with groups of algae which can be seen to be composed predominantly of a single species.

3. Sampling of macrophytes

Macrophyte sampling method is based on the STAR protocol (DAWSON, 2002), which can be accorded with KOHLER (1978) method.

The sampling for macrophytes is carried out minimum once per year, mainly in the main vegetation period: (June) July–September (October), depending on the elevation and the annual weather condition.

Macrophytes have to identify for species level.

4. How to carry out macrophyte survey

During vegetation sampling all macrophyte present in the sampling area are recorded, together with the estimated plant mass (EPM) quantity scores. The quantity score is a 5 level scale: 1 = rare 2 = occasional; 3 = frequent, 4 = abundant, 5 = very abundant according to KOHLER (1978).

It is important to note that unlike the estimates reported only on the surface (the common meaning of "abundance"), the EPM takes into account the vertical development of plants, not just horizontal.

The macrophyte flora and physical character of the survey length are then surveyed by wading, boat or walking along the bank. Sampling aids are used when necessary. Representative samples (*Ranunculus*, algae, *Callitriche* and *Potamogeton* species) and species which is uncertain should are taken for laboratory identification and preparation of herbarium specimens. Physical parameters and additional data of the survey length are estimated and photographs taken. If possible, a GIS sketch with the location of the survey length also taken by PDA.

Sampling area is different in rivers and lentic waters.

The assessment of macrophyte in lakes and reservoirs is based on transects, located parallel to the shore. The length of transects is 100m (seldom more in certain situations). The number of the required parallel transects is determined after Schaumburg et al., (2007) according to "lakes" surface area (<0,5 km^2 - 1-5 transects; 0,5 – 2,0 km^2 - 4-8 transects; 2,0 – 5,0 km^2 - 5-10 transects; 5,0 - 10 km^2 - 6-12 transects; >10 km^2 - 8-15 transects).

Along each parallel to the shore transect a minimum of 4 belt transects have to take right angle to the shore. The width of these belt transects have to be 2m.

> *(Within the belt transect depth zones can be differentiated, if appropriate (usually 0 – 1m, 1 – 2 m, 2 – 4 m, 4 – 8 m, and deeper than 8 m). This may be of great help in discerning human impact e.g. from sport boating, which has the greatest impact in the upper layers, or from man-induced increase in turbidity, which affects most the lower parts of the littoral.)*

For either the full lengths of the belt transect (*or in case of using depth zones then within each depth zone*) plant mass (EPM) have to estimates for each species occurring. These estimates form the field data set for each transect.

Species list of the 4 belt transects have to rally on, the estimated quantity scores of the common species need to average.

Picture 1. Lake transects position.

Indispensable tools for monitoring

- Safety and health equipment
- Maps
- Sketch habitat map from sampling sites
- Copy of previous survey sheets
- Survey sheets
- Pen and pencil
- GPS/PDA
- Grapnel on min. 10 m long rope
- Rubber boot / fishing wader
- Plastic or paper bags, labels and tubes for specimens
- Tape measure
- Identification and field guides
- Camera
- Hand lens (10x)
- Polarising sunglasses
- Boat (optional)

5. Assessment

5.1. Calculation of reference index (RI)

The calculation of RI is based on the submerged, free floating pondweeds, amphyphytes and also the hygro- and helophytes on the shores are taken into account. Hygro- and helophytes take only if they develop below the water table or in moist surface.

The upper extension of species list compared to the German, Bulgarian etc. similar lists were necessary because of (1) the natural eutrophy of EC-GIG waters, which makes hard to detect eutrophic stressor responses by the method.

5.1.1. Transformation of abundance into quantity units

The determined in situ abundance of each species (from 1 to 5, according to KOHLER, 1978) is converted into quantity units according to the mean values of Braun-Blanquet cover classes (ENGLONER 2012):

Kohler's states (Kohler's scale)	Metric values	Conversion (mean values of Braun-Blanquet's cover classes[a])
Rare	1	3 (0 < x ≤ 5%)
Occasional	2	15 (5 < x ≤ 25%)
Frequent	3	37.5 (25 < x ≤ 50%)
Abundant	4	62.5 (50 < x ≤ 75%)
Very abundant	5	87.5(75 < x ≤ 100%)

[a] Ranges of cover classes in parentheses

5.1.2. Incorporation of species to groups

The species, described at each sampling station are divided into three specific groups.

Group A includes species with high abundance in reference sampling sites and absence or low abundance in any other circumstances. Those species are assigned to the characteristic or typical species of the type specific reference biocenosis.

Group C includes taxons, described seldom at in reference sampling sites. They usually have high abundance at sites where Group A is absent or is with very low abundance.

Group B includes species with no apparent preference to reference or other conditions. Usually they are found with either Group A or Group C representatives.

Species groups were assessed using several databases, literature and expert judgement. For this purpose we used social behaviour type (SBT), water (WB), salt (SB) and nutrient (NB) indicator values (BORHIDI, 1995). Indicator values indicate species salt, water and nutrient tolerance or demands, while social behaviour types, based on Grime's C-S-R strategy (GRIME, 1979), express species ecological habits (competitors, specialists, ruderals etc) in general. All species were analysed with regard to their preferences in relation to TP, TN and chl-a pressure indicators.

5.1.3. Calculation of the total group quantity.

The individual species quantity units are summed in a total Group quantity. If in a new assessment additional species are found, which are not mentioned in the following species list, these taxa should not be considered for index calculation. If the number of unlisted (=non indicative) species is high, this most likely will falsify the calculated index. Consequently, if the percentage of non indicative species is ≥ 25 %, the index value cannot be considered reliable.

5.1.4. Criteria for the reliable assessment

Species are not taken as reliable species if

- They have not got a category in a given column
- If a species are not in Table 1. or 2.

Lakes, Ponds and Reservoirs

- The total quantity of macrophytes must be at least 55, except alkali ponds where the minimum total quantity must be 15, otherwise the RI cannot be taken as representative, and can be used only as an addition to other BQE. If natural causes can be eliminated for these reason/conditions, then the lake is assigned as "very bad" ecological state or potential.

Additional criteria

- If the number of indicator species less than 3 and the plant quantity less than 27 the assessment cannot be reliable, EQR is set to be 0.
- If the following species are dominant (at least 80% quantity) the RI is reduced by 50:
    - *Amorpha fruticosa*
    - *Elodea canadensis/ nuttallii or*
    - *Myriophyllum spicatum or*
    - *Najas marina or*
    - *Potamogeton pectinatus or*
    - *Ceratophyllum demersum or*
    - *Ceratophyllum submersum*
- If due to application of these criteria RI < -100, it is set to be -100

5.1.5. Calculation of RI

For all types of lakes/ reservoirs and rivers the RI is calculated according to the following formula:

$$RI = \frac{\sum_{i=1}^{n_A} Q_{Ai} - \sum_{i=1}^{n_C} Q_{Ci}}{\sum_{i=1}^{n_g} Q_{gi}} * 100 \quad ,$$

where:

RI = reference index;
QAi = "quantity" of the i-th taxon of Group A;
QCi = "quantity" of the i-th taxon of Group C;
Qgi = "quantity" of the i-th taxon of all Groups;
nA = number species in Group A;
nC = number species in Group C;
ng = total number species (A+B+C).

If the value of RI drops below -100, due to application of several additional criteria (type specific), a fixed value of -100 is set for the RI.

5.2. Final assessment (EQR)

The transformation of the RI to a 0 to 1 scale unit is done according to the following formula:

EQR = {(RI + 100) * 0,5}/100

In the cases, in which the absence of macrophytes from the sampling station is due to changes in physico-chemical parameters, morphology or other anthropogenic influences, the station is assessed as "very bad" ecological state or potential.

6. Boundary Setting

For the boundary setting, the species composition of least disturbed sites (E-C1 lakes) as alternative benchmark sites was used (see least disturbed site selection below). High/good boundary was considered as the 75th percentile of the HURI index-value in the least disturbed sites (so called alternative benchmark lakes). Good/moderate boundary was considered as the 25th percentile of the reference index in the benchmark lakes. This approach corresponds to that applied in the EC-Lake-GIG Phytoplankton group.

The bad status means a loss of macrophytes. Therefore the boundary for the bad status is 0. The moderate/poor boundary was considered as the arithmetic mean of the good/moderate boundary and the poor/bad boundary. The comparison with the WFD Annex V normative definitions is given in Table 2. The boundaries for each HURI index-value are listed in Table 3.

Table 2. The comparison with WFD Annex V, normative definitions for for the Hungarian assessment system.

Ecol.status	Normative definition (WFD)	Boundary setting procedure
High EQR = 0.87–1.0	The values of the biological quality elements for the surface water body reflect those normally associated with that type under undisturbed conditions, and show no, or only very minor, evidence of distortion.	In high status species of group A represent more than half of the overall plant quantity. H/G boundary is the point at which A-species loss their overall dominance. For the boundary setting, the least disturbed sites as "alternative benchmark" sites were used. H/G boundary corresponds to the 75th percentile of "alternative benchmark" lakes EQR's.
Good EQR = 0.67–<0.87	The values of the biological quality elements for the surface water body type show low levels of distortion resulting from human activity, but deviate only slightly from those normally associated with the surface water body type under undisturbed conditions.	In good status A-species still overweight C-species. A- and B-species represent clearly more than half of the overall plant quantity. G/M boundary was determined according to the relative abundance distribution of 'A' and 'C' species along the EQR range. This is the turning point among 'A' and 'C' species: the relative amount of 'A' and 'C' species are changed toward 'C' species. This corresponds to the 25th percentile of the reference index in the HU benchmark lakes.
Moderate EQR = 0.33–<0.67	The values of the biological quality elements for the surface water body type deviate moderately from those normally associated with the surface water body type under undisturbed	In moderate status A- and B-species represent clearly more than half of the overall plant quantity, C-species start to overweight A-species.

	conditions. The values show moderate signs of distortion resulting from human activity and are significantly more disturbed than under conditions of good status.	The M/P boundary was considered as the arithmetic mean of the G/M boundary and the P/B boundary. This is the point where C species start to become overall dominant.
Poor EQR = >0.00–<0.33	Waters showing evidence of major alterations to the values of the biological quality elements for the surface water body type and in which the relevant biological communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions, shall be classified as poor.	In poor status C-species represent more than the half of the overall plant quantity. Few species and low abundances. The P/B boundary represents the loss of macrophytes due to anthropogenic pressure.
Bad EQR =0.00	Waters showing evidence of severe alterations to the values of the biological quality elements for the surface water body type and in which large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent, shall be classified as bad.	No macrophyte vegetation.

Table 3. EQR values for macrophytes in EC-1 lakes

	Ecological state	EC-1
		EQR
High	5	1.00 – 0.87
Good	4	0.86 – 0.67
Moderate	3	0.66 – 0.33
Poor	2	0.32 – 0.00
Bad	1	-

Least disturbed sites were selected according to hydro-morphological alterations and fishing/fish-farming stressors. Some more or less unimpacted lakes could be identified in the Hungarian database. These lakes were selected and analysed with regard to eutrophication-parameters.

Out of this pool of lakes the lowest impacted lakes with regard to nutrient- and eutrophication indicating parameters were selected as least disturbed sites or "alternative benchmark" lakes. For the common intercalibration type EC1 lakes all in all 5 least disturbed sites or "alternative benchmark" lakes could be identified.

7. Pressure response relationships

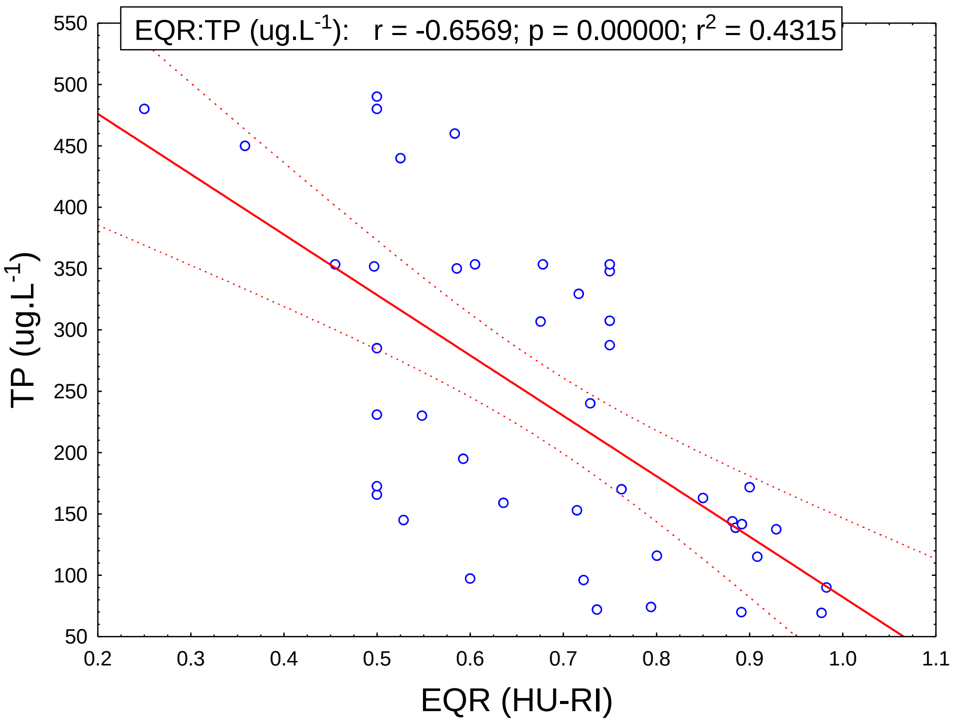

Figure 1. Relationship between TP and EQR. Red line is linear regression with confidence intervals.

