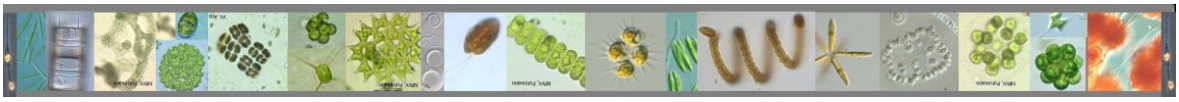

XGIG Large River Intercalibration Exercise

– Milestone 6 Report

Intercalibrating the national classifications of ecological status for very large rivers in Europe

Biological Quality Element: Phytoplankton

2. Version – November 2016

Ute Mischke

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Version information on this report

This document is the full final version checked by all participating countries.
In this version, Romania and Poland approved the necessary boundary adjustments.

The GAP justifications of the Nordic countries (FI, NO, SE) and of Italy not to use phytoplankton in large rivers are attached in Annex X and XI.

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Introduction

The report at hand was compiled by the XGIG large river group as part of the exercise to intercalibrate the national classifications of good ecological status for very large rivers (> 10,000 km^2 catchment size) using phytoplankton. The process specified in the intercalibration guidance (EC 20111) covers various steps to be completed by the Member States, documenting specific features of their biological assessment methods prior to the actual intercalibration analysis. Here, we provide an overview of the national methods participating in the exercise, demonstrate their pressure-impact relationships and check their compliance with the WFD-criteria. This document does not deal with the issues of intercalibration feasibility and the comparison of national class boundaries.

The aim of the large river exercise is to intercalibrate the national methods that classify the ecological status of large rivers. It already became obvious that most methods assess the main channel habitats, i.e. the integration of floodplain habitats into an integrative status assessment of large rivers is currently not practised. The intercalibration group thus focus on methods to assess the main channel habitats of large rivers.

It has to be noted, that the exercise gained a common view which pressures has to be reflected. Beside the eutrophication due to nutrient loads, some Member States also included parameters for reflecting saprobic conditions. This is in line with the EU-WFD. The normative definition of good status the Annex IV of EU-WFD (2000) includes boundaries for secondary effects: “not indicate any accelerated growth of algae resulting in undesirable disturbances to the balance of organisms present in the water body or to the physico-chemical quality of the water or sediment.”

A common view for reference conditions of these pressures for phytoplankton might be best addressed in a targeted research effort at European scale.

[1] European Commission (2011) Guidance document on the intercalibration process 2008–2011. Guidance Document No. 14. Implementation strategy for the Water Framework Directive (2000/60/EC). Technical report-2011-045.

Part A

A.1. Overview of national assessment methods for large rivers using phytoplankton

This report addresses the details of 13 national assessment methods for large rivers using phytoplankton (see table below) and are participating in the intercalibration exercise. Specific details of the assessment methods can be found in the completed questionnaires (see annex I) and in the detailed method descriptions attached to this document (see annex II).

Cyprus, Denmark, Ireland, Luxembourg, Malta and United Kingdom do not hold (a significant part of) large rivers exceeding 10,000 km^2 catchment area.

France, Greece, Netherland, Slovenia and Spain lack national assessment methods.

Finland, Italy, Norway and Sweden provide justification for excluding phytoplankton in the assessment of very large rivers (Gap 4; see Annex X and XI).

Belgium (Wallonia) identified their large rivers as heavily modified and thus revoked their participation in the exercise (information provided for IC of macroinvertebrates in LR-XGIG). The phytoplankton expert group made no final decision on exclusion criteria from IC.

Table A.1: List of all national assessment methods nominated in the context of the large river intercalibration exercise of phytoplankton.

Member State	Method name	Status
Austria	German PhytoFluss-Index 4.0	finalised
Belgium (Flanders)	German PhytoFluss-Index 2.0	finalised
Bulgaria	German PhytoFluss-Index 4.0	finalised
Croatia	HRPI - Hungarian River Phytoplankton Index	finalised
Czech Republic	CZ - Assessment method for ecological status of rivers based on phytoplankton	finalised
Estonia	EST_PHYPLA_R - Assessment system for rivers using phytoplankton	finalised
Germany	German PhytoFluss-Index 2.2 (finalized) or in decision process: PhytoFluss-Index 4.0 (not officially finalized)	finalised
Hungary	HRPI - Hungarian River Phytoplankton Index	finalised
Latvia	Modified HRPI - Hungarian River Phytoplankton Index	finalised
Lithuania	German PhytoFluss-Index 2.2 for lowland rivers of type 15.2	finalised
Poland	IFPL metric - Method for large rivers assessment using phytoplankton	finalised
Romania	ECO-FITO - Assessment Method for Ecological Status of the Water Bodies based on Phytoplankton	finalised
Slovakia	Phytoplankton-SK - Slovak assessment of phytoplankton in large rivers	finalised

A.2. Pressure-impact relationships of national methods

Introduction

The theoretical base for expecting a relationship between the main pressure "nutrients" (e.g. TP, TN) to BQE phytoplankton is taken over from the relationship established for lakes. WFD methods to assess lakes bases on phytoplankton are successfully established and intercalibrated in the first and second round of the intercalibration (IC) exercise.

Still, "(…) in streams, more excessive than in lakes, a complex interplay among factors can occur that influences the trophic state. Phytoplankton sensitivity to nutrient loads differs with catchment size, and the autotrophic state can be decoupled from nutrients by water residence time. (…)" and by light limitation (Mischke et al. 2011).

Borics et al. (2007) stated that "for the development of any algal population at a given place three basic criteria need to be present simultaneously:

1. Inocula of the species,
2. Appropriate environmental variables (temperature, light, nutrients),
3. Sufficient time.

If any of these is missing, the population has no chance to develop."

In very large rivers an inocula of species and sufficient time is provided in most of them, but light might be a limit almost as frequent as nutrients.

"(…) River-type-specific analysis shows a strong correlation between phytoplankton biomass to a certain nutrient supply for select fast flowing lowland rivers (MOSS et al., 1989; BASU and PICK, 1996; VAN NIEUWENHUYSE and JONES, 1996; LOHMANN and JONES, 1999; BEHRENDT and OPITZ, 2001; CHETELAT et al., 2006). (…)"

In order to take into account multi-factor limitations, different strategies to improve the demonstrating of the relationship were used by the MS in LR-XGIG:

a) Some countries split the very large rivers in groups with different nutrient sensitivity (DE; high or low area specific run-off; <>10L s^{-1} km^{-2}; see table A.2, and Annex III).

b) They exclude all samples from pressure-impact analysis, in which the phytoplankton biomass remain below a certain threshold (e.g. LV; chl_a < 18µg/L), assuming that other factors than nutrient limit phytoplankton (e.g. light limitation by occasionally high amount of suspended solids.

c) Declare an exclusion criterion e.g. for regions with wash-out effects due to regular occurring frequent high flow events (N >5) during vegetation period (e.g. example Sava river).

Besides multi-factor limitation of phytoplankton growth, the second obstacle to establish pressure-impact relationships is the narrow window of the nutrient gradient, which was highlighted by all countries.

Thirdly, some MS methods do include the pressure "organic pollution" (parameter oxygen demand (BOD)) because they found a common relationship between phytoplankton in saprobic index by Puntle-Buck Index (e.g. RO) and by the increase of Euglenophyta (SK) to BOD.

Table A.2: Percentiles and maxima of chlorophyll a concentrations (annual means) found in very large rivers (XGIG data) split into those with low area specific run-off and those with high run-off.

	Chla in **low** run-off [µg/L]	Chla in **high** run-off [µg/L]
25% perc	6,0	5,0
75% perc	33,0	14,9
max Chla	185,5	67,8
Chla median	**13,5**	**9,1**
N (annual mean)	***556***	***191***

Austria

Austria applied the German PhytoFluss-Index for large streams with high specific run-off (DE Type 20.1) on sections of Austrian large mountain and lowland rivers (Danube, March, Thaya). Austria provides a demonstration of pressure impact relationship together with data of German and Bulgarian sites.

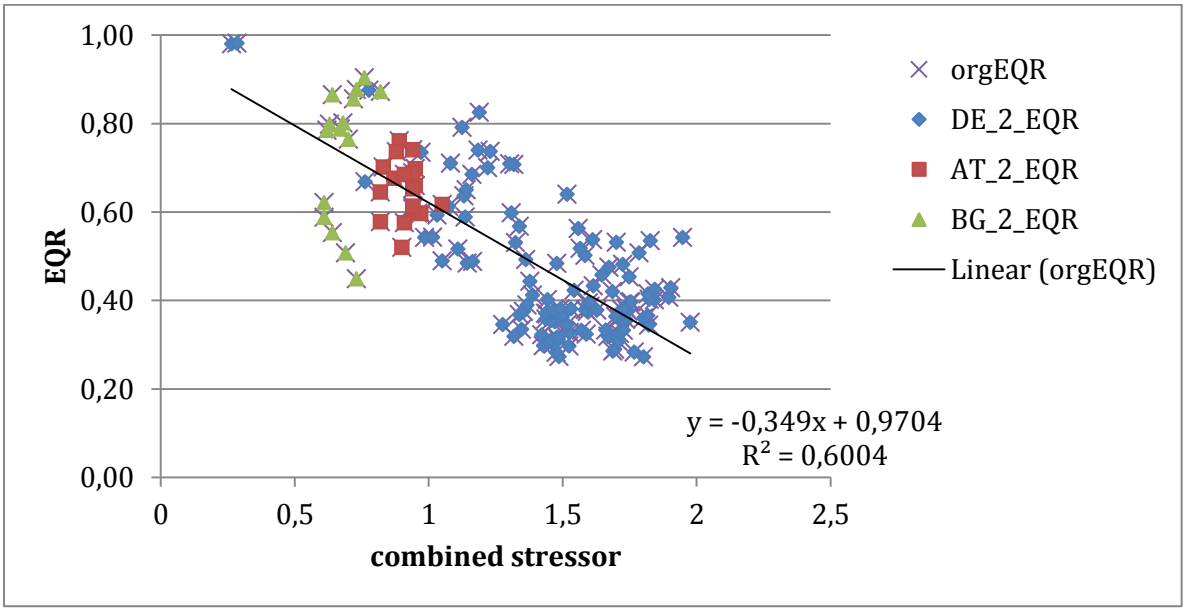


Figure AT_1: EQR (PhytoFluss 4.0) to combined stressor (TP, TN and Cl-1 used in IC exercise) for phytoplankton data from Austrian (red symbols), German (blue symbols) & Bulgarian (green symbols) very large rivers

Belgium (Flanders)

Belgium (BE_FL) applied the German PhytoFluss-Index for large streams with low specific run-off (DE Type 20.2) on **one Belgium large lowland river**. Belgium (FL) provides no demonstration of pressure impact relationship (not applicable with one site), but this was done in the XGIG analysis with pooled data for rivers with low specific run-off (see Fig. BE_FL_1). Sensitivity of phytoplankton biomass to stressor TP is detectable along the 75th percentiles for rivers with low run-off.

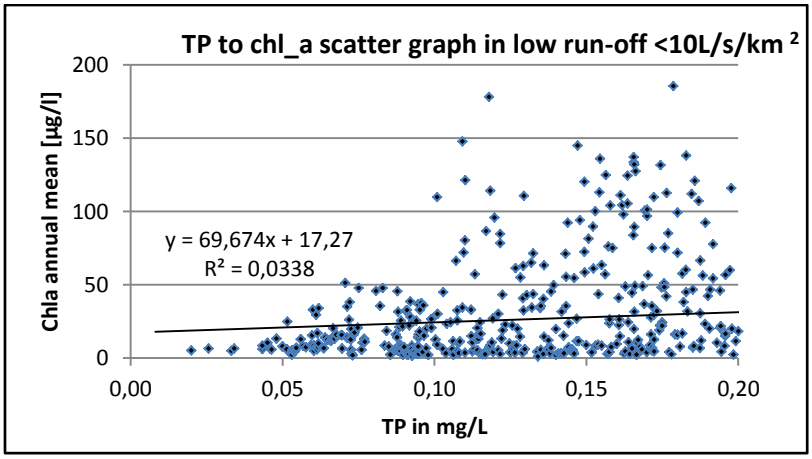


Figure BE_FL_1: Distribution of PP biomass (as chlorophyll a) to total phosphorus (TP) as scatter graph in very large rivers redrawn from XGIG data base (N = 556); all values in annual means.

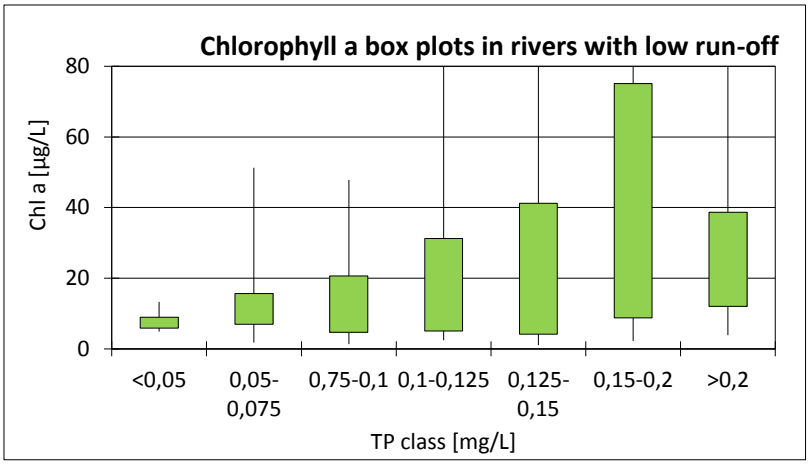


Figure BE_FL_2: Distribution of PP biomass (as chlorophyll a) to total phosphorus (TP in classes) as box plots in very large rivers redrawn from XGIG data base (N = 556); all values in annual means.

Bulgaria

Bulgaria applied the German PhytoFluss-Index for large streams with high specific run-off (DE Type 20.1 and Danube indicator list TIP_2013 see Annex III) on sections of **6 stations of the Bulgarian Danube river**. Bulgaria provides no demonstration of pressure impact relationship (narrow range of pressure). Bulgaria decided to use version 4.0 of PhytoFluss of German method.

As a first test the Bulgarian phytoplankton data in 2014 where assessed by the prototype PhytoFluss 3.0 (high to moderate status) and were plotted against the pressure total phosphorus (figure BG_1) with pooled German data.

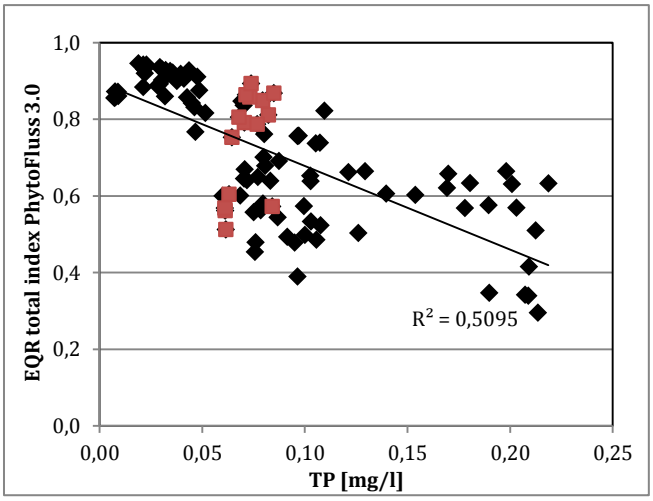


Figure BG_1: EQR (PhytoFluss 3.0) to total phosphorus (TP) in German (black symbols) & Bulgarian (red symbols) very large rivers with high run-off (>10L s^{-1} km^{-2})

The same Bulgarian phytoplankton data where assessed by PhytoFluss 4.0 (high to moderate status) and were plotted against the IC combined pressure scale of TP, TN and chloride (description in Part B) with German and Austrian data (see figure AT_1). The main difference between PhytoFluss 3.0 and 4.0 is that the metric Pennales is no longer used, and the single metrics are averaged to total index by a weighting factor for this river type ((chla * 1 + TIP_2013 *3)/4). Both modifications improve the pressure-impact relationship.

Croatia

Croatia applied the HRPI - Hungarian River Phytoplankton Index on sections of **Croatian large lowland rivers**. Data used for this analysis were from 2010 in PhD thesis Stanković, I. 2013. PHYTOPLANKTON AS INDICATOR OF ECOLOGICAL STATUS OF LARGE LOWLAND RIVERS IN CROATIA. University of Zagreb, Croatia.

There were 6 samples per sampling site (April-September) on 9 sampling sites (1 in Mura River, 4 in Drava River, 2 in Danube River and 2 in Sava River). Kendal tau and Spearman Correlation Coefficients were calculated for relationship of HRPI and BOD, COD, NO3, TN, DOP, TP and Qs (average monthly discharge) and they are presented in the following table:

Table A.2_HR: Statistic characters for relationship of pressure parameters to HRPI

	Kendall's tau				Spearman's rho			
	Mura River	Drava River	Danube River	Sava River	Mura River	Drava River	Danube River	Sava River
	τ	τ	T	τ	P	ρ	ρ	ρ
BOD	0,138	-0,121	**-,636****	-0,254	0,232	-0,152	**-0,805****	-0,348
COD	0,333	-0,146	-0,419	**-0,708****	0,486	-0,176	**-0,608***	**-0,821****
NO3	0,600	**0,550****	0,394	-0,242	0,771	**0,682****	0,441	-0,350
TN	0,600	**0,454****	0,121	**-0,455***	0,771	**0,580***	0,140	**-0,622***
DOP	0,067	-0,132	**0,606****	**-0,727****	-0,029	-0,225	**0,678***	**-0,874****
TP	-0,200	-0,296	0,321	**-0,636****	-0,257	-0,433	0,431	**-0,797****
Qs	-0,067	-0,098	0,229	0,394	-0,029	-0,152	0,291	**0,587***

Since demonstrated statistical analysis doesn't clearly indicate pressure impact relationship, further analysis was done with use of multiple pressures.

After normalization of pressure data (BOD, COD, NH3, NO2, NO3, TN, DOP, TP and TOC), PCA analysis was performed and Axis 1 and Axis 2 data were brought to linear regression with EQR of river phytoplankton. Three analyses (options) were done:

- All sampling sites no matter chlorophyll a concentration or hydrological impact,
- Sampling sites where three year average chlorophyll a concentration is >10 µg/L (1 sampling site on Drava River and 2 sampling sites on Danube River),
- Sampling sites where three year average chlorophyll a concentration is <10 µg/L (1 sampling site on Mura River, 3 sampling sites on Drava River and 2 sampling sites on Sava River).