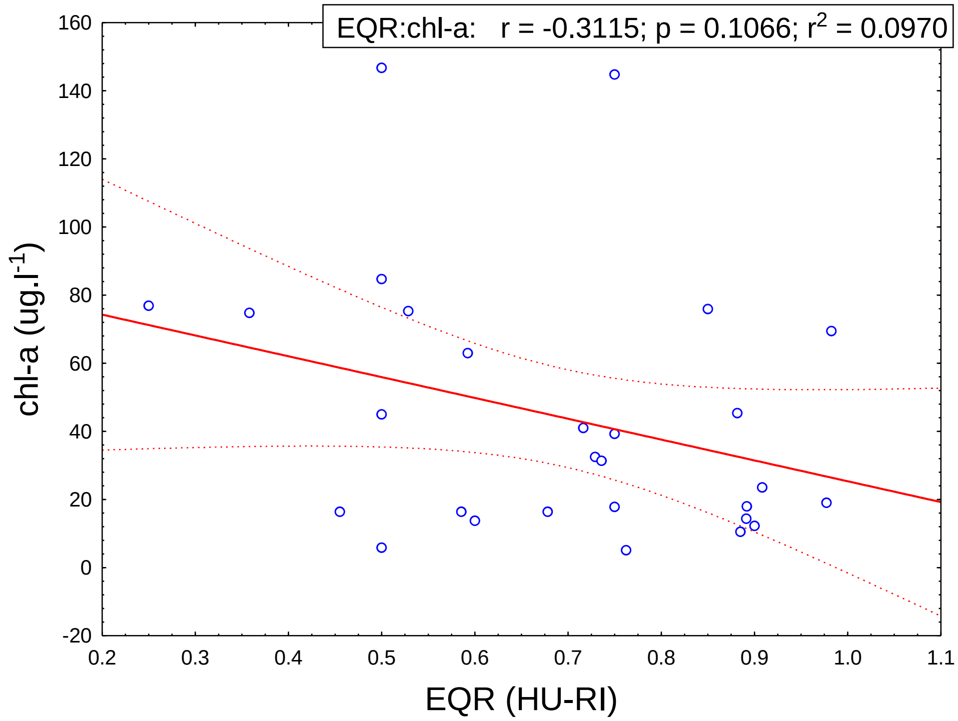

Figure 2. Relationship between chl-a and EQR. Red line is linear regression with confidence intervals.

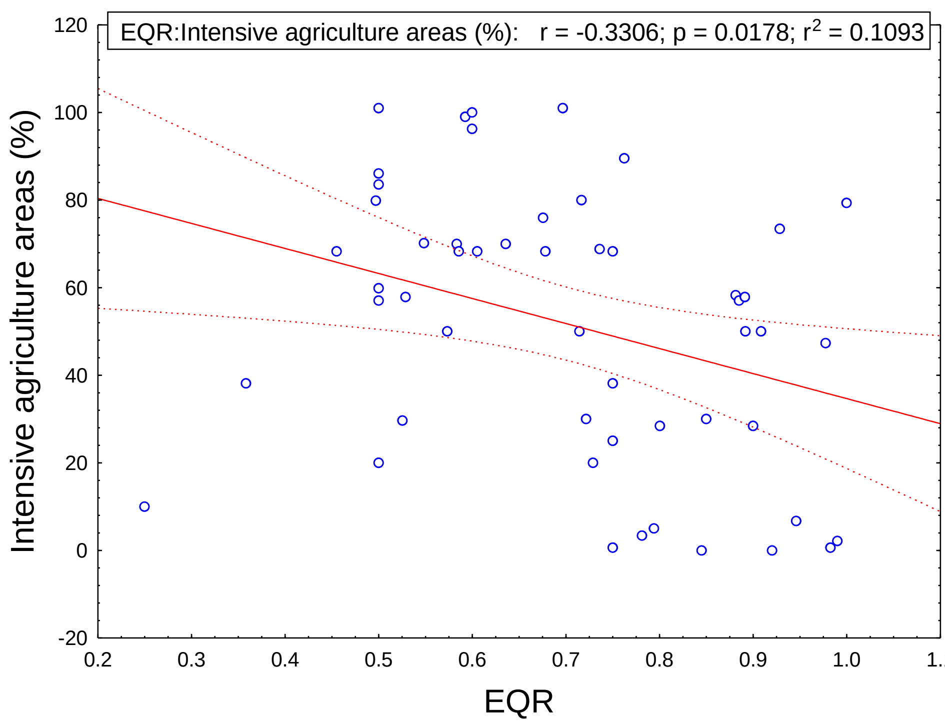

Figure 3. Relationship between the area of intensive agricultural ares and EQR. Red line is linear regression with confidence intervals.

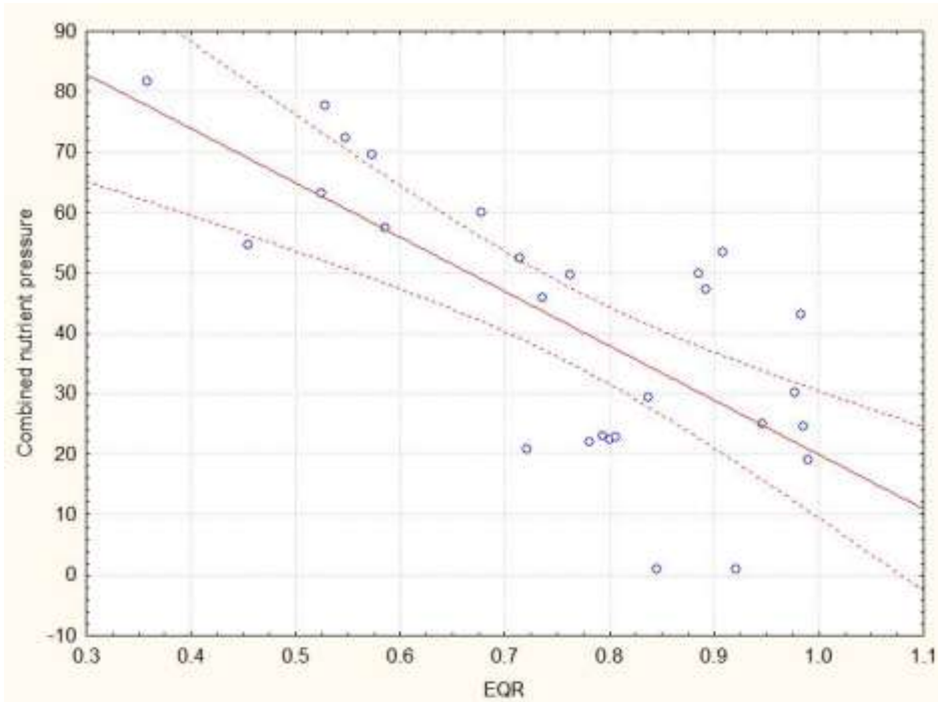

Figure 4. Relationship between combined pressure metric and EQR.

B Romanian macrophyte-based assessment system MIRO

1) PRINCIPLE OF THE METHOD – SUMMARY

The Romanian macrophyte-based assessment system for lakes MIRO (Pall et al., 2015)is an adaption of the German Reference-Index (Schaumburg et al., 2006).

The data for the method development were provided by the National Administration "Romanian Waters". The dataset comprised macrophyte-, abiotic-, and pressure-data from 48 natural Romanian lakes out of the years 2006 to 2014. They have been sampled according to a WFD-compliant and national agreed field protocol.

As highly relevant pressures nutrient enrichment/eutrophication and hydro-morphological alterations could be identified. In order to derive reasonable pressure-response relationships not only hydrophytes but also amphiphytes and helophytes as well as selected other water-related species were selected to enter the assessment.

In the dataset no real "reference lakes" in the sense of "anthropogenic unimpacted lakes" could be identified. Therefore a set of least impacted lakes was identified. These lakes were used on the one hand as benchmark for a WFD-compliant boundary-setting and, on the other hand, as precondition to derive a macrophyte-based typology for Romanian natural lakes.

Within the benchmark dataset 5 macrophyte-based lake types could be distinguished. Altitude and alkalinity turned out to be the most important differentiation factors.

Concerning the assessment concept it was the explicit request of the National Administration "Romanian Waters" to try an improvement of the original German "Reference-Index" Method as the first choice. This method was widened in terms of macrophyte groups and pressures considered. Furthermore the calculation rules were modified and optimised for conditions in Romanian lakes.

The new method "MIRO" (Romanian Macrophyte Index; working name proposal) delivered sound pressure-response relationships. This applies especially for the nutrient enrichment/eutrophication pressure. Even for the pressure hydro-morphological alterations promising correlation with the index values resulted. However, this metric has the potential to be strongly improved by using optimising and widen the stressor data base.

For boundary setting a statistical approach, using percentiles of benchmark lakes, was used.

The values of the pressure parameter for all quality classes set are presented as box-plots.

The method presented fulfils the requirements of the Water Framework Directive and it is ready for intercalibration within the Eastern-Continental GIG.

2) DATASET USED

The data were provided by the National Administration "Romanian Waters". The dataset comprised macrophyte-, abiotic-, and pressure-data from 48 natural Romanian lakes out of the years 2006 to 2014.

2.1) Lakes considered

The lakes are situated in 9 different river basins and belong to 10 different Romanian lake types.

2.2) Chemical/physical and pressure data

The following abiotic parameters were provided:

Table 1: Abiotic Parameters.

Typology	RO Lake type
	Type characterisation
	Ecoregion
	Lake altitude [m.s.l.]
	Lake geology
	Average depth [m]
	Lake area [km²]
	Catchment area [km²]
	Retention time
	Sediment type
	Bank structure
Physical / chemical features	Water transparency [m]
	Water temperature [°C]
	Conductivity [uS/cm]
	Alcalinity [mmol/l]
	pH
	Dissolved oxygen [mg/l]
	Oxygen saturation [%]
	BOD5 [mgO2/l]
	DOC [mg/l]
	TOC [mg/l]
	TP [mg/l]
	PO4-P [mg/l]
	TN [mg/l]
	NO3-N [mg/l]
	NO2-N [mg/l]
	NH4-N [mg/l]
	Calcium [mg/l]
	Magnesium [mg/l]

	Total hardness [mg/l $CaCO_3$]
	Suspended solids [mg/l]
	TDS [mg/l]

Table 2: Pressure Information.

Pressures	Shoreline degradation
	Shipping
	Water abstraction
	Recreation
	Water level fluctuations
	Fishing pressure
	Fish farms
	Eutrophication, diffuse
	Point source discharge, wastewater
	Intensive agricultural use
	Corine artificial surfaces [%]
	Corine intensive agriculture [%]
	Corine low intensive agriculture [%]
	Corine natural lands [%]
	Population density in the catchment area
	Alien species (fish and macrophytes, Benthic fauna)

2.3) Macrophyte data

Macrophytes have been mapped according to the Romanian field protocol (SÂRBU et al., 2009). Following this, Hydrophytes, Amphiphytes and Helophytes as well as some other relevant water-related plants are considered. All in all 144 species were identified.

3) ESTABLISHING PRECONDITIONS

3.1) Identifying relevant anthropogenic Pressures

3.1.1) Nutrient enrichment – Eutrophication

Macrophytes as photo-autotrophic organisms first of all respond to eutrophication. The use of macrophytes as indicator organisms for the degree of this pressure in lakes already has a long tradition. Mostly the focus was on the assessment of the trophic condition of the littoral and, as help for water protection institutes, particularly on the exact localization of pollution sources along the lakeshores.

Regarding the requirements of the Water Framework Directive (WFD) macrophytes have thus to be consulted for the assessment of the trophic condition of lakes and the detection of pollution sources. In this connection it is a big advantage that aquatic plants do not show a sudden reaction to changes of the trophic conditions, but it usually takes the macrophyte vegetation some years to adapt to the new conditions.