Linear regression of multiple pressures PCA Axis 1 and Axis 2 vs. EQR for all three options is shown on Figures HR1.-3. Although there is no clear connection between EQR and TP in Croatian rivers, it is clear that pressure impact relationship between multiple pressures and EQR exists. The strongest relationship is in rivers with three year average chlorophyll a > 10 µg/L (Fig. HR2).

When rivers with chlorophyll a < 10 µg/L are separated (Fig. HR3), it is clear that those samples are weakening pressure impact relationship between multiple pressures and EQR in all Croatian rivers linear regression (Fig. HR1).

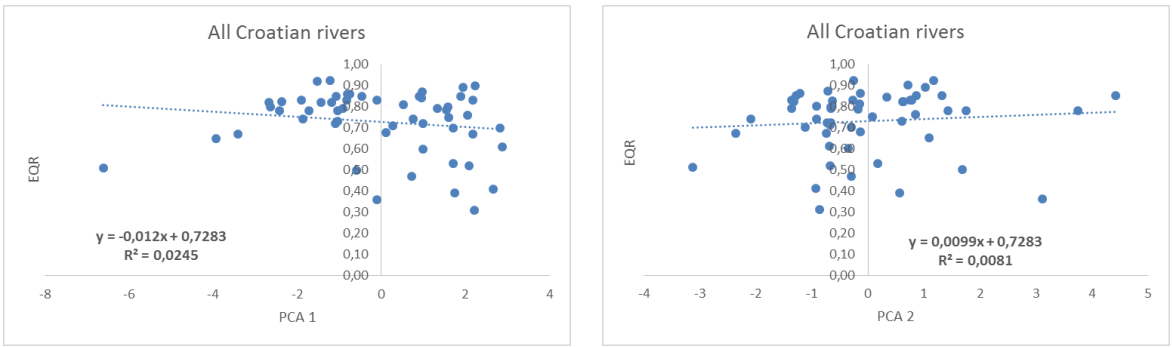


Figure HR_1. Linear regression of multiple pressures PCA Axis 1 and 2 vs. river phytoplankton EQR in all Croatian rivers.

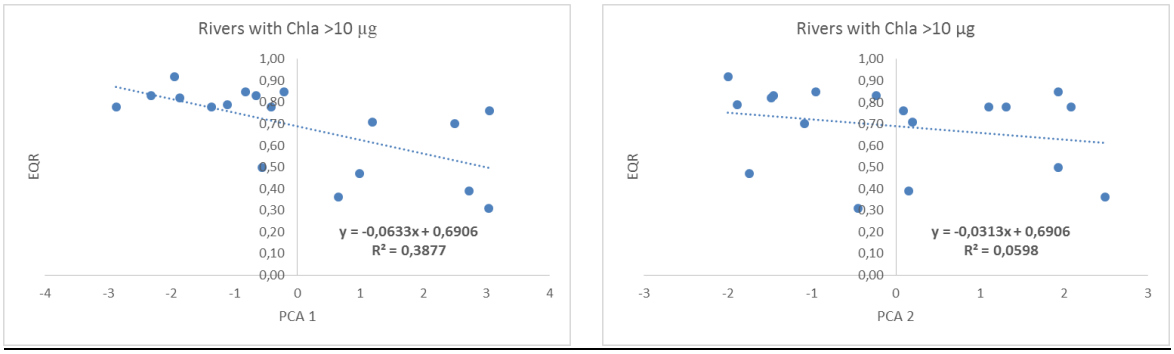


Figure HR_2. Linear regression of multiple pressures PCA Axis 1 and 2 vs. river phytoplankton EQR in Croatian rivers with three year average chlorophyll a > 10 µg/L.

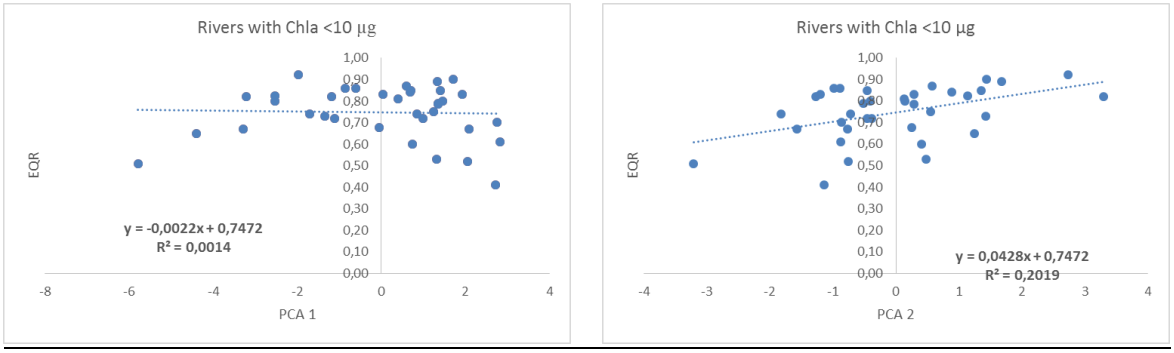


Figure HR_3. Linear regression of multiple pressures PCA Axis 1 and 2 vs. river phytoplankton EQR in Croatian rivers with three year average chlorophyll a < 10 µg/L.

Czech Republic

The Czech Republic applied the CZ - Assessment method for ecological status of rivers based on phytoplankton on very large rivers (8 stations, Labe/Elbe, Dyje, Vltava) and including also rivers larger than 5000km^2 (Berounka , Morava).

Testing the relationship between values of biological metrics and nutrients in sampling sites was carried out on a dataset that included 131 samples; however, only 24 samples were taken at 9th Strahler stream order. Data was tested in three ways.

Statistical factor analysis for the search and testing of relationships between datasets (biological metrics and nutrient values) was used already at the stage of selecting metrics appropriate for inclusion in the multimetric index.

Furthermore, differences between values of selected metrics on the best available sites and impacted sites were tested (Figure CZ1). In the case of phytoplankton a site cannot be selected that could be declared to be a reference in terms of quality and quantity of phytoplankton in the Czech Republic, primarily because it is assessed in lower reaches of large impacted rivers. Therefore, the selected sites represent the best available ecological status. They were selected based on the expected lower nutrient content and expert judgement.

Finally relationship between values of multimetric index and selected nutrients (total phosphorus, P-PO4, N-NO3, N-NH4, N-NO2) was examined. Significant relationship was statistically significant mainly for total phosphorus. Here, the Spearman correlation coefficient using the whole dataset (rivers of 7th – 9th Strahler order) was around 0.6 (Figure CZ2), but for large rivers (9th Strahler order) the correlation coefficient was significantly lower especially due to the small number of data (24 samples) and short gradient.

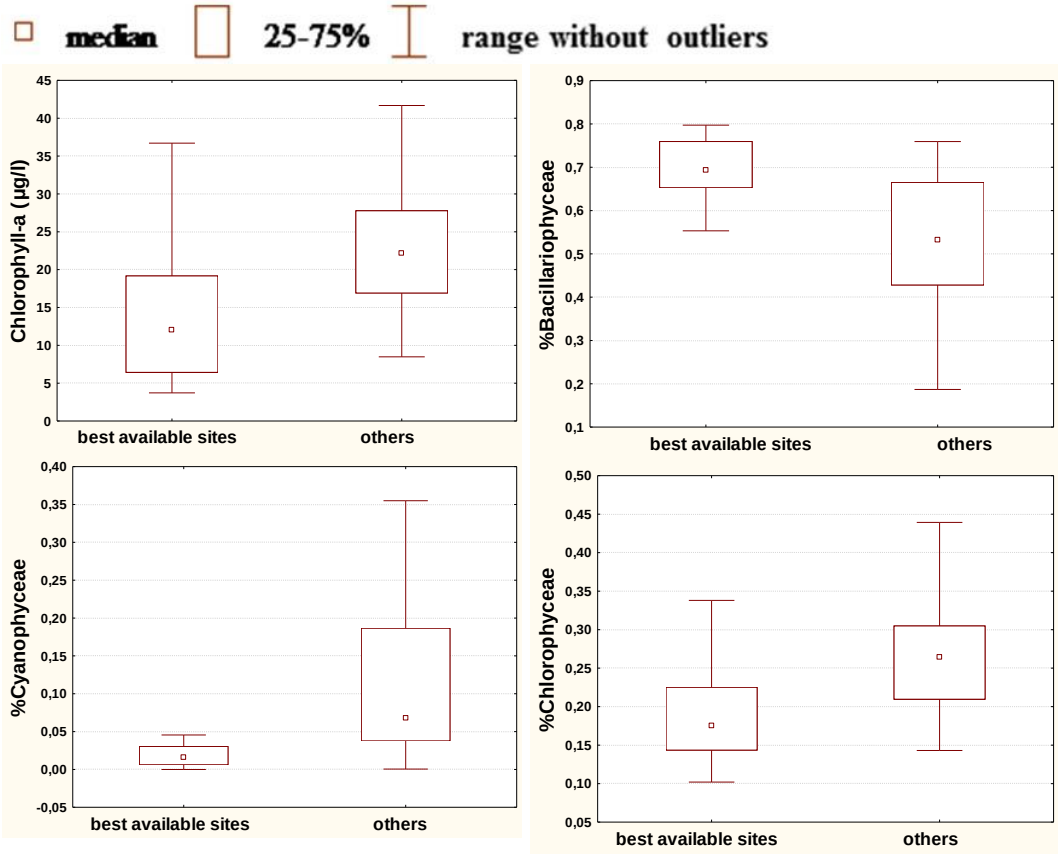


Figure CZ_1: Values of metrics selected for the CZ multi-metric index on best available and impacted sites (Chlorophyll-a - F(1;64) = 6,3381; p = 0,0143; %Bacillariophyceae - F(1;64) = 22,8944; p = 0,00001; %Cyanophyceae - F(1;64) = 13,9718; p = 0,0004; %Chlorophyceae - F(1;64) = 19,5178; p = 0,00004)

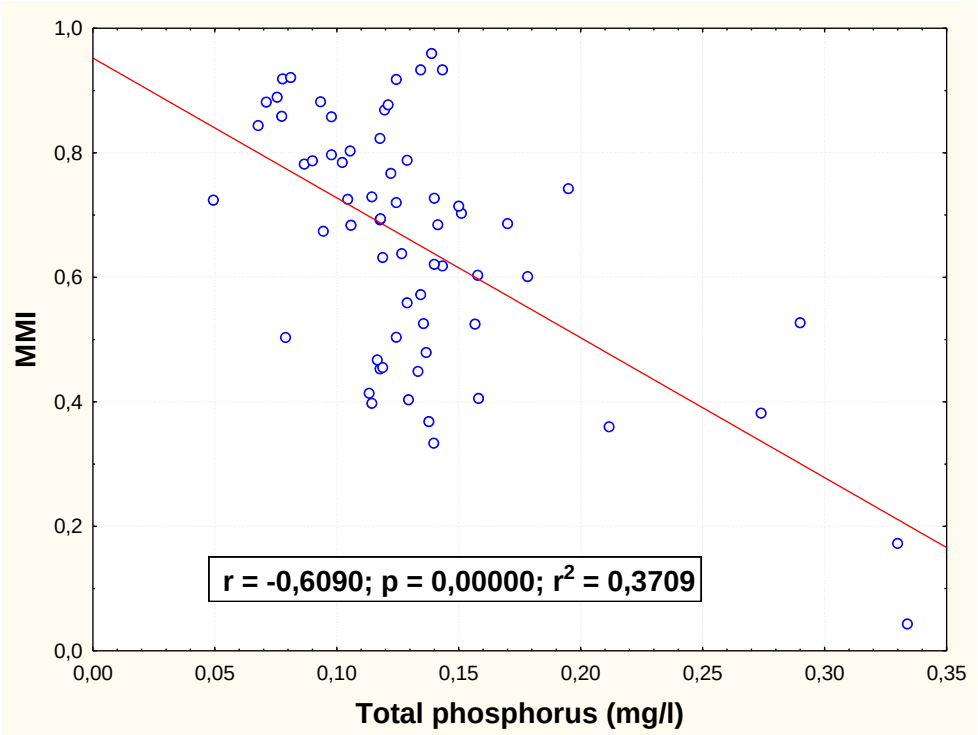


Figure CZ_2: Relationship final CZ multimetric index values and total phosphorus

Estonia

Estonia applied the HRPI - Hungarian River Phytoplankton Index on sections of **Estonian large lowland rivers**. The detailed method description is available **Annex II – C** of this report.

The share of X2 group (representative species *Rhodomonas lacustris*) was negatively correlated with TP r=-0,58, P<0,05, in 15 samples.

The whole index result is not significantly correlated to TP (r^2=0.28) or TN (r^2=0.174) in the few samples for Narew river (N=20). It has to be noted that the concentrations of the pressure parameters TP, TN and Chloride are low in comparison to other European Large Rivers; so the range of pressure is small, which additionally hampers the establishment of metrics.

Germany

Germany has recently pooled all assessment results (EQR of total index) for station data of very large German rivers (N = 299) and tested against total phosphorus (TP; period 1993-2014). The EQR results are shown in the following figures on the updated German data set for very large rivers and calculated with official version PhytoFluss 2.2 and with the updated method PhytoFluss 4.0.

The phytoplankton data (seasonal means of at least 5 samplings) were assessed with status boundaries accordingly the German river type to which the station belong. Very large river sites occur in four German river types with different altitudes and area specific run-off types (see Mischke et al. 2011):

10.1+20.1 -streams with area specific high run-off

10.2+20.2 -streams with area specific low run-off

9.2 -large highland rivers; mainly high run-off

15.2+17.2 -large lowland rivers; low run-off

Using PhytoFluss 2.2, the total index is weakly negative correlated against TP (r^2 = 0.246) in the official method version, and shows better correlation with the updated method 4.0 (figures DE_1 and DE_2).

Germany conclude from analysis, that the pressure-impact relationship is sufficient demonstrated.

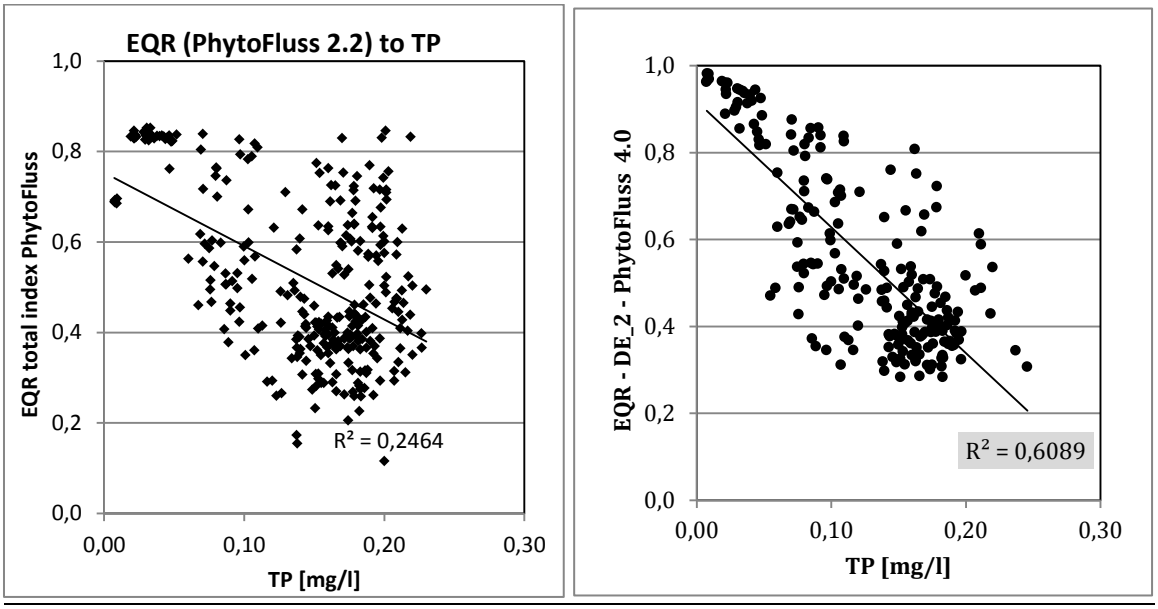


Figure DE_1: Pressure-impact relationships between total phosphorus (TP vegetation means; Apr-Oct) and the EQR of total index PhytoFluss in pooled data for German very large rivers (area >10,000km^2) in two method versions (PhytoFluss 2.2 (= official method); PhytoFluss 4.0 (= updated method).

Development of the official PhytoFluss-Index (version 2.2) was dated in year 2007, therefore only data before the year 2006 were analysed (Mischke et al. 2011). Germany tested the pressure-impact relationship by pooling qualitative data for very large rivers with low specific run-off (10.2, 20.2) plus tributary rivers to Baltic Sea (type 23). This was done for chlorophyll concentration against total phosphorus (107 years of investigation; from this 82% were very large rivers with low area specific run-off).

Composition metrics were tested against five pre-classified trophic status classes according boundaries for total phosphorus and chlorophyll a (see Mischke et al. 2011, figure 4)

- % Chlorophytes (96 years of investigation; 80% very large rivers in this pooled data)
- % Pennales (171 years of investigation; 14% very large rivers in this pooled data)
- % Cyanobacteria (83 years of investigation; 29% very large rivers in this pooled data)

Trophic Index potamal (TIP) indices based on indicator taxa were available for 314 years of investigation with a share of 37% very large rivers included. Metrics were selected when increasing or decreasing trends for at least 3 status classes.

The version PhytoFluss 4.0 is the updated method and is based on more recent data. This version is on decision to get officially accepted on national level until end of February in 2017. The expert group for river assessment on behalf of the German Federal States (LAWA) requested to intercalibrate this updated method in parallel to the official version. A detailed description of the method modifications is in Annex II – D-2.

The EQR index PhytoFluss show a significant better linear relationship to TP (r^2 = 0.609; see figure D-1, left graph). For rivers with high area specific run-off (DE type 10.1+20.1; type 9.2) the TP to index relationship is strongly improved with new method version (figure DE-2).

An improved indicator taxa list (metric TIP_2013) is available in the prototype tool PhytoFluss 4.0, and the indicators are specific for the three regions: lowland, highland and Danube with tributaries. The trophic scores are calibrated solely against TP. A taxonomic level of species is needed to apply the new indicator list.

The three algal class metrics were not significant correlated to TP in the recent German data set, and therefore assessment without algal class metrics is realized in PhytoFluss 4.0. For international harmonisation, this version 4 use corrected chlorophyll a values (acc. DIN and ISO) instead of uncorrected values in the biomass metric. The biomass boundaries for status classes are as stringent as for PhytoFluss 2.2.