Aquatic plants are thus particularly well suitable for the long-term assessment of trophic conditions. In contrast to more quickly reacting elements, as for example phytoplankton, the quality element macrophytes offers the advantage, that even from a unique mapping within the vegetation period, a founded and temporally-integrated information about the water quality and the state of eutrophication and re-oligotrophication processes can be derived.

3.1.2.) Hydro-morphological Alterations

However, macrophytes do not reflect solely the trophic conditions in a lake, they also respond very sensitively to other impacts on their environment, especially changes of the hydrological regime (alteration of the natural water level fluctuations) or hydrodynamic conditions (e.g. change of the wave climate by motorboats and navigation). Beside the submerged vegetation and the floating leafed plants these affect especially the ecologically important habitat of the amphibious vegetation and the reeds. Last but not least the structural alterations of the shoreline and/or the water-land-linkage can be judged by including the amphibious vegetation and the reeds.

3.1.3) Fishing and Fish-Farming

Especially in shallow lakes, besides eutrophication and hydro-morphological alterations, intensive fishing activities and especially fish-farming have an influence on the macrophyte vegetation. However, this results from a combination of nutrient enrichment, hydro-morphological alterations of the shoreline and mechanical damage.

3.2) Selection of Benchmark Lakes

Following the requirements of the WFD the assessment has to reflect the degree of deviation of the current macrophyte vegetation from the reference condition. This presupposes knowledge of the reference conditions.

However, within the Romanian dataset no real "reference lakes" in the sense of "anthropogenic unimpacted lakes" could be identified. This applied especially with respect to nutrient enrichment and eutrophication, respectively. Phosphorus- as well as Nitrogen-concentrations in the considered lakes are anyhow higher than they should be expected in reference conditions. Even the values of other parameters as e.g. Oxigen-saturation, BOD, transparency can be interpreted as signs for a favourable nutrient enrichment in many cases.

Only with respect to hydro-morphological alterations and fishing/fish-farming some more or less unimpacted lakes could be identified in the database. These lakes were selected and analysed with regard to eutrophication-parameters.

Out of this pool of lakes the lowest impacted lakes with regard to nutrient- and eutrophication indicating parameters were selected as "benchmark lakes". For 8 out of the 10 Romanian lake types in the database benchmark lakes could be found:

Table 3: Selected benchmark lakes.

Benchmark lake name	Romanian type
Bentul Latenilor Brat Dunarea Veche Victoria Geormane	ROLN01
Somova	ROLN02
Razim	ROLN03
Puiu Rosu Gorgostel	ROLN04
Bodi Mogoșa Știucilor	ROLN07
Balea Lacu Rosu	ROLN08
Lata Tarova	ROLN09
Snagov	ROLNPM01

The lakes selected were used on the one hand as benchmark for a WFD-compliant boundary-setting and, on the other hand, as precondition to derive a macrophyte-based typology for Romanian natural lakes.

3.3) Lake Typology

3.3.1) Romanian Typology for natural Lakes

Romania has developed a national typology for natural lakes, based on abiotic parameters (ANAR, 2013). According to this the dataset provided comprises ten different lake types.

3.3.2) Macrophyte-based Typology for natural Romanian Lakes

In order to derive macrophyte-based lake types for Romanian natural lakes the macrophyte data sets were aggregated from transect-data-sets to lake-data-sets, whereat investigations of the same lake in different years were treated separately. Finally 22 datasets entered a cluster-analysis. All macrophyte species were included.

On the basis of the cluster-analysis 5 macrophyte-based lake types could be distinguished (Figure 1):

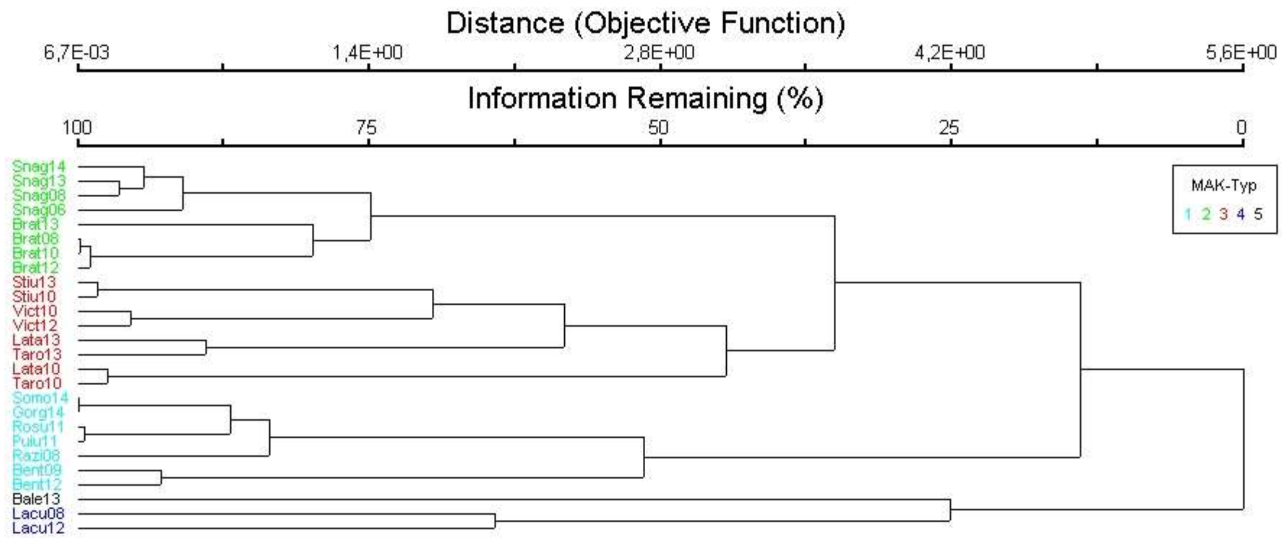

Figure 1: Cluster-Analysis of benchmark lakes.

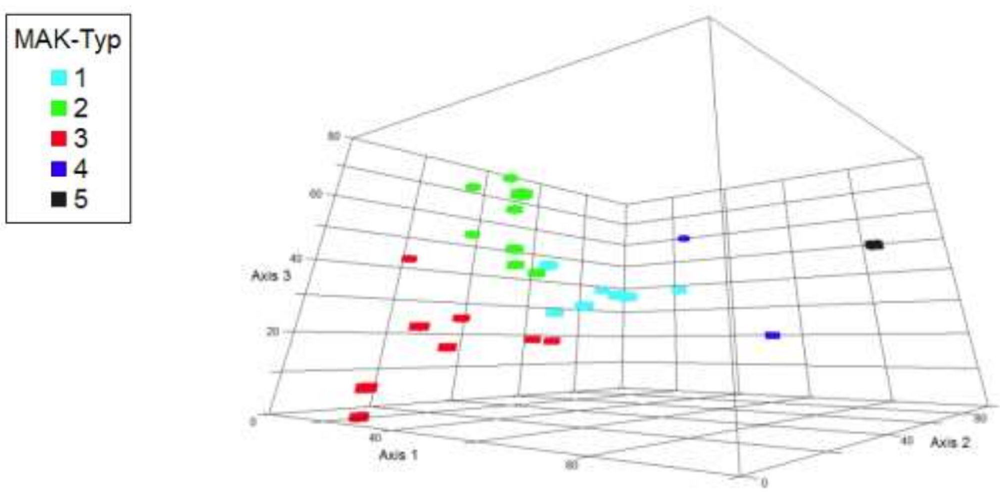

Figure 2: DCA of benchmark lakes – axes 1, 2 and 3.

As expected, not all of the defined Romanian lake types within the selected set of benchmark lakes showed remarkable differences concerning their macrophyte vegetation. Contrariwise some Romanian lake types had to be split up.

Analysing the results it turned out that altitude and alkalinity were the most important differentiation factors.

4) OPTIMISING THE REFERENCE INDEX METHOD FOR ROMANIA

4.1) The Reference-Index – Principle of the Method

The Reference-Index in its original form was developed by SCHAUMBURG et al, (2004) for German lakes (see this paper for details). The method is based on the type-specific relative abundance of macrophyte species of three different ecological species groups:

- Reference taxa,
- Indifferent taxa, and
- Degradation indicators.

The idea is that the ecological status classes result from the share of the three different groups:

• Predominantly reference taxa (group A)	high status
• Predominantly reference taxa and indifferent taxa Taxa of species group A have higher abundances than Taxa of species group C	good status
• Predominantly indifferent taxa Taxa of species group C equal or slightly outweight taxa of species group A	moderate status
• Predominantly degradation indicators Taxa of species group A are nearly replaced by taxa of species group C	poor status
• Depopulation of macrophytes Very low abundances without natural reasons	bad status

The pressures addressed by the original method are

- Eutrophication and
- General degradation.

In the original method only hydrophytes enter the assessment. Required are quantitative macrophyte-data on species level. For that several transects per lake have to be sampled. The quantity of each species has to be given in one of five abundance classes:

1 = very rare, 2: = rare, 3 = common, 4 = frequent, 5 = abundant/predominant.

Prior to performing any calculations, the nominally scaled values of plant abundance are converted into metric quantities using the following function:

Equation 1: Deriving Macrophyte Quantity

$$\text{Abundance Class}^3 = \text{Quantity.}$$

The taxa occurring at the sampling site will be assigned to the defined type specific species groups. The quantities of the different species will be summed up separately for each group and for all submerged species of a sampling site.

The Reference Index is calculated according to the following formula:

Equation 2: Calculation of the Reference Index

$$RI = \frac{\sum_{i=1}^{n_A} Q_{Ai} - \sum_{i=1}^{n_C} Q_{Ci}}{\sum_{i=1}^{n_g} Q_{gi}} * 100$$

RI =	*Reference Index*
QAi =	*Quantity of the i-th taxon of species group A*
QCi =	*Quantity of the i-th taxon of species group C*
Qgi =	*Quantity of the i-th taxon of all groups*
nA =	*Total number of taxa in group A*
nC =	*Total number of taxa in group C*
ng =	*Total number of taxa in all groups*

The RI is an expression of the "plant quantity" ratio of type-specific sensitive taxa, dominating at reference conditions, compared to the "plant quantity" of insensitive taxa and is therefore a tool for estimating the deviation of observed macrophyte communities from reference communities. The resulting index values range from +100 (only species group A taxa) to –100 (only species group C taxa).

The Reference Index value is to be converted in an EQR-value as follows:

Equation 3: Converting Index-values to EQR-values

$$\boldsymbol{EQR = \frac{(RI + 100) * 0,5}{100}}$$

RI =	*Reference Index*
EQR =	*Ecological Quality Ratio*

4.2) Macrophyte Index for Romanian Lakes "MIRO" (adapted Reference-Index)

4.2.1) Pressures adressed

The aim of this study was first of all to optimise the Reference Index assessment system for its applicability for Romanian lakes. In the original system the main pressure addressed is eutrophication. Accordingly the classification of species in the indicator list was based mainly on the ranking of species along a Phosphorus gradient. However, the Phosphorus concentration in the Romanian lakes considered is so high that solely species adapted to these high levels occur and that there are only minimal differences in the species sets along the remaining Phosphorus gradient.

Nevertheless, **nutrient enrichment** can be regarded as the main pressure to be detected by macrophytes in Romania, too. As abiotic indicators for this pressure we considered not only Phosphorus- (TP and PO_4-P) but also Nitrogen-concentrations (TN, NO_3-N, NO_2-N, NH_4-N) as well as Secchi-depth, DOC and TOC. The composition of the macrophyte vegetation was analysed along the single parameters as well as along a "combined nutrient pressure".

The second important pressure to be detected and assessed using macrophytes is **hydro-morphology.** We used the information about the degree of shore degradation, shipping, water level fluctuations, recreation activities and bank fixation and analysed the macrophyte composition along these gradients. Additionally an analysis was performed for a "combined hydro-morphological pressure".

Analogous analyses were performed for the **fishing/fish-farming** pressure. We could find some correlations between several species and pressure degree, especially for fish-farming. However, the results don't allow a proper development of a related index. Therefor more data would be needed.

4.2.2) Species considered

Most macrophyte-based assessment system currently used for WFD purposes concentrate on eutrophication at not to high degree und thus deal preferably with Hydrophytes. However, especially under highly eutrophic conditions, occurrence and distribution of Amphiphytes and Helophytes can provide valuable hints concerning nutrient enrichment. This applies also for some additional "water-related" species. Furthermore are these plant-groups highly relevant indicators for the hydro-morphological pressure. The method proposed here therefore includes all above mentioned plant-groups, namely **Hydrophytes**, **Amphiphytes**, **Helophytes** and "**other water-related species**".

4.2.3) Classification of Species

All species were analysed with regard to their preferences in relation to the above mentioned abiotic pressure indicators. This was done using several databases, literature and expert judgement.