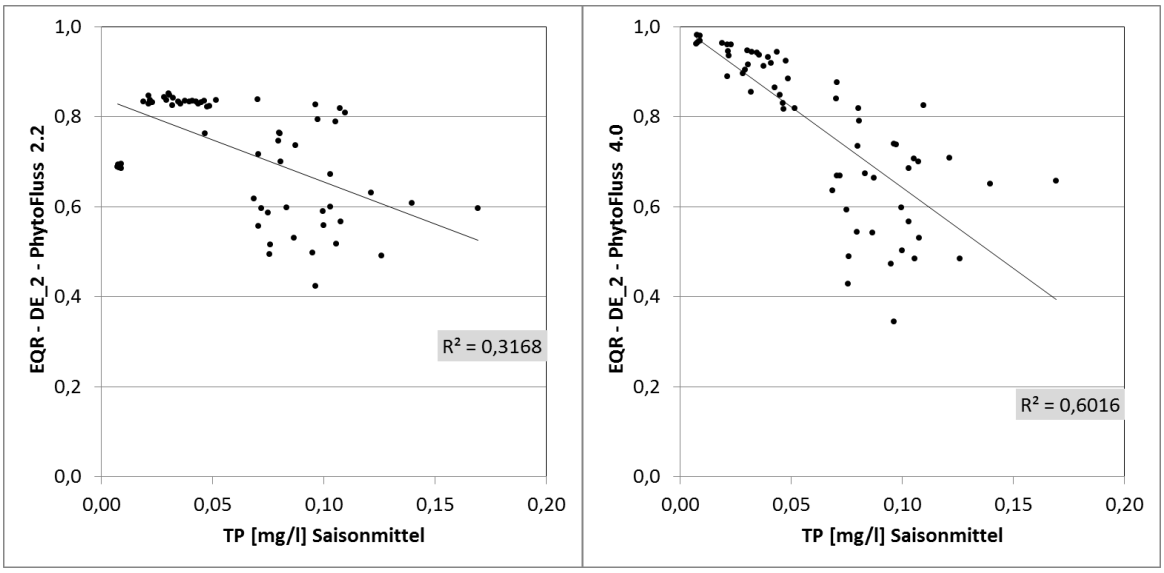


Figure DE_2: Pressure-impact relationships between total phosphorus (TP vegetation means; Apr-Oct; N =63) and the EQR of total index PhytoFluss in selected German data rivers (catchment area >10,000km^2) with high run-off type shown in two method versions (PhytoFluss 2.2 official method; PhytoFluss 4.0 (update)).

Hungary

Hungary applied the HRPI - Hungarian River Phytoplankton Index on each sample of stations from Danube (Duna), Tisza, Maros, Mura, Hármas Körös,and Sajó.

Significant relationship was found, besides of the narrow window of pressures (both TP and TN values were in the eutrophic range).

Including also smaller lowland rivers with potamal character (N = 384), the pooled data show a correlation to total phosphorus (TP), COD, BOD and the land use index in the catchment area (see figure HU_1, A-D).

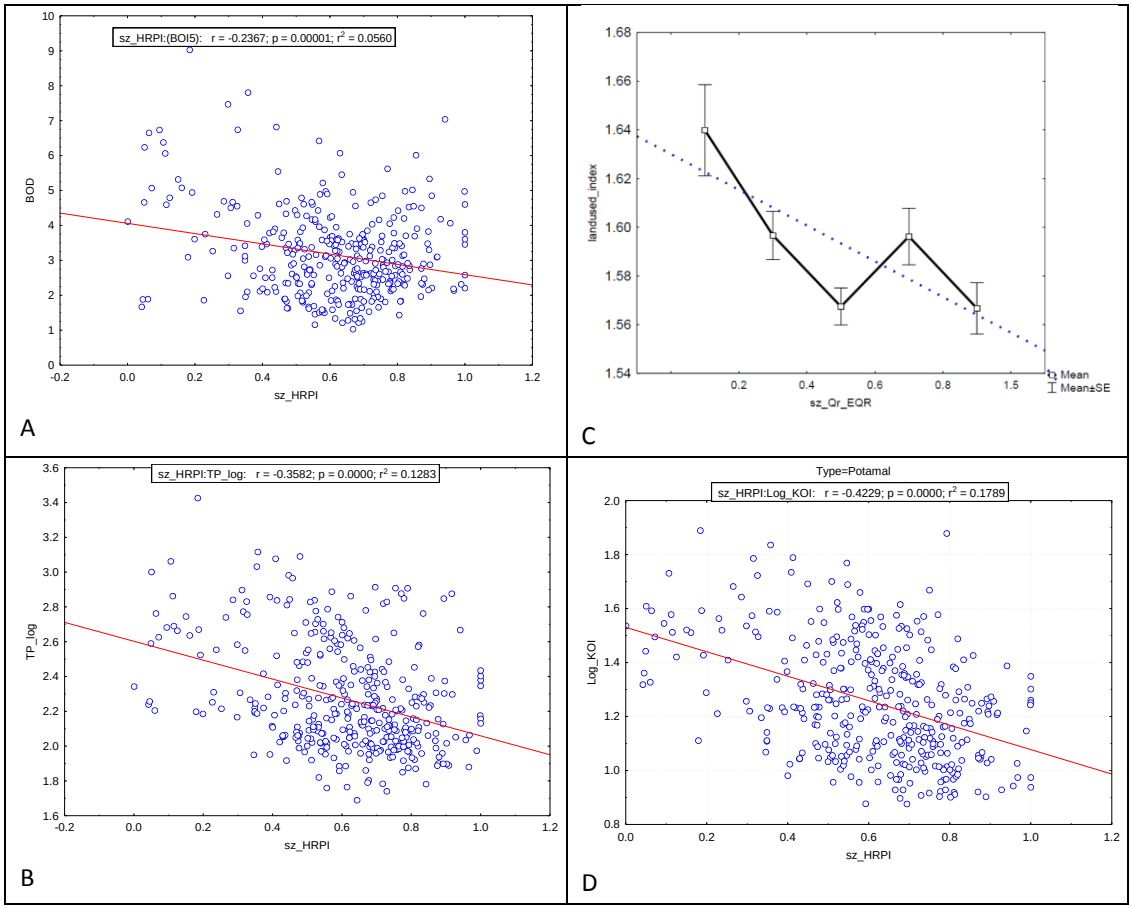


Figure HU_1: Pressure-impact relationships of BOD (graph A), total phosphorus (graph B). landuse_index (graph C: figure redrawn from XGIG presentation) and COD (graph D) the Hungarian Qr-EQR for pooled Hungarian potamal rivers Large Rivers

Latvia

Latvia uses the index LatRPI, an adapted version of Hungarian Large River Phytoplankton Index that uses two parameters to assess the ecological quality of the phytoplankton: chlorophyll a and species composition metric Q (see **Annex_II_A**). Latvian method modifies the Hungarian biomass index and it takes over the boundaries for HU river type 3 for composition metric.

The Latvian assessment method was tested against the chlorophyll-a concentration at seven sampling sites, demonstrating a significant negative relationship (see Figure LV_1).

Latvia used LatPRI of 27 samples to demonstrate sensitivity against the pressure TP (see figure LV_2). The linear correlation was high (r^2 = 0.4377). In the updated version of the Latvian method chlorophyll a values are obligatory required and therefore the pressure-impact relationship was updated accordingly (19.02.2016).

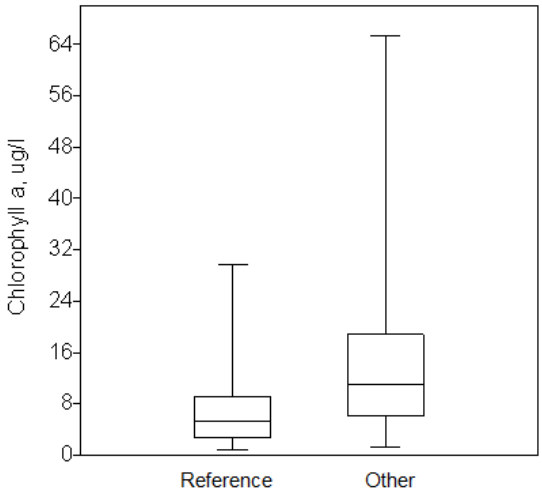


Figure LV_1: Range of chlorophyll a-values at least disturbed sites (reference) and impacted sites in Latvia.

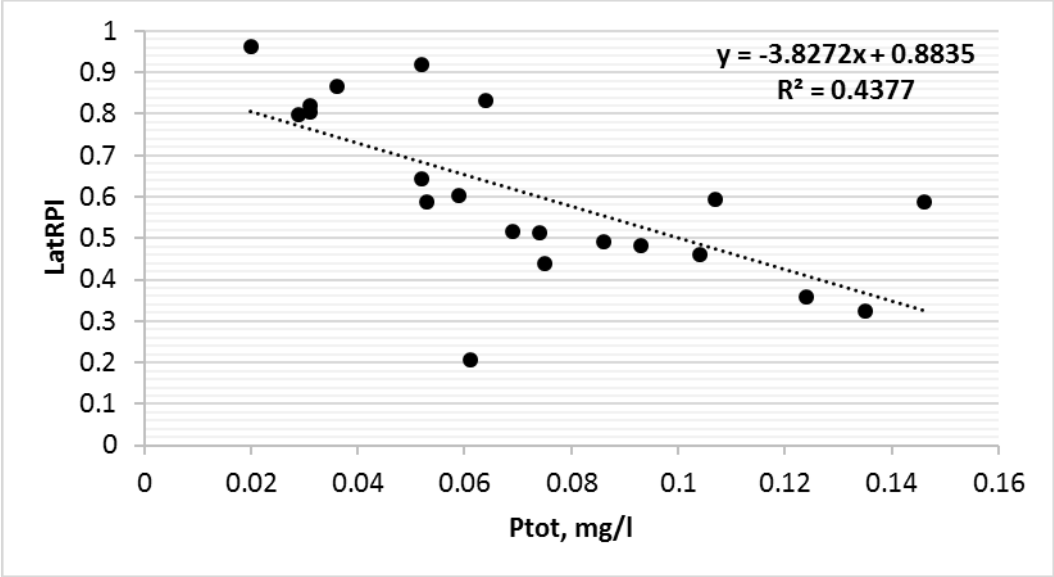


Figure LV_2: Pressure-impact relationship of national LatRPI to total phosphorus (Ptot) in very large rivers of Lativa (yearly average data, N=27).