4.2.4) Calculation Rules

4.2.4.1) Aggregation of Macrophyte Data

As a first step the data from the single sections of a single transect have to be combined to one macrophyte dataset per transect. This has to be done in the way that for each species the maximum abundance value is taken. After this, the resulting abundance values for all species are to convert in plant quantities (see equation 1).

As a second step the data from the single transects of one lake (one investigation period/year) have to be combined to one macrophyte dataset per lake. For this, for all species the arithmetic mean of the plant-quantities of all transects is to be calculated. Following this the cubic root is to be calculated. Resulting values >0 and <1 are to be rounded up to 1. All other values are to be rounded down to the next integer.

4.2.4.2) Calculation of the Index-Values and Conversion in EQR-Values

The calculation of the conversion in EQR-values can be performed according to the formulae of the original version of the Reference-Index (see equation 2 and 3).

The calculations have to be done separately for the nutrient enrichment/eutrophication-metric and the hydro-morphological alteration metric.

4.2.4.3) Additional criteria

The Reference Index can only be calculated in case there are at least 3 different indicator species in a quantity of at least 27.

4.3) Pressure-Response-Relationship

4.3.1) Pressure Nutrient Enrichment/Eutrophication

Figure 3 shows the pressure-response-relationships between the considered parameters of the pressure "nutrient enrichment/eutrophication" and the corresponding metric of MIRO.

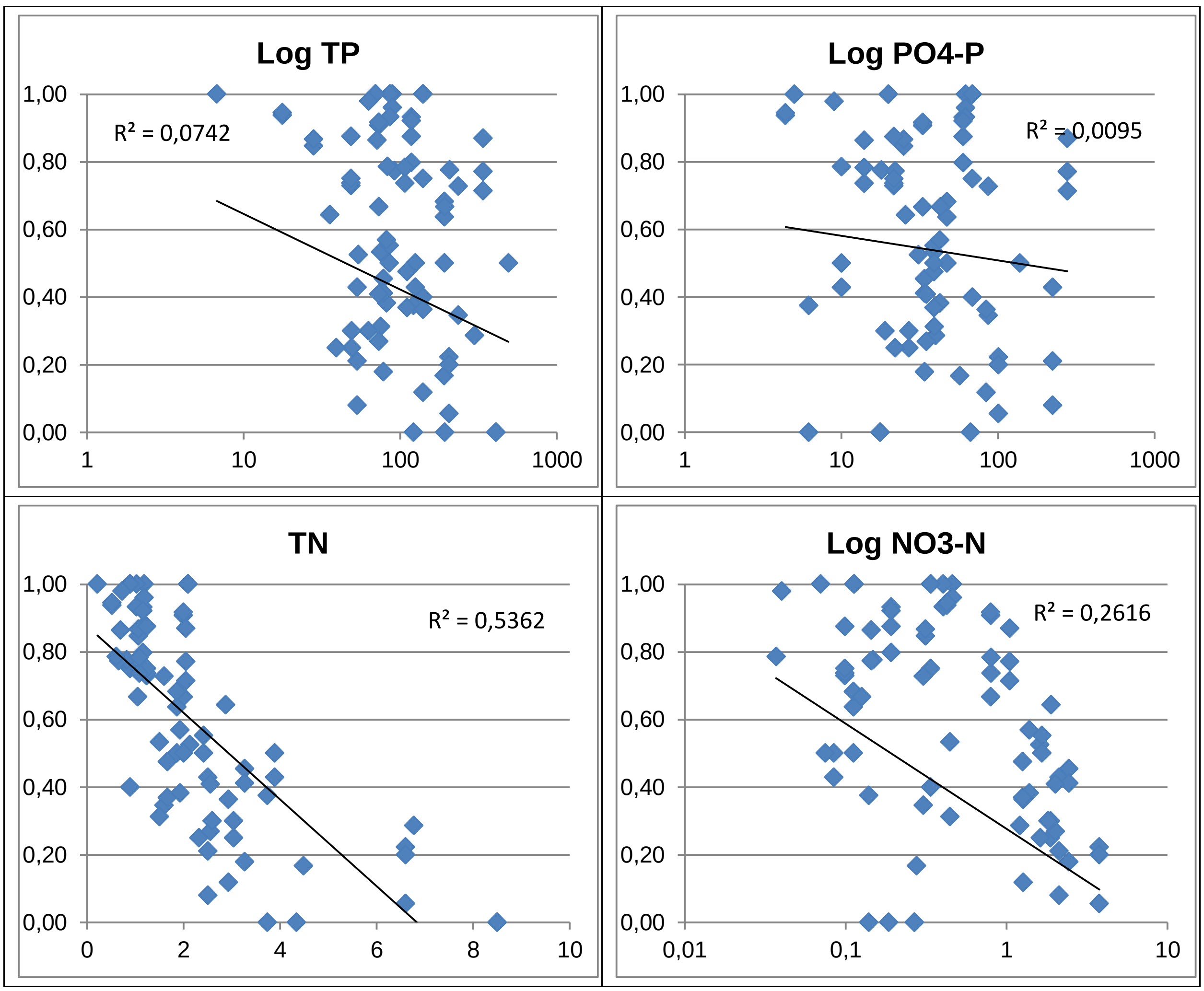

Figure 3: Pressure-response-relationship: Nutrient-enrichment/eutrophication.

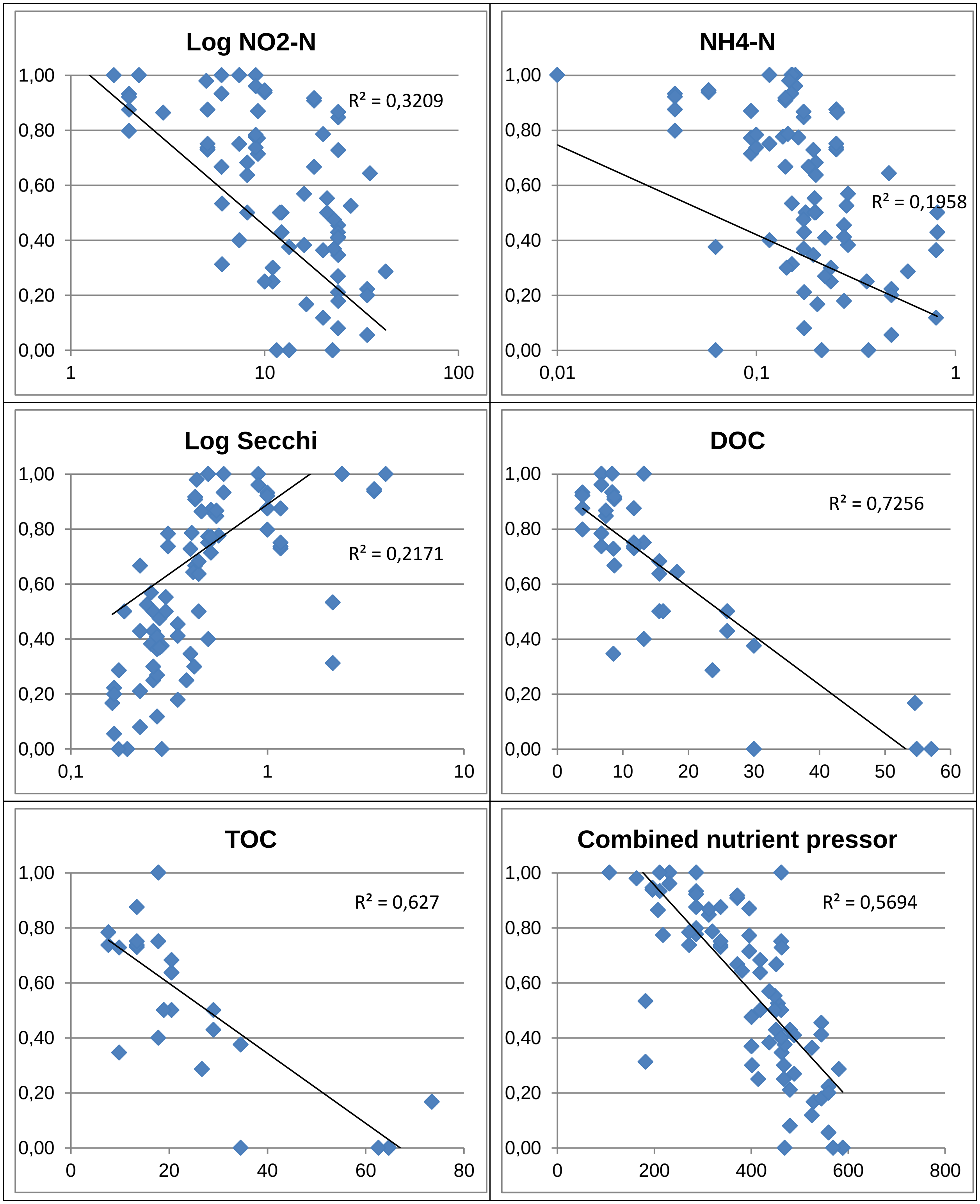

Figure 3, continued: Pressure-response-relationship: Nutrient-enrichment/eutrophication.

(The combined nutrient pressure represents the rank sum of all eutrophicaten relevant physic-chemical and landuse parameters).

4.4) Boundary Setting

For the boundary setting, the species composition of least disturbed sites (E-C1 lakes) as alternative benchmark sites was used. High/good boundary was considered as the 75th percentile of the MIRO index-value in the benchmark lakes. Good/moderate boundary was considered as the 10th percentile of the reference index in the benchmark lakes. The bad status means a loss of macrophytes. Therefore the boundary for the bad status is 0. The moderate/poor boundary was considered as the arithmetic mean of the good/moderate boundary and the poor/bad boundary. The boundary setting procedure is specified in Table 4, the boundaries for each MIRO index-value are listed in Table 5.

Table 4. The comparison with WFD Annex V, normative definitions for the Romanian assessment system

Ecol.status	Normative definition (WFD)	Boundary setting procedure
High EQR = 0.86–1.00	"The taxonomic composition corresponds totally or nearly totally to undisturbed conditions. There are no detectable changes in the average macrophytic […] abundance. […]"	In high status the quantity of group A species represents more of the half of the overall species quantity, species of group C have only a minor share. H/G boundary is the point where A-species loss their dominance over B- and C-species. For the boundary setting, the least disturbed sites as alternative benchmark sites were used. H/G boundary was considered as the 75th percentile of the reference index in the benchmark lakes. This approach corresponds to that applied in the EC-Lake-GIG Phytoplankton group.
Good EQR = 0.66–<0.86	"There are slight changes in the composition and abundance of macrophytic […] taxa compared to the type-specific communities. […]"	In good status species of group A and B represent clearly more than half of the plant quantity, the share of A-species overweigths the share of C-species. G/M boundary is the point where C-species start to overwight A-species. This corresponds to the 10^{th} percentile of the reference index in the RO benchmark lakes.
Moderate EQR = 0.33–<0.66	"The composition of macrophytic […] taxa differ moderately from the type specific communities and-are significantly more distorted than those observed at good quality. Moderate changes in the average macrophytic […] abundance are evident. […]"	In moderate status species of group A and B represent more than half of the plant quantity, the share of C-species starts to overweigth the share of A-species. M/P boundary is the point where C species start to become dominant over A- and B-species.

		This corresponds to the arithmetic mean of the good/moderate boundary and the poor/bad boundary.
Poor EQR = >0.00–<0.33	"Macrophyte communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions".	In poor status species of group C represent more than the half of the overall plant quantity. Very low abundances of macrophytes and very low number or species. The P/B boundary was set at the point where macrophytes completely disappear due to anthropogenic pressure.
Bad EQR =0.00	"Large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are missing"	Bad status: No macrophyte vegetation due to anthropogenig pressures.

Table 5: Class boundaries for the MIRO, metric nutrient-enrichment for E-C1 lakes.

MIRO Class boundaries	Pressure nutrient enrichment/ eutrophication
high / good	0.86
good / moderate	0.66
moderate / poor	0.33
poor / bad	0.00

4.5) Status classes versus pressure

The following graph shows for each quality class the median, the 25th and 75th percentiles, the 10th and 90th percentile as well as minima and maxima of the combined nutrient pressure.

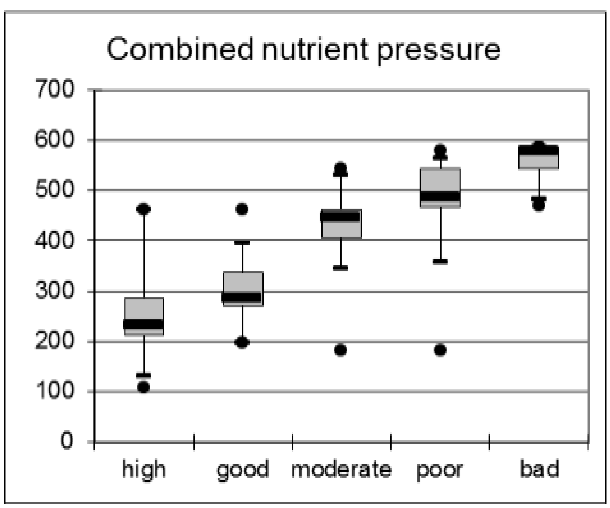

Figure 4: Status classes versus combined nutrient-pressure.