All data set (54 samples) was tested against different pressures and impacts. Pearson correlation index showed statistically significant negative correlation between abundance of Cyanophyta and species composition metric Q (R=-0,832, p<0,01), LatRPI index (R=-0,512, p<0,05) and ecological quality assessed using national quality criteria (R=-0,328, p<0,05). This indicates successful assessment of water bodies based on functional groups. Increase in biomass of blue-green algae correlates with reduction in value of LatRPI index and quality assessment.

Lithuania

Lithuania applied the German PhytoFluss-Index on 4 sites in 2 large lowland rivers in 7 years of investigation. LT select the German river type 15.2+17.2 as most similar to its very large rivers (e.g. in Germany lower Saale river (area >10.000km^2) is also assessed with this river type). Lithuania provides no demonstration of pressure impact relationship, but was done in the XGIG analysis with pooled data for rivers (N = 556 annual means) with low specific run-off (see Fig. 1 in chapter Belgium). Sensitivity of phytoplankton biomass to stressor TP is detectable along the 75th percentiles for rivers with low run-off.

Poland

Poland applied the IFPL metric - Polish Method for large rivers assessment using phytoplankton on lowland rivers of different PL river types but all with catchment area> 5000 km^2.

Ecological data from 102 sites (12 sites type 19, 3 sites type 20, 77 sites type 21, 9 sites type 24 and 1 type 25) were examined to establish pressure-impact relationship between phytoplankton metric and eutrophication gradient.

The relationship between phytoplankton metric and TP (average from vegetation season) showed significant correlation (R^2=0,451; figure PL_1 redrawn from Polish presentation at XGIG meeting in Berlin, 2014); between phytoplankton metric and PO4; TN and nitrate correlation was weak (R^2 respected 0,206; 0,288 and 0,146).

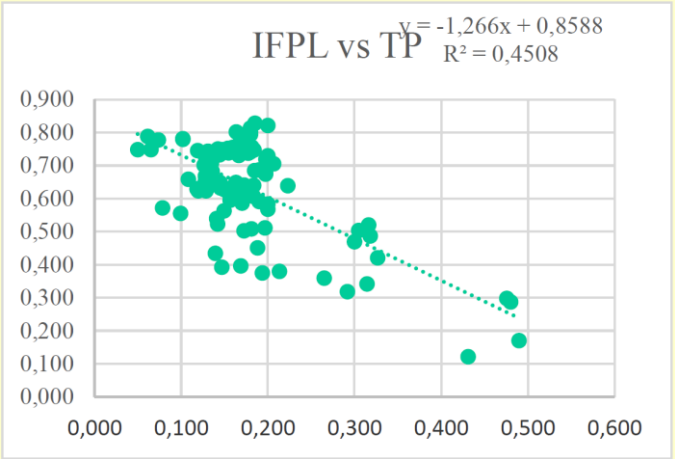


Figure PL_1: Pressure-impact relationships of total phosphorus the Polish index IFPL for pooled Polish lowland rivers with catchment area larger than 5000km^2 (figure redrawn from XGIG presentation).

Romania

Romania has produced correlation factors for each of the five metrics of ECO-FITO –" Assessment Method for Ecological Status of the Water Bodies based on Phytoplankton". All correlations are based on the same set of data (Statsoft 7.0). The indices used were Pantle Buck Saprobic Index, Simpson Diversity Index, Chlorophyll a, taxa no, numeric and biomass abundance of different algal groups. All metric were significant correlated (highlighted in re in table RO-1) to chemical oxygen demand (COD-Cr) and two to nitrate concentration, other nutrient parameters correlate for 1 of the metrics.

The metrics were tested for nutrient pollution, organic pollution and general degradation and the analysis is comprehensively carried out for large Romanian river types RO11, RO12, RO13, RO14.

This evaluation method based on phytoplankton communities described is applied to all Romanian water courses and is in accordance with the principles of Water Framework Directive. The method elaboration takes into account the main pressures to which the phytoplankton/algal communities respond. The phytoplankton is sensitive at: nutrient load, organic pollution, general degradation.

The reference guidance values have been described for each typology and each metric selected (see details here in ANNEX II, Romanian phytoplankton method).

Table RO-1: Correlation matrix between physico-chemical and biological variables for simultaneous sampling date (Marked correlations – in red are significant at p < 0.05000, N = 244, Case wise deletion of missing data)

	Bacillariophyceae abundance	Chlorophyll a	Simpson Diversity Index	Saprobic Index	Taxon no Index
Water temp	**-,3511**	**,2060**	**-,1402**	-,0528	**-,2605**
	p=,000	**p=,001**	**p=,029**	p=,412	**p=,000**
DO (conc)	**,1979**	,0119	-,0390	-,0519	,0135
	p=,002	p=,854	p=,544	p=,420	p=,834
DO (sat)	-,0986	**,2229**	**-,1636**	**-,1283**	**-,1723**
	p=,124	**p=,000**	**p=,010**	**p=,045**	**p=,007**
BOD5	-,0735	**,1471**	**-,2601**	-,0786	**,1818**
	p=,252	**p=,022**	**p=,000**	p=,221	**p=,004**
COD-Cr	**-,1382**	**,2902**	**-,6129**	**-,1535**	**-,4245**
	p=,031	**p=,000**	**p=0,00**	**p=,016**	**p=,000**
N-NH4	-,0124	-,0966	-,0405	,1130	**-,2047**
	p=,847	p=,132	p=,529	p=,078	**p=,001**
N-NO3	**,2950**	,0698	**-,1589**	,0391	-,0529
	p=,000	p=,277	**p=,013**	p=,543	p=,411
N (inorganic)	**,2649**	,0396	**-,1502**	,0601	-,0856
	p=,000	p=,539	**p=,019**	p=,350	p=,183
Total N	**,2909**	-,0066	-,0646	,1051	,0319
	p=,000	p=,919	p=,315	p=,101	p=,620
P-PO4	**,2133**	-,1104	,0794	,1254	,0799
	p=,001	p=,085	p=,217	p=,050	p=,214
Total P	,0704	-,0392	,1167	**,1710**	,1246
	p=,273	p=,543	p=,069	**p=,007**	p=,052

Slovakia

Slovakia has tested the Phytoplankton-SK - Slovak assessment of phytoplankton in large rivers.

The classification of phytoplankton evaluation was carried out on total phosphorus (TP). According to Slovak method for ecological status assessment 90 percentile of TP is used for evaluation. Boundaries between first and second class of ecological status are set for large/ very large rivers as 0.1 or 0.2 mg/l depending on the type, and for boundaries between second and third class as 0.3 or 0.4 mg/l. We have one common method for phytoplankton in large and very large rivers, therefore we used the mean values between above mentioned boundaries (0.15 and 0.35 mg/l respectively).

Overall Slovakia used 28 results from seven lowland localities in rivers (Danube n=3, Váh n=1, Ipeľ n=1, Hron n=1, Morava n=1) of Panonian ecoregion classified as large or very large rivers, representing mean year values mainly of seven samples of variables (chlorophyll-a and percentage of four phytoplankton groups- Cyanophyta, Chlorophyta, Chromophyta and Euglenophyta).

The EQR of Slovak method correlates with TP by correlation coefficient of $r^2 = 0.3196$.

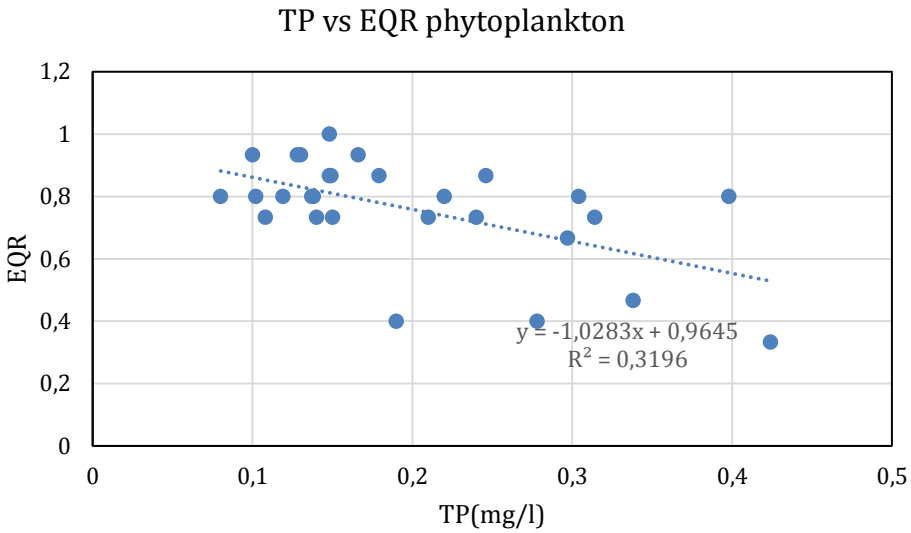


Figure SK_1: Relationship between EQR of Slovak method and TP

The EQR was estimated equidistant from the relationship of TP and EQR
TP in I/II =0.15, EQR =0.8
TP in II/III=0.35, EQR=0.6
The boundaries for Chromophyta were set from the boundaries of Chlorophyta, because they are mainly complementary to the percentage cover of Chlorophyta. In the Slovak national assessment were used the Danube river as very large river and also the mouth of the rivers Ipeľ , Váh, Hron and the Morava river which are tributaries of the Danube river, because we have only narrow gradient in case of three localities on the Danube river.
The following graphs confirm that the results of each of the indices are in relationship with TP.