C Bulgarian macrophyte-based assessment system RI-BG

Overview of the assessment method

The macrophyte survey is carried out once during the main vegetation period (end of June until September). In each sampling site a belt transect of 20–30 m width orthogonal to the shoreline and positioned within an ecologically homogenous section of the littoral is surveyed (Fig. 1). The transect number is in correlation to the lake size. At each transect four different depth zones are sampled (0–1 m, 1–2 m, 2–4 m and >4 m). The abundances of all single species in all depth zones are registered using a five-level scale (see below).

For assessment the species are designated to three different groups: "reference taxa", "indifferent taxa" and "degradation indicators". The relative share of these different groups decides of the ecological class of the investigated transect.

The ecological quality class of the water body results from averaging the results of the single transects.

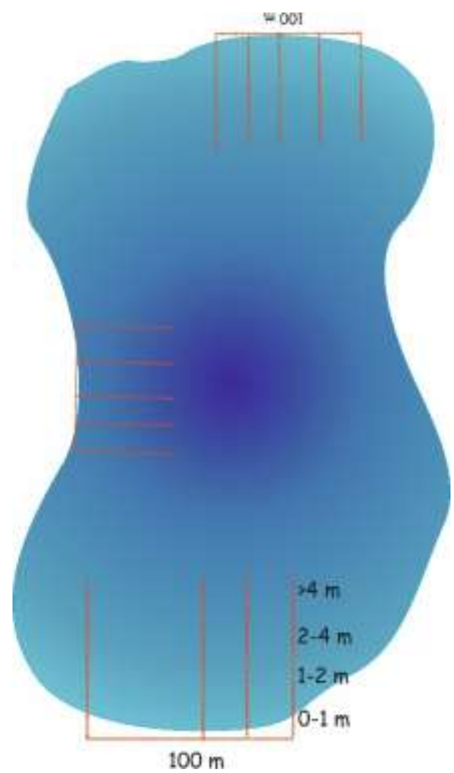

Fig. 1. Location of the transects.

Indicators used

Macrophyte taxonomic composition:

The taxonomic composition of aquatic macrophytes (Characeae, mosses and vascular plants) is assessed on a species level, except for Chara and Sphagnum, which are determined on genus level.

All submerged growing species as well as those with floating leaves and amphiphytic taxa are taken into account if they are submerged or floating.

Macrophyte abundance:

The abundance of each species is recorded according to KOHLER'S five class scale (1978, Table 1). The species composition uses 5 classes of abundance (Table 1).

For all four depth zones (0-1 m; 1-2 m; 2-4 m; > 4 m) the abundance of the species is recorded separately.

Table 1. Relative abundance scale after KOHLER (1978).

1	very rare
2	rare
3	common
4	frequent
5	abundant/predominant

How are these indicators monitored?

Sampling strategy

Selected transects are investigated (in Table 2 are given examples of different sized lakes and the required transect number).

Table 2. Recommended number of transects in correlation with the lake size.

Surface of water body	N of transects	Examples
<0.5 km^2	1-5	**Arkutino, Velyov vir**
0.5 – 2.0 km^2	4-8	Reservoirs Asenovets, Aheloy, Studena, Ognyanovo
2.0 – 5.0 km^2	5-10	Reservoirs Yastrebino, Beli Lom, Belmeken, Vacha
5.0 - 10 km^2	6-12	**Srebarna**
>10 km^2	8-15	Resrvoirs Iskar, Mandra, Ogosta, Kardzhali

Mapping is established with a rake. A belt transect of 20–30 m width orthogonal to the shoreline and positioned within an ecologically homogenous section of the littoral is surveyed in four depth zones (0–1 m, 1–2 m, 2–4 m and >4 m). The depth of the vegetation limit is noted as well as the species occurring in the greatest depth. The registered relative abundance of each indicator species is converted into metric quantities using the following function:

macrophyte abundance^3 = macrophyte quantity (see e.g. Melzer et al, 1986 or Schaumburg et al., 2011).

When is monitored and with which frequency?

Samples are taken once in the middle of growing season i.e. 15th June-30th September.

Use of equipment

Sampling can be done by boat, using a water viewer (aqua-scope) in combination with a double-sided rake connected to a rope. Sampling bags and cool bags are used to store species for later determination.

Analysis of sample and level of determination

Most plants are determined to species level in the field, and partly validated in the laboratory. Samples are taken for further determination under the stereo or light microscope if necessary.

Way of reporting basic data

All data are entered from the field protocol into its electronic version. Reference index is calculated per transects, as well as overall Reference index of the water body.

Assessment

Data requirements

Software: Microsoft Excel.

Methods of calculation

The registered relative abundance of each indicator species is converted into metric quantities using the following function: macrophyte abundance^3 = macrophyte quantity.

Taxa are assigned to indicator groups A (reference taxa) B (indifferent taxa) and C (disturbance indicators). Many species are treated different for growing in different depth zones, as the indicator value is improving as deeper is their occurrence. Thus, taxa found in differing depth zones are treated as different taxa (e.g. taxon A in 0–1 m, taxon A in 1–2 m, etc.).

The list of indicators, additional type-specific criteria and class boundaries were specified for national conditions as follow:

(i) the accuracy of the RI system in ecological assessment was evaluated (in relation to the rest of the BQEs, physico-chemical and hydromorphological parameters);

(ii) bioindicative capabilities of individual plant species was assessed based on literature data, expert knowledge, analysis of lake database;

(iii) the list of indicators was verified in relation to its application in Bulgaria;

(iv) a number of alternative species observed locally in Bulgaria was proposed (which need further verification).

The quantities of the different species are to be summed up separately for each group and for all submerged species of a sampling site. The Reference Index is calculated according to the following formula (Equation 1):

$$RI = \frac{\sum_{i=1}^{n_A} Q_{Ai} - \sum_{i=1}^{n_C} Q_{Ci}}{\sum_{i=1}^{n_g} Q_{gi}} * 100$$

RI = *Reference Index*

QAi = *Quantity of the i-th taxon of species group A*

QCi = *Quantity of the i-th taxon of species group C*

Qgi = *Quantity of the i-th taxon of all groups*

nA = *Total number of taxa in group A*

nC = *Total number of taxa in group C*

ng = *Total number of taxa in all groups*

Equation 1. Calculation of the Reference Index.

The RI is an expression of the "plant quantity" ratio of type-specific sensitive taxa, dominating at reference conditions, compared to the "plant quantity" of insensitive taxa (both "indifferent" and "degradation" taxa) and is therefore a tool for estimating the deviation of observed macrophyte communities from reference communities. The resulting index values range from +100 (only species group A taxa) to –100 (only species group C taxa).

Additional criteria

No additional criteria for EC1 lakes

Criteria for assessment

a) The total quantity of submerged macrophytes should be at least 21, otherwise the RI is considered to be unreliable and can only be used as additional information for the assessment of other BEC. If they can be excluded due to natural factors for the absence of macrophytes, that results in a "bad" status.

b) The number of indicator species must be at least three and above 50% compared to the total number of species established at the specific point.

In order to obtain EQR values, the index values must be transformed. A unified scale from "0" to "1" is suitable. The value "1" represents the best ecological status according to the WFD, i.e. status class 1. The value "0" stands for the highest degree of degradation of a water body, i.e. status class 5. The transformation for the module „Macrophytes" (Reference Index, RI) is carried out according to Equation 2.

$$M_{MP} = \frac{(RI_{Seen} + 100) * 0{,}5}{100}$$

M_{MP} = Module Macrophyte Assessment

$RI_{Seen/Lakes}$ = type specifically calculated Reference $Index_{Seen/Lakes}$

Equation 2. Transformation of the module $RI_{Seen}/_{Lakes}$ (Reference Index$_{Seen}/_{Lakes}$ Macrophytes) on a scale from 0 to 1.

Description of boundary setting procedure

The classification of the EQR values into the categories of ecological status is based on the definitions for ecological status, given by Annex V of the Water Framework Directive (Table 3).

Table 3. Classification of the EQR values into the categories of ecological status

Ecol.status	Normative definition (WFD)	Boundary setting procedure
High EQR = 0.83–1.00	"The taxonomic composition corresponds totally or nearly totally to undisturbed conditions. There are no detectable changes in the average macrophytic […] abundance. […]"	The HG boundary was assumed as the point at which sensitive species (Group A) were in quantity approximately more than 50% of the quantity of all groups. Because of lack of such sites in the dataset, one quarter (0.25) was added to the highest EQR value assessed in the dataset. At high status macrophyte communities of the national type L5 should feature a high diversity of growth forms, dominated parvopotamids (=*Potamogeton natans, P. praelongus*), *Hydrocharis morsus-ranae, Stratiotes aloides*, and, to a lesser extent, bryophytes (e.g. *Riccia fluitans*) and pteridophytes *(e.g. Wolffia arrhiza).*
Good EQR = = 0.58–<0.83	"There are slight changes in the composition and abundance of macrophytic […] taxa compared to the type-specific communities. […]"	The highest EQR value assessed in the database was applied as GM boundary. It was the point at which Group B (indifferent taxa) was dominant and/or Group A and C were with equal quantities. The good-moderate boundary position is characterised by the disappearance of bryophytes and pteridophytes. In contrast, water lilies and large emergents such as *Sparganium erectum* increase in abundance from high throughout the good status, but also decline when conditions are worsening.
Moderate EQR = 0.21–<0.58	"The composition of macrophytic […] taxa differ moderately from the type specific communities and-are significantly more distorted than those observed at good quality. Moderate changes in the average macrophytic […] abundance are evident. […]"	MP boundary was accepted as the lower EQR assessed. The MP boundary was set as the average where the community is dominated by disturbance indicators (Group C). It is characterised by disappearance of water lilies and dominance of elodeids.
Poor EQR = >0.00–<0.21	"Macrophyte communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions".	EQR values are very low. The PB boundary is a point at which macrophyte species are extinct due to anthropogenic pressures.

Bad EQR =0.00	"Large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are missing"	Macrophyte depopulation due to anthropogenic pressure.

Following the requirements of the WFD the assessment has to reflect the degree of deviation of the current vegetation from the reference condition. This presupposes knowledge of the reference conditions.

Due to the commented above absence of existing near-natural monitoring sites, as well as historical records, reference values for the macrophyte assessment were derived based on expert judgement and the list of macrophyte scoring taxa that was generated resembling undisturbed biological communities. Scoring taxa of Group A, i.e. reference indicators represented 23% of the total share of scoring taxa.

In the Bulgarian IC dataset mostly lakes in moderate status were collected (due to small number of lakes from the common EC1 type in Bulgaria). Only one lake was classified in good status. Macrophyte communities at lakes in moderate status were species-rich, with high diversity of growth forms and high total abundance, dominated by indifferent taxa.

During a common intercalibration-project between Bulgaria and Austria in the years 2014 and 2015 it became clear that Bulgarian classification system was too relaxed. The new classification system (Table 4) was elaborated on the basis of the scientific dataset, which comprises lakes surveyed during 2014-2015. H/G boundary was determined as the assessed highest EQR value from the dataset plus one quarter (0.25), while the highest EQR value itself was applied as G/M boundary; M/P boundary was accepted as the lower EQR assessed.

Table 5 presents dominant scoring taxa. Average concentrations of selected water chemical parameters at the assessed in good status site were: NH_4 = 0.09 mg N/l, NO_3 = 0.1 mg N/l, TP = 0.03 mg/l, PO_4 = 0.01 mg P/l, BOD_5 = 1.67.

Table 4. Final class boundaries for RI-BG.

Ecological status	EQR
High	1 - 0,83
Good	0.82 - 0,58
Moderate	0.57 – 0.21
Poor	0.20 – 0
Bad	Macrophyte depopulation

Table 5. Dominant macrophyte species of the national lake type L5. The table lists relative cubed abundance (%) of taxa recorded at the site classified in good status by national assessment method and Groups. * Depends on the depth zone.

Taxon	Group	%
Ceratophyllum demersum	C/B*	5.2
Nymphaea alba	B	17.6
Spirodela polyrhiza	C	0.7
Lemna minor	C	0.7
Hydrocharis morsus-ranae	A	17.6
Salvinia natans	B	5.2
Lemna trisulca	C	17.6
Utricularia vulgaris	B	17.6
Stratiotes aloides	A	17.6

The relationship between scoring taxa (represented as cubed abundance at studied sites) and RI-BG (represented as EQR) was examined with multiple regression (Table 6) and revealed significant correlation. Bivariate correlations between EQR and 3 indicator groups: A (reference taxa) B (indifferent taxa) and C (disturbance indicators) are shown at Fig. 2 -4.

Table 6. Coefficient of determination (R^2), probability (p) for the correlation and Beta Coefficient of EQR based on RI-BG with scoring taxa.

EQR (based on RI-Bulgarian)	R^2	p	Beta Coefficient
Scoring taxa of Group A, B and C	0.832	<0.001	
Scoring taxa of Group A			0.676
Scoring taxa of Group B			0.376
Scoring taxa of Group C			**-0.91**

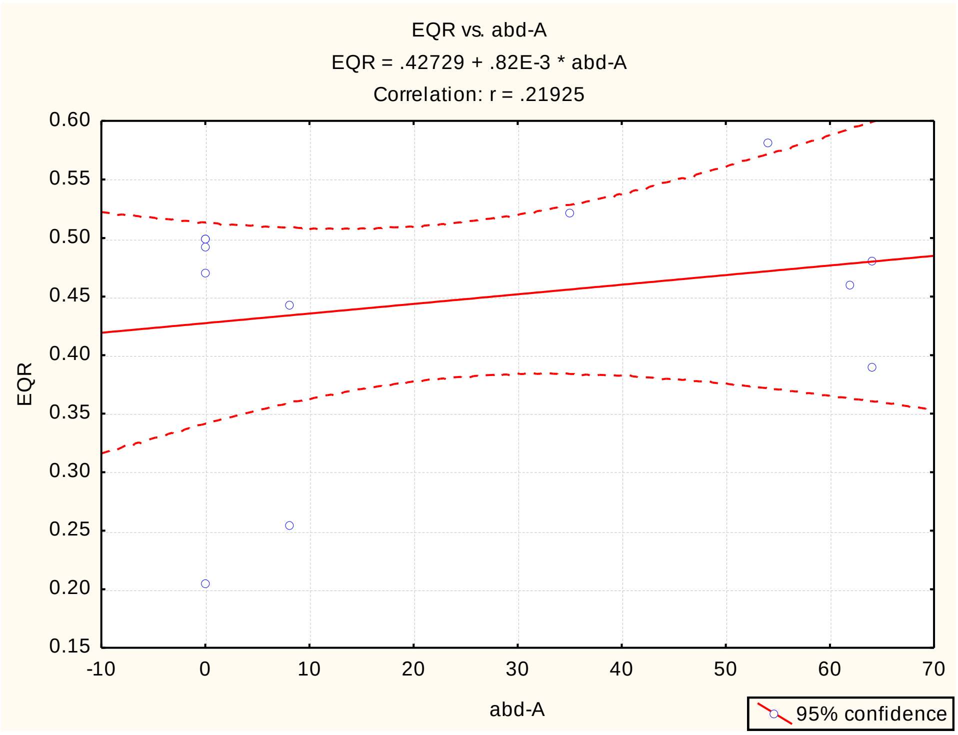

Fig. 2. Correlation between EQR based on RI-BG and abundance of scoring taxa from group A [reference indicators] in BG lake type L5.

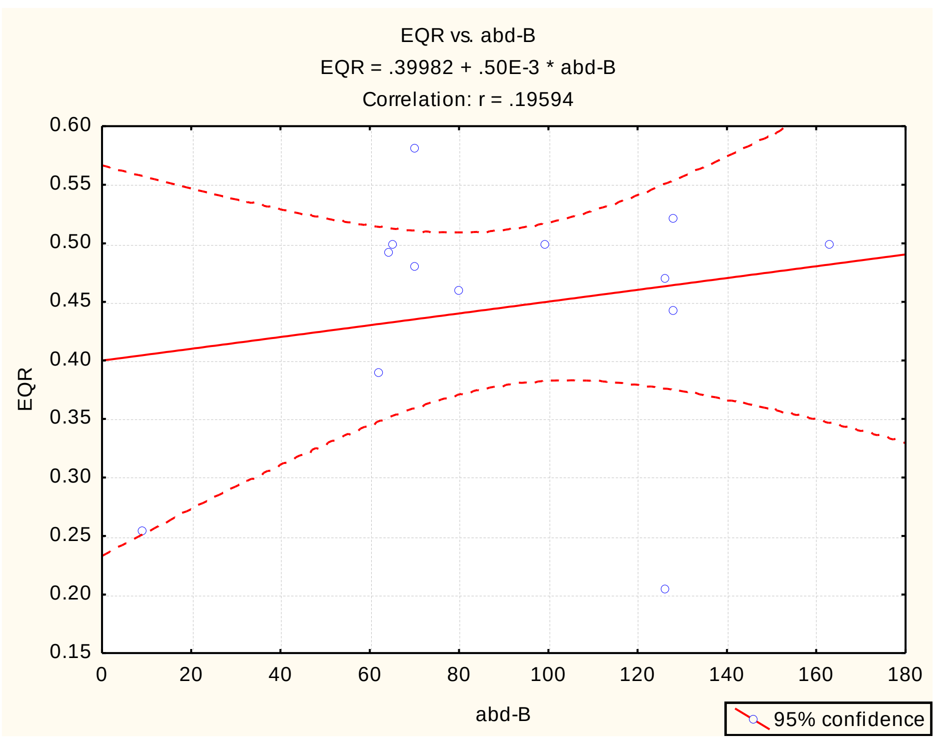

Fig. 3. Correlation between EQR based on RI-BG and abundance of scoring taxa from group B [indifferent taxa] in BG lake type L5.

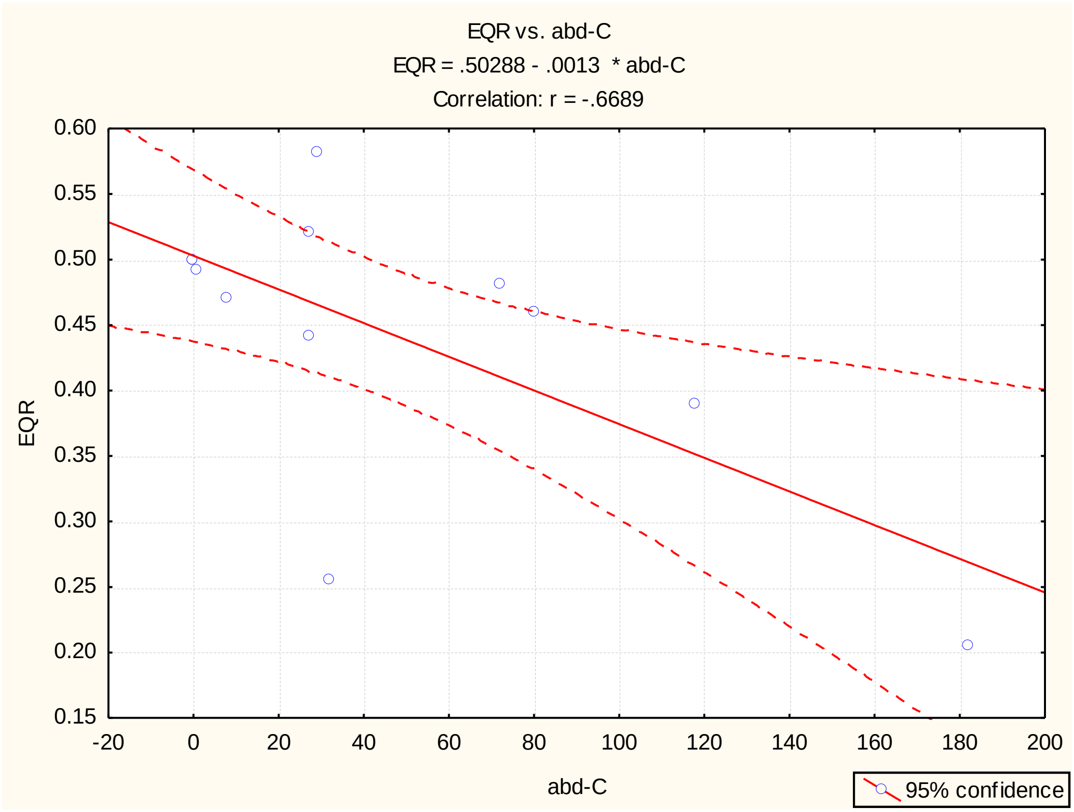

Fig. 4. Correlation between EQR based on RI-BG and abundance of scoring taxa from group C [degradation indicators] in BG lake type L5.

How well correlate the indicators with pressure indicators?

Table 8 below lists the pressures addressed by the national method and pressure-impact-relationships of national method and selected pressures. Bivariate correlations are illustrated at Fig. 5-8.

Table 8. Overview of the sensitivity to pressures and pressure-impact-relationship of national method and selected pressures (multiple regression).

Pressure		**Indicator tested**	**Sample size**	**r**	**r^2**	**p**
Eutrophication and general degradation						
	Chla, TP, BOD_5, NO_3-N	Macrophyte-based EQR	6	0.542	0.294	0.541

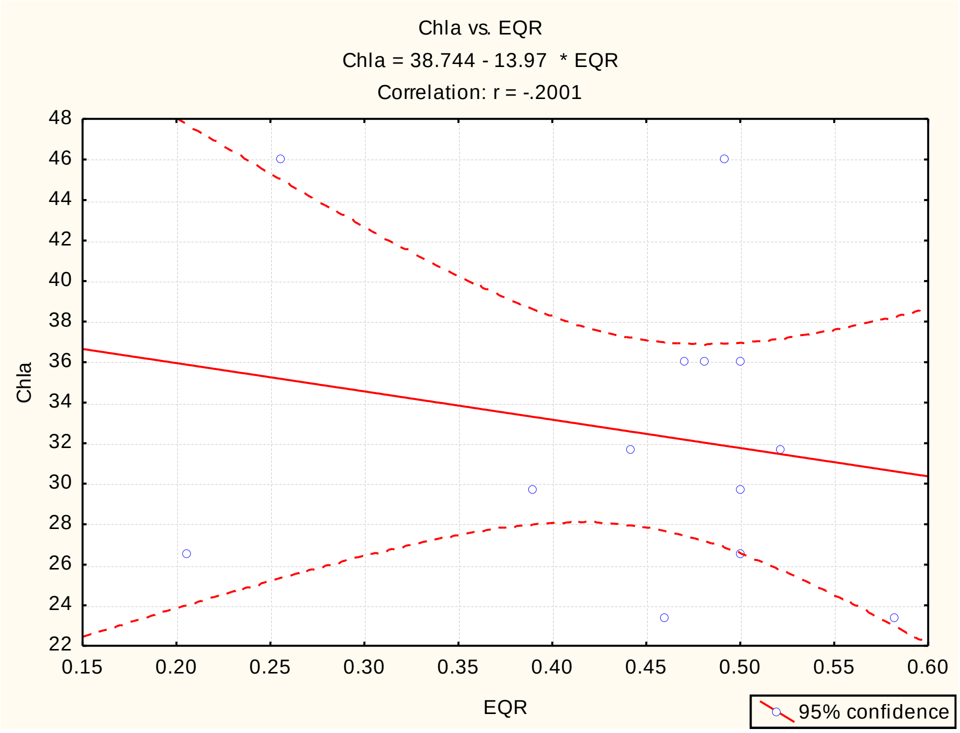

Fig 5. Correlation between EQR based on RI-BG and total chlorophyll a [mg L^{-1}] in BG lake type L5.

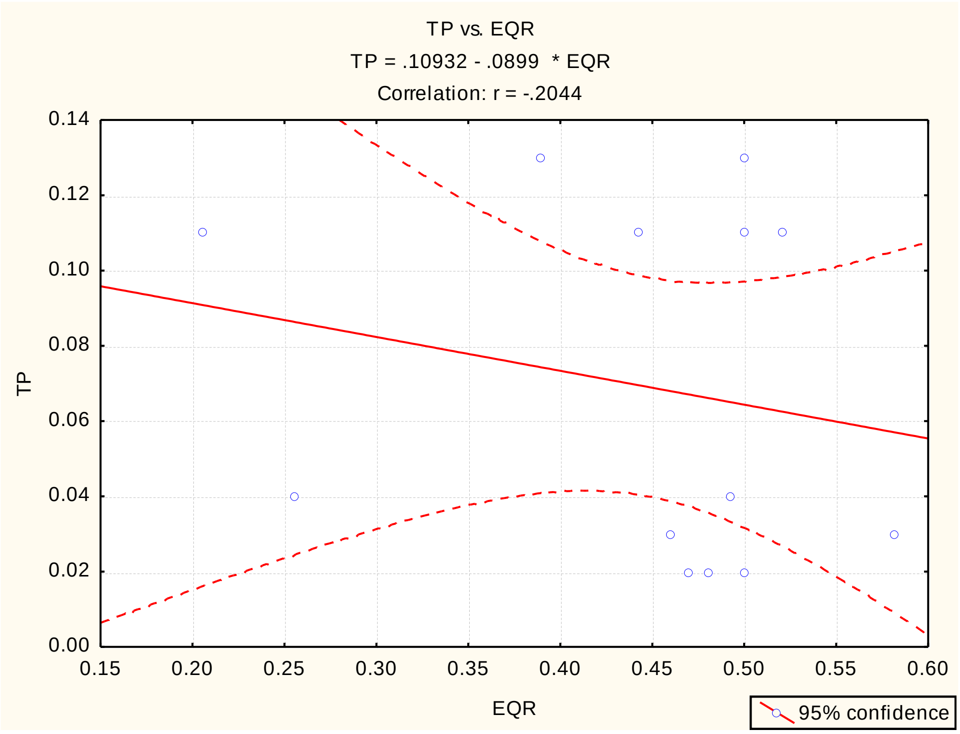

Fig 6. Correlation between EQR based on RI-BG and total phosphorus [mg L^{-1}] in BG lake type L5.