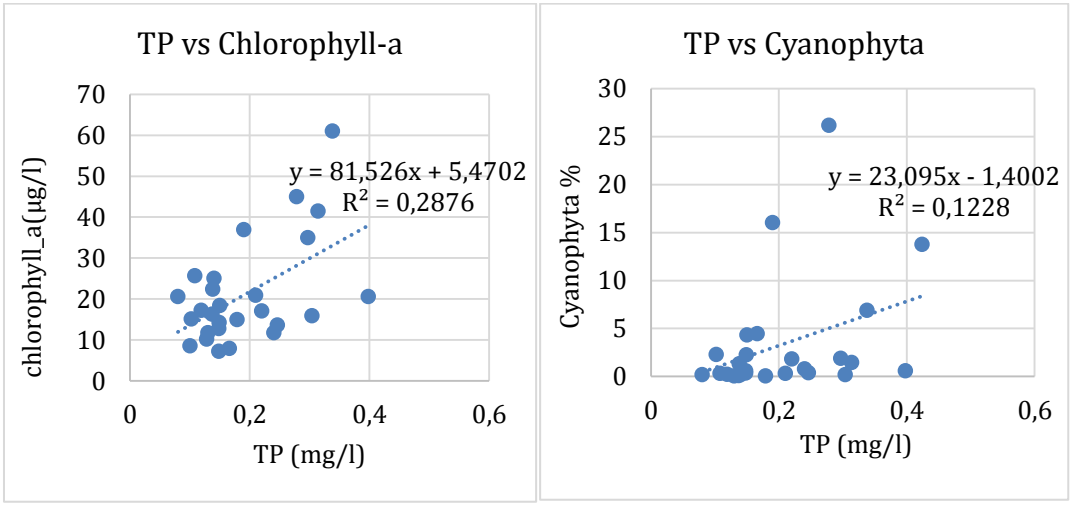


Figure SK_2 (left graph): Relationship between chlorophyll_a and TP
The boundaries for chlorophyll_a were modified to (I/II)TP= 0,15, chlorophyll_a=15 ;
(II/III) TP =0,35, chlorophyll_a =30

Figure SK_3 (right graph): Relationship between Cyanophyta and TP
The boundaries for % of Cyanophyta were modified to (I/II)TP= 0,15, % Cyanophyta=2,5
(II/III) TP =0,35, % Cyanophyta =5

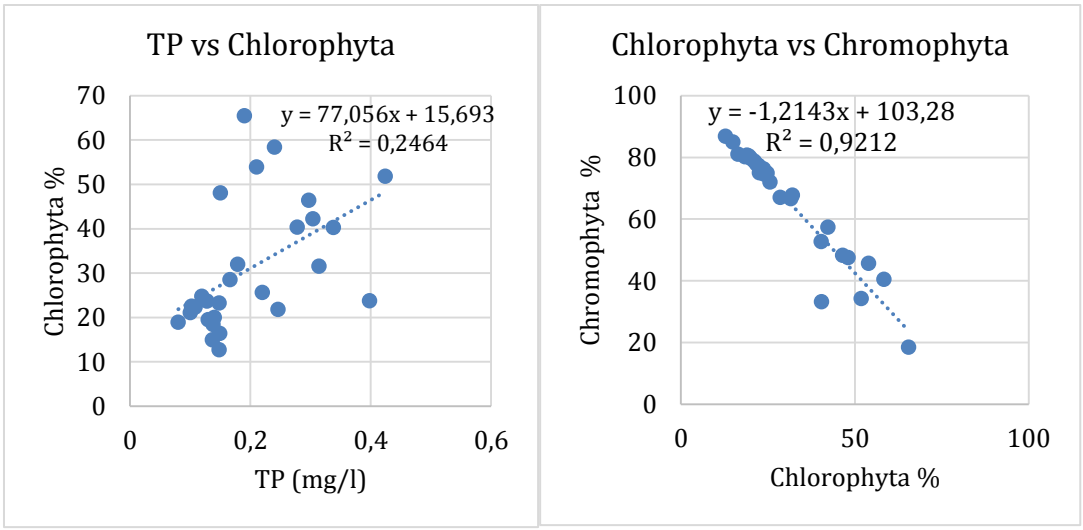


Figure SK_4: Relationship between Chlorophyta and TP
The boundaries were modified to (I/II)TP= 0,15, % Chlorophyta=30
(II/III) TP =0,35, % Chlorophyta=40

Figure SK_5: Relationship between Chlorophyta and Chlorophyta
The boundaries were modified to (I/II) % Chlorophyta=30, Chromophyta 66
(II/III) % Chlorophyta=40, Chromophyta 50

5., % Euglenophyta, which represent organic pollution was present in samples only in very small amount, but it was suggested, that they could be present in samples, and in this case, the boundaries will be nearly as strict as in case of % Cyanophyta. Therefore were the boundaries set by expert judgment as (I/II) = 2 % and (II/III)= 5%.

Checking of compliance with the WFD requirements

According to EC (2011) only assessment methods meeting the requirements of the WFD can be intercalibrated. An important step in the intercalibration procedure is the checking of the national methods considering various WFD compliance criteria. The WFD compliance criteria are specified in the reporting template for milestone reports (Annex VI of EC 2011). We referred to this template to document the compliance of the national assessment methods in the following.

A.3. Checking of compliance with the WFD requirements

A.3.1 Compliance criterion 1 "five classes"

"Ecological status is classified by one of five classes (high, good, moderate, poor and bad)." (EC 2011)

Compliance statement of the XGIG IC group

All methods classify the ecological status by one of five classes (high, good, moderate, poor and bad). Therefore, *compliance criterion 1* is considered to be fully met by all national methods.

A.3.2 Compliance criterion 2 "boundary setting"

"High, good and moderate ecological status are set in line with the WFD's normative definitions (boundary setting procedure)." (EC 2011)

Compliance statement of the XGIG IC group

Most Member States have set their status boundaries against a continuous gradient of anthropogenic pressure, justifying statistical approaches in national boundary setting. Equidistant division of the EQR gradient is used most frequently, combined with good status boundary setting using best available sites, and boundary calibration against pre-classified river sites.
Compliance criterion 2 is considered to be fully met by all national methods.

Table A-3.2.1 provides an overview of national boundary setting procedures.
Table A-3.2.2 provides details on the national boundary setting procedures for assessment methods applied on very large rivers based on phytoplankton.

Table A-3.2.1: Overview of national boundary setting procedures. (X) = MS take over method and boundary setting from another method and MS without checking with own data.

Member State	Using discontinuities in the relationship of anthropogenic pressure and the biological response	Using paired metrics that respond in different ways to the influence of the pressure	Good status boundaries derived from metric variability at best available sites	Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2)	Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement)
Austria				(X)	(X)
Belgium (Flanders)			X	(X)	(X)
Bulgaria				(X)	(X)
Croatia				X	X
Czech Republic			X	X	X
Estonia				X	
Germany	X			X	X
Hungary				X	X
Latvia	X			X	X
Lithuania				(X)	(X)
Poland				X	X
Romania			X		
Slovakia				X	X
SUM					

Table A-3.2.2: Details on the national boundary setting procedures (BSP)

Member State	Explanation
Austria	AT adopts the BSP of Germany Using system developed for the most similar German river type (type 20.1)
Belgium (Flanders)	Setting of ecological status boundaries: • *Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).* Originally, equidistant division of the EQR gradient was applied (boundaries at 0.8, 0.6, 0.4, 0.2); these values were modified (to respectively 0.9, 0.7, 0.5, 0.3) parallel to the changes applied to smaller types as a result of the intercalibration exercise (assuming pressure-impact relationship is similar for all types). The EQR gradient is assumed to represent a continuous trend with general degradation.
Bulgaria	BG adopts the BSP of Germany Using system developed for the most similar German river type (type 20.1)
Croatia	Setting of ecological status boundaries: • *Using paired metrics that respond in different ways to the influence of hydromorphological alterations.* Four boundary values were set where characteristic shifts in the community were observed along the gradient: a) High/Good boundary was defined where the portion of tolerant taxa begins to increase (tolerant < sensitive). b) Good/Moderate boundary was defined where the portion of tolerant taxa reaches the portion of sensitive taxa (tolerant ≈ sensitive). c) Moderate/Poor boundary was defined where the portion of tolerant taxa exceeds the portion of sensitive taxa (tolerant > sensitive). d) Poor/Bad boundary was defined where portion of tolerant taxa starts to dominate (tolerant >> sensitive). • *Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2) for the Saprobic Index*
Czech Republic	Setting of ecological status boundaries: • *Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).* The reference value was defined as 25% (or 75%) quantile of metric values at the best available sites for each stream order separately and in some cases expertly adjusted. The lower limit (for EQR calculation) was determined as the average of 99% (or 1%) quantile of metric values at all sites of each stream order. The EQR range 0-1 was divided into five categories in the same range (= ecological status classes). Final assessment results from the tested dataset were subjected to expert judgement and subsequently limits for the EQR calculation were revised so as to achieve a compromise between statistical calculations and expert opinion. For more detailed analyses the sufficient dataset was not available.
Estonia	Setting of ecological status boundaries: • *Calibrated against pre-classified sampling sites.* Setting of ecological status boundaries phytoplankton: Using system developed for the most similar Hungarian river type (type 5)
Germany	Setting of ecological status boundaries phytoplankton: • *Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).* • *Calibrated against pre-classified sampling sites.* River types were placed into three nutrient response groups using empirical analysis (low, high and very high response in phytoplankton biomass). To set the "high/good" status class boundary (H/G), in the first step, a TP background level of 0.05 mg L^{-1} was applied to all river types (background modelling see Behrendt et al., 2003) . The boundaries for the biomass metric approximately fit the different nutrient response curves of the phytoplankton biomass, measured as chlorophyll a uncorrected for phaeophytin a (chla_uncorr), along the 75% percentiles within the five TP classes. Regression curves were mathematically fitted to cross the boundaries along the parameter responses, and were transformed to index "calculation functions" operating between 0.5 to 5.5. To set the "poor/bad" status class boundary (P/B), we used the "point of no further response of biomass to TP". When TP concentrations exceeded 0.25 mg L^{-1}, the 75% percentile of chla_uncorr concentration did not further increase in nutrient sensitive rivers, but was assumed to be more influenced by saproby. The "poor/bad" boundary of TP was set higher (at 0.30 mg L–1) for those river types with an overall low slope of the response curve between phytoplankton biomass and TP concentration. To set the remaining two boundaries the range of the TP scale between H/G and P/B was fitted

Member State	Explanation
	to a linear or an exponential curve, and divided into 3 equal parts, and the resulting TP values were finally rounded. Metrics taxa composition: The boundaries for the taxonomic composition metrics Pennales, Chlorophytes and Cyanobacteria were derived also from the 75% percentile values, when the parameter distribution was plotted in box-plots and grouped by a pre-classification of all sites in five eutrophication classes, based on the biomass boundaries combined with those for TP according to pre-set boundaries (trophic status assessment).
Hungary	Setting of ecological status boundaries phytoplankton: • *Good status boundaries derived from metric variability at best-available sites.* • *Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).* • *Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).* For river type group 3 (Middle-sized and large colline and lowland rivers with coarse -fine substrate) 50rd, 75th, 90th and 95th percentiles were used calculated from all available data for boundary setting of chlorophyll a. In case of river type group 4 and 5 (Danube) 33rd, 45th, 66th and 80th percentiles were used calculated from all available data for boundary setting of chlorophyll a. The functional groups of algae were evaluated on basis of their ecological characteristics (expert judgement). Nutrient status, tolerance of turbulent conditions, time sufficient for development of the given assemblage and general risk. All the groups were given a factor number (1-5). All the boundaries were set by the relative abundance of the reference (F=5) and good (F=4) taxa. Criteria for the selection of least disturbed sites (LDS): lack of impoundments and off-river reservoirs, BOD<5 mg l^{-1}, (~5000 data).
Latvia	Setting of ecological status boundaries phytoplankton: Using system developed for the most similar Hungarian river type (HU type 3) The functional groups of algae were evaluated on basis of their ecological characteristics. Nutrient status, tolerance of turbulent conditions, time sufficient for development of the given assemblage and general risk. All the groups were given a factor number (1-5). All the boundaries were set by the relative abundance of the reference (F=5) and good (F=4) taxa.
Lithuania	LT adopts the BSP of Germany Using system developed for the most similar German river type (type 15.2)
Poland	Setting of ecological status boundaries phytoplankton: • *Good status boundaries derived from metric variability at best-available sites.* • *Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).* In the beginning, equidistant intervals of class boundaries were applied. In the years 2009-2011, more than 80 localities on large rivers were examined. Those with average values (from vegetation season) of TP concentration not higher than 0.13 mgP/l were selected and 95 percentile of IFPL index data set was calculated to obtain the boundary value between high and good status classes. The 95 percentile equalled 0.812 and therefore the value 0.8 of H/G boundary was accepted. The rG/M, M/P and P/B boundaries were indicated by dividing the remaining value on equidistant intervals.
Romania	Setting of ecological status boundaries phytoplankton: • *Good status boundaries derived from metric variability at best-available sites.* Organic pollution are reflected by Pantle Buck Saprobic Index (modified). Boundary for phytoplankton between High/Good status for large rivers (real value = 2,3) represents 10% from existing data from less impacted sites. Boundary between good/moderate for large rivers (real value = 2,5) represents 30% from existing data from less impacted sites
Slovakia	Setting of ecological status boundaries phytoplankton: Boundaries between first and second class of ecological status are set according to chemical boundaries for H/G and G/M status for large/ very large rivers as 0,15 and 0,35 mg/l TP respectively. This boundaries were used for chlorophyll_a and Cyanophyta and Chlorophyta. Chromophyta were calculated as complementary to percentage of Chlorophyta, because these are mostly the most abundant taxonomic group. In the case of Euglenophyta, which represent organic pollution was present in samples only in very small amount, but it was suggested, that they could be present in samples, and in this case, the boundaries will be nearly as strict as in case of % Cyanophyta. Therefore were the boundaries set by expert judgment nearly as strict as Cyanophyta.

A.3.3 Compliance criterion 3 "relevant parameters"

"All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A combination rule to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole." (EC 2011)

Compliance statement of the XGIG IC group

All relevant parameters indicative of the biological quality element are covered by almost all methods: Taxonomic composition, abundance, and frequency and intensity of planktonic blooms.
The parameter "frequency and intensity of planktonic blooms" is missing in all methods. The Member States with abundance assessment (e.g. chlorophyll a) based in more frequent sampling (fs) than on taxonomic composition declare that their methods are sufficiently indicative of the phytoplankton status as a whole (marked with (fs) in the table); see explanation below.
The GIG group also agreed that it is necessary to have at least 6 samples within vegetation season to cover algal blooms (see also A.3.7). *Compliance criterion 3* is considered to be fully met by all national methods.

Table A-3.3.1 list all metrics used in the national methods.

Table A-3.3.2 provides Information about combination rules for metrics in national methods.

Table A-3.3.1: Overview of metrics used in the national methods.

Country	Metrics	Taxonomic composition	Abundance	frequency and intensity of planktonic blooms
Austria	Total biomass of phytoplankton (chlorophyll a) TIP Index (PhytoFluss 4.0): Composition of indicator taxa Weighted averaging with (1 *chla + 3 * TIP) /4	ok	ok	(fs)
Belgium (Flanders)	Total biomass of phytoplankton (chlorophyll a + (phaeophytin a/1.7)) TIP Index: Composition of indicator taxa Chloro Index: Relative biovolume of class Chlorophytes (applied type 10.2 and 20.2) Cyano Index: Relative biovolume of class Cyanobacteria (applied type 10.2 and 20.2) Averagred metric score	ok	ok	(fs)
Bulgaria	Total biomass of phytoplankton (chlorophyll a) TIP Index (PhytoFluss 4.0): Composition of indicator taxa Weighted averaging with (1 *chla + 3 * TIP) /4	ok	ok	(fs)
Croatia	chlorophyll a and species composition metric Q Weighted averaging with (2* chla + Q)/3	ok	ok	(fs)
Czech Republic	Chlorophyll_a concentration Relative proportion of Bacillariophyceae (%Bacillariophyceae) Relative proportion of Cyanophyceae (%Cyanophyceae) Relative proportion of Chlorophyceae (%Chlorophyceae) Averaged metric score	ok	ok	(fs)
Estonia	chlorophyll a and species composition metric Q (applied HU type 5 for metric Q)	ok	ok	(fs)
Germany	Total biomass of phytoplankton (chlorophyll a + (phaeophytin a/1.7)) TIP Index: Composition of indicator taxa Pennales Index: Relative biovolume of order Pennales (applied type 9.2, 10.1 and 20.1, 15g) Chloro Index: Relative biovolume of class Chlorophytes (applied type 10.2 and 20.2) Cyano Index: Relative biovolume of class Cyanobacteria (applied type 9.2, 20.2, 15g) Averaged metric score In case PhytoFluss 4.0 become official method: Total biomass of phytoplankton (chlorophyll a) TIP_2013 Index (PhytoFluss 4.0): Composition of indicator taxa Weighted averaging with (1 *chla + 3 * TIP) /4 for type 10.1&20.1 (high run-off) Weighted averaging with (2 *chla + 1 * TIP) /3 for type 10.2&20.2 (low run-off)	ok	ok	(fs)

Country	Metrics	Taxonomic composition	Abundance	frequency and intensity of planktonic blooms
Hungary	chlorophyll a and species composition metric Q Weighted averaging with (2* chla + Q)/3	ok	ok	(fs)
Latvia	chlorophyll a and species composition metric Q (applied HU type 3 for metric Q) Averaged metric score	ok	ok	(fs)
Lithuania	Total biomass of phytoplankton (chlorophyll a + (phaeophytin a/1.7)) TIP Index: Composition of indicator taxa Pennales