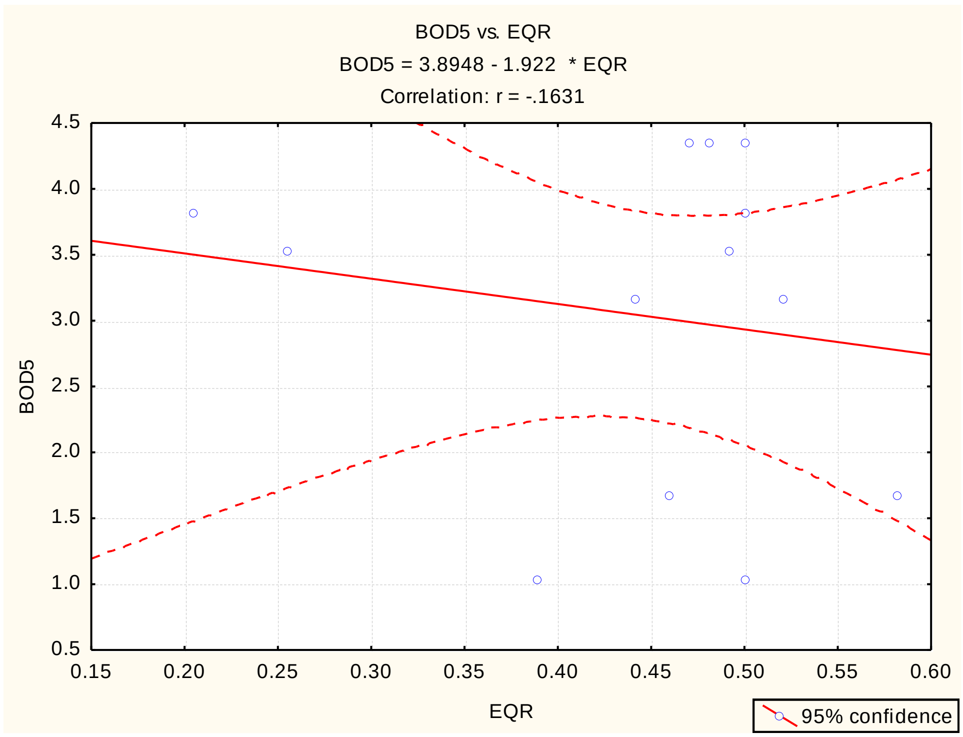

Fig 7. Correlation between EQR based on RI-BG and BOD_5 [mg L^{-1}] in BG lake type L5.

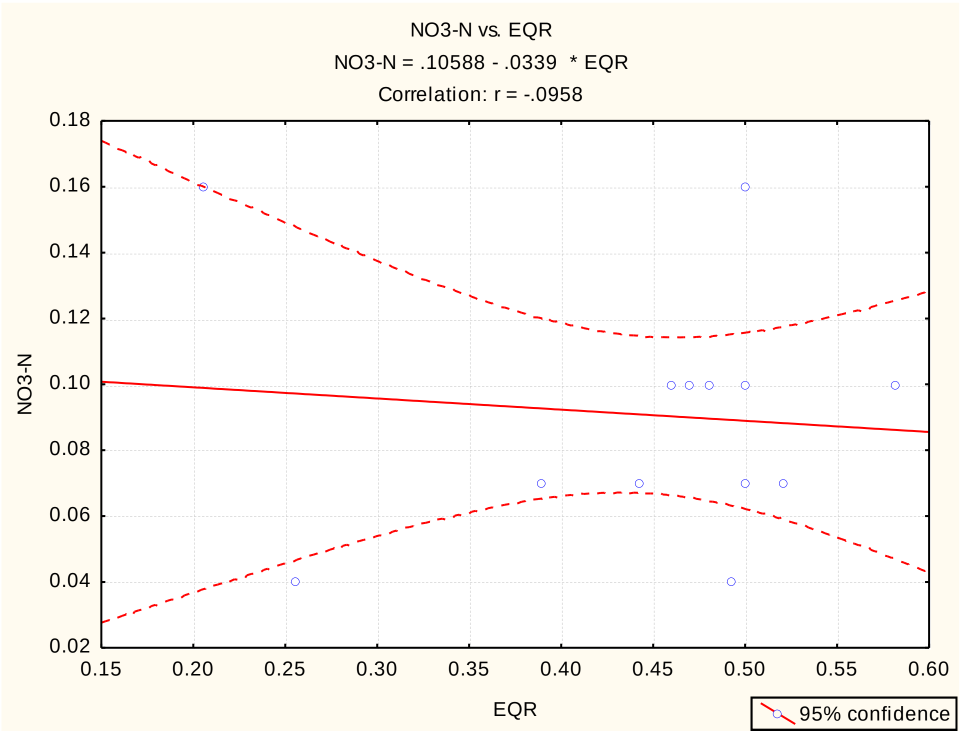

Fig 8. Correlation between EQR based on RI-BG and NO_3-N [mg L^{-1}] in BG lake type L5.

D Answers to the IC review

We have got the review of the EC GIG macrophyte intercalibration in April 2016. Considering the main concerns of the reviewer we identified the main tasks. These were the followings:

1. The application of the RI methods when species richness is very low
2. The classification of species into different response groups.
3. Relaxed classification of sites. Compare EC-GIG sites with its nearest counterparts (i.e. Polish lakes).
4. References, pressure-response relationship and stressor gradient.
5. Species composition in high status lakes
6. Natural eutrophic characteristic of EC-GIG lakes.

The application of the RI methods when species richness is very low - (Nigel Willby)

We proposed to apply a filter to sort out sites that have low or very low species number or low abundances due to we accept the problematic application of the method and the potential misclassification effect related to this issue. The following rule was applied (and taken over in the national assessment systems): The calculation of RI is only allowed in case at least 3 indicator species can be found in a quantity of at least 27 (corresponding to abundance class 3, meaning "common" occurrence).
However the size of our dataset decreased dramatically (n=52 vs n=117) the stressor response correlations got a bit stronger.

1. The classification of species into different response groups - (Nigel Willby)
To validate the classification of the species into different response groups we compared the TP optima (mean values) of our macrophytes species with the N-GIG and CB-GIG lake macrophyte dataset.
38 hydrophyte species were common among the GIG's in the dataset. The comparison revealed that there is a significant (Spearman, R= 0.454) correlation of species TP optima among the GIG's.

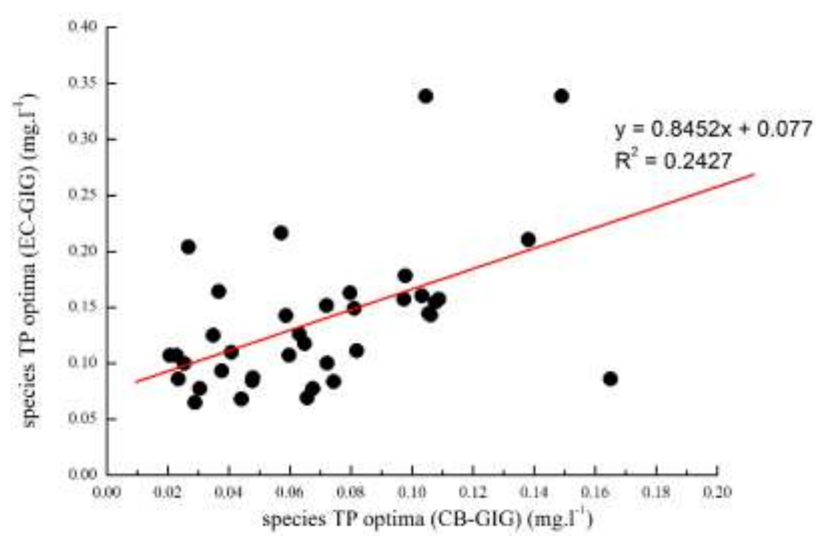

Figure 2.1. Correlation of hydrophyte species TP optima between CB-GIG and EC-GIG. Red line is linear regression.

According to this comparison we found that the difference between species TP optima increased in the higher TP range and near the same in the lower TP range (Fig. 2.1). Species can tolerate higher TP have a bit lower TP optima (0.12-0.2 $mg.l^{-1}$) in the CB-GIG.

2. Relaxed classification of sites. The comparison of EC-GIG lakes with its nearest counterparts the Polish lakes - (Nigel Willby)

We compared EC-GIG lakes and Polish lakes according to the ICM scores in a TP, TN and Chl-a gradients. Questions were:

- Does the classification of EC-GIG lakes are relaxed or not?
- Which part of the stressor gradient EC-GIG lakes lies in? (i.e. validating our stressor gradient)

ICM species scores for missing species were determined by the relationship between Ellenberg's N score and Polish ICM scores (ICM score = 1.0591* Ellenberg score + 0.5609). Species having no Ellenberg score and occasional species (appeared in less than 10% of the dataset) were excluded.

The correlation of ICM scores vs. stressors reveals that the EC-GIG lakes have high overlaps with Polish lakes in the scatterplots; EC-GIG lakes basically positioned in the moderate zone of the ICM gradient while most of the Polish lakes lies in the good and moderate zones (Fig. 3.1). If we assume that Polish lakes are reference lakes to EC-GIG sites we can assess that EC-GIG lakes not differ from its nearest counterparts in terms of species composition. It also suggests that Polish lakes are generally in a more pristine condition, which corroborates with our premises (i.e. reference lakes). Moreover fig 3.1 demonstrates that EC-GIG and Polish dataset are not relaxed; EC-GIG stressor gradient can be compared with its Polish (i.e. CB-GIG) counterpart, which can also means high and good status are assessed and classified correctly.

Comparison by pseudo-RI corroborates with the results above mentioned; we found high overlaps between GIGs lakes. It indicates that Polish and EC-GIG dataset have the same species composition and GIG's boundaries can be also comparable (we do not indicate boundaries in this scatterplot due to it was calculated from presence-absence data).

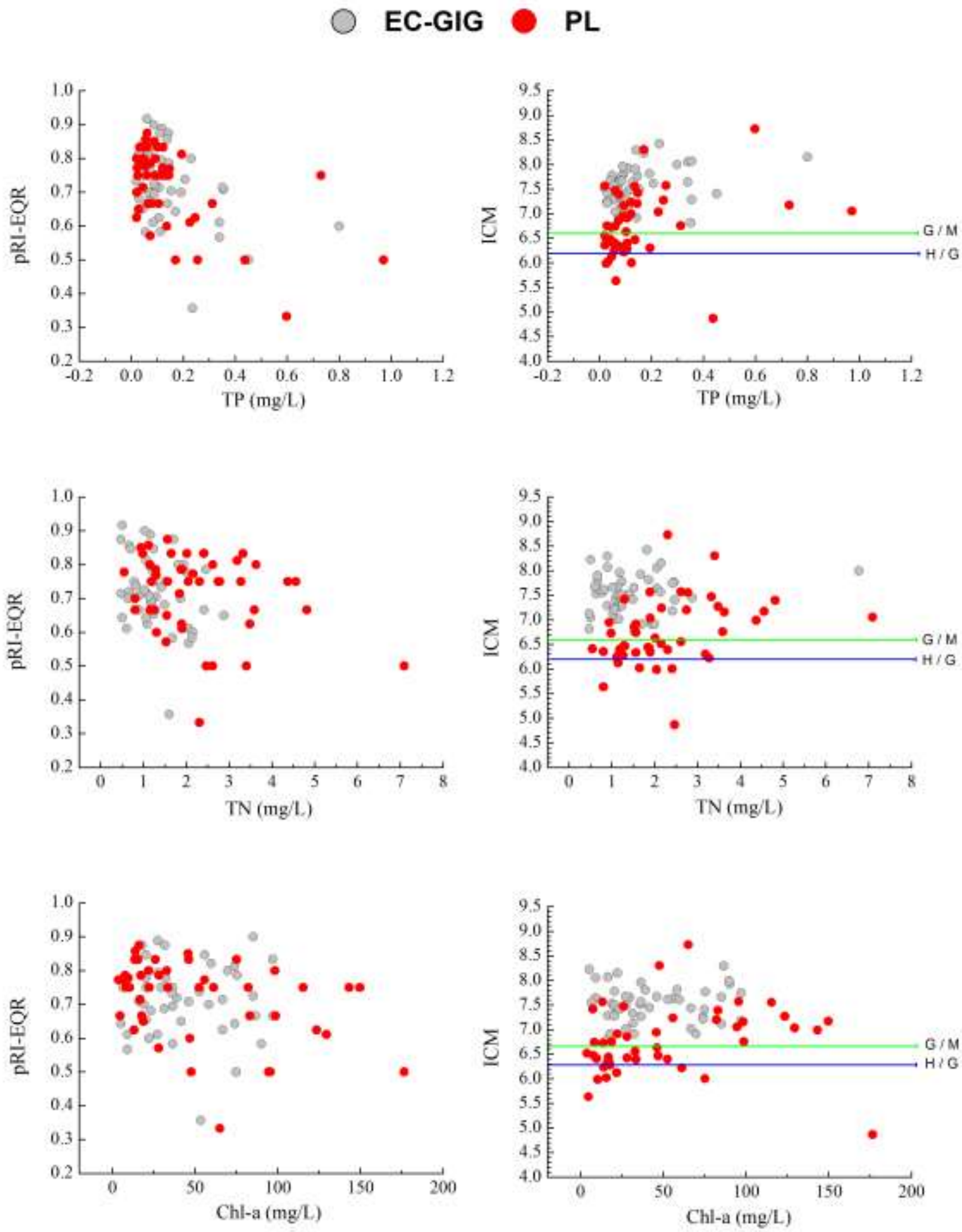

Figure 3.1. The correlation between ICM, pseudoRI and stressors (TN, TP, Chl-a) using EC-GIG (n=52) and Polish (n=48) dataset. Provisional H/G and G/M boundary highlighted according to the CB-GIG intercalibration. Pseudo-RI is

calculated from species presence-absence data, therefore it cannot be compared with real RI values and boundaries.

3. Pressure-response relationship, reference and stressor gradient

It was created a new "combined pressure metric", as the "sum of Spearman ranks" apparently leaded to some confusion. We now use as "combined pressure metric" the sum of normalised values of the parameters conductivity, Log BOD_5, Log TP, Log TN and degree of Corine intensive agriculture. The correlations with the national EQRs are as follows:

Table 4.1. Correlation of national EQRs with the combined pressures metric (whole GIG [HU+RO+BG] dataset).

Relationship with pressure		Pressure indicators / Strength of relationship (Pearsons R)		
		HU	**RO**	**BG**
Combined nutrient pressure	Sum of normalised values of the parameters conductivity, Log BOD5, Log TP, LOG TN and degree of Corine intensive agriculture	**-0.81***	**-0.83***	**-0.80***

*) **p <0.001**

Furthermore, in accordance with the statements of the reviewers, each Member State critically revised its opinion about reference conditions or the status of its benchmark lakes, respectively. The class boundaries of every Member State's method were adjusted accordingly. However, the GIG shares the opinion, that EC-GIG lakes are naturally eutrophic (see chapter 5).

With the new view on reference/benchmark conditions the pressure gradient is covered as follows:

Table 4.2. Overview of the EC-GIG dataset.

Parameter	Specification
Coverage per ecological quality class	High 7% Good 32% Moderate 49% Poor 11% Bad 1%

4. Species composition in high status lakes

In sites commonly assessed in high status the following species composition was detected (Tab. 4.3):

Table 5.1. Species composition in lakes commonly assessed to be in high status.

% Abundance	Species
11	*Typha latifolia*
10	*Lemna trisulca*
8	*Myriophyllum verticillatum* *Nymphaea alba* *Trapa natans* *Typha angustifolia*
6	*Salvinia natans* *Utricularia vulgaris*
3	*Hydrocharis morsus-ranae* *Myriophyllum spicatum* *Nymphoides peltata* *Oenanthe aquatica* *Potamogeton gramineus* *Potamogeton perfoliatus*
2	*Eleocharis palustris* *Potamogeton natans* *Stratiotes aloides*
>=1	*Alisma gramineum* *Azolla filiculoides* *Carex riparia* *Ceratophyllum demersum* *Lemna minor* *Najas minor* *Nuphar lutea* *Persicaria amphibia* *Potamogeton pectinatus* *Schoenoplectus lacustris* *Sparganium erectum*

5. Natural eutrophic characteristic of EC-GIG lakes (Laurence Carvalho)

The referee of a previous EC-GIG macrophyte intercalibration report deemed necessary to clarify the difference between the lakes considered to represent high status in this study and the true reference lakes, in addition to demonstrate that the lakes considered to be in high status are assessed and classified correctly. (That is, these lakes are really in high status and are not just the best available sites in good status). To clarify this concern we overviewed the paleolimnological results, collected historical data, and estimated the natural nutrient load to the lakes belong to EC-1 lake type.

6.1. Overviewing paleolimnological evidences for the region (Trophic reconstruction of EC lakes by paleolimnological evidences)

Several paleolimnological investigations were done in the Central-Eastern part of Europe in the recent decades focusing primarily on the climate reconstruction of the area and prehistoric human impacts on the landscape. However, besides these fashionable research areas, studies focusing on the trophic reconstructions of ancient lakes were not conducted. The other difficulty is that strata studied by paleolimnologists are much better preserved and remain undisturbed in deep lakes as compared to shallow water bodies, thus much more efforts were focused on deep lakes than on shallow ones. Despite these facts, several studies contain information on the biota in the lakes and the in-lake processes, from which we can infer to the trophic conditions of lakes in the prehistoric times. Although the aims of the authors of these studies were to tell the real story of the climate changes, or describe the human impacts on the landscape, they published algological and paleolimnological records, or results on macrofossils or chemical constituents which can also be used for trophic reconstructions. In reviewing the literature we focused on studies:

(i) in which the authors explicitly drew connections between their findings and the trophic state of the studied water bodies;
(ii) in which detailed description of the prehistoric biota is presented, including species of aquatic macrophytes, algae, or zooplanktic taxa.

Evidences for natural eutrophication were reported in four studies (Table 1). In two studies only references to the development of eutrophic state of the lakes could be found (Willis et al 1995; Sümegi et al. 2003; Lascu et al. 2014). In the other studies, based on macro- and micro-fossils transition from mesotrophic to eutrophic state was reconstructed.

Table 6.1. Paleo-ecological evidences for natural eutrophication process in the Carpathian-Basin (Romania and Hungary).

Lake studied	Development of eutrophic	Evidences	Literature
Shallow buried lakes at Nádasdladány (Hungary)	9500-4500	Occurrence of floating marsland dominated by *Thelypteris palustris.*	Sümegi et al. 2008
Kolon-tó (Hungary)	9500	Disappearence of *Chara* vegetation, occurrence of other aquatic macrophytes, development of eutrophic lake sediment.	Sümegi et al. 2008
Sarlóhát Tisza oxbow (Hungary)	10000	Appearance of *Pediastrum* spp. *Gloeotrichia Botryococcus* in the lake sediment. Developmentof aquatic macrovegetation dominated by *Nymphaea*, *Nymphoides*, *Potamogeton*, *Salvinia*, *Trapa*, *Nuphar.*	Magyari et al. 2012
Paleo-lake at Sajómagyarós (Romania)	13000	Increase of organic matter in the lake sediment	Lascu et al. 2014
Bátorliget láp (Hungary)	2000	Appearence of eutrophic lacustrin sediment (radiocarbon investiagations indicated macrophyte induced shift in the composition of carbon isotops).	Sümegi et al. 2003 Willis et al. 1995

It has been demonstrated that the late glacial paleolakes in the Carpathian Basin were oligotrophic Chara-lakes (Sümegi 2008). However, more or less simultaneously with the transition in the terrestrial macrovegetation, when coniferous forests were replaced by deciduous ones (9-13000 years BP), the *Chara* vegetation was replaced by aquatic phanerogams. Based on the macrofossils observed in the core samples (Table 1.), it can be argued that at that period the aquatic macrophyte species were identical with those that can be observed in protected bog-lakes and oxbows of low human impact in these days.

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6.2. Rough estimation of the natural nutrient load to the lakes

Ageing of lakes is a natural process. Newly formed lakes are generally oligotrophic, but during ageing they undergo a number of functional and structural changes, which are triggered by the nutrients entering the systems. The main natural sources of nutrients are the river inflows, erosion of nutrient rich soil, leaf litter fall, atmospheric load, ground water input and aquatic birds. The rate of accumulation of nutrients in the lake basin is strongly influenced by the perimeter and volume of the lake, the water retention time, topographic relief and climatic impacts. Shallow lowland lakes with long water retention time are subjects to rapid eutrophication, because the high surface to volume ratio enables the rapid increase of nutrients, and these nutrients cannot be removed from the system. It is especially true for the closed basins, which develop in those regions where evaporation exceeds precipitation. In the eastern part of Europe sandhill ponds and oxbows are the most frequently occurring forms of standing waters. Most of these do not have natural outflow or if they have, water as surface outflow can leave the lake basin only in exceptionally humid years. (We note that ground water movements which can be also important processes that shape the nutrient regime of lakes can be independent of the actual meteorological situation)

Although there are some sources of nutrients which are difficult to assess quantitatively (like soil erosion, the impacts of birds, ground water input), the impact of the most important sources can be at least be roughly estimated. Empirical measurements of leaf litter fall, and atmospheric load (wet and dry deposition) allow developing a simple calculation to give a rough estimation for the annual load.

Nutrient load = leaf litter fall + atmospheric load

Leaf litter fall is a typical perimeter related process and thus depends on the length of the shoreline. Field measurements were done to measure this kind of nutrient input in lakes; the load was given as dry mass of leaf litter per shoreline length (g m^{-1}).
For the atmospheric load reliable data are available in the literature (Anderson & Downing, 2006).

$TP_{Ann} = L_{Sh} \times TP_{LLF} + A \times TP_{Atm}$

TP_{Ann}: Annual TP load (mg a^{-1})
L_{Sh}: Length of shoreline (m)
TP_{LLF} : TP load resulting from leaf litter fall, given as mg m^{-1}
TP_{Atm} : Atmospheric TP load (mg m^{-2}) (dry and wet deposition)
A: Lake area (m^2)

The annual TP load entering the system can be illustrated on an example of a circular lake of 0.5 km^2 (L_{Sh} =2506m) with TP_{LLF} = 333mg m^{-1} (Gasith and Hasler, 1976); TP_{Atm} =1mg m^{-2} (Tabatabai et al., 1981); NLE=0.1 m, and, TP_{GW} = 0.01mg m^{-3} (the lowest value measured by the Hungarian groundwater monitoring system).
TP_{Ann} = 2506 ×333 +500000 × 1 + 500000 ×0.1 × 0.01 = 834498 + 500000 = 1334498mg.

TP_{Ann}/Lake volume gives the annual increase of TP in mg m^3 (i.e. in μg L^{-1}). Calculating with an average depth of 3m the volume of the lake is 1.5 million m^3 and the annual increase of TP is ~ 1μg L^{-1}. Although during the calculation of the TPAnn the lowest possible values were considered, (the lake basin was circular, thus the length of the shoreline was minimal) LE was the the), the 1μg L^{-1} annual increase of phosphorus means that the closed basins formed in deciduous forests become eutrophic quickly, perhaps within a century.

We would like to emphasise that this approach is a very rough simplification of the real processes and the calculations above are considered as a broad estimation of the nutrient load. Several important processes like co-precipitation of the nutrients, e.g. phosphorus precipitating with iron, or with calcite, other sediment related processes, incorporation of nutrients into macrophytes, or flying out of insects were neglected, although these processes can be crucial in changes of nutrient regime of lakes. Reliable calculations to estimate the nutrient input can be given if the circumstances specific to the subtypes of lakes and/or each lakes individually are considered.

Although the shallow lakes in the Carpathian Basin are naturally eutrophic it does not mean that these are not influenced by anthropogenic nutrient loads. Enhanced nutrient concentration results in the accelerated ageing of lakes, however, if it is not coupled with intensive stocking of fish the lakes become dominated by macrophytes, and appear to be similar to those described in paleolimnological studies.

References

Anderson, K. A., & Downing, J. A. 2006. Dry and wet atmospheric deposition of nitrogen, phosphorus and silicon in an agricultural region. – *Water, Air, and Soil Pollution* 176(1-4): 351–374.

KJ-NA-29336-EN-N

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doi:10.2760/242521
ISBN 978-92-79-92968-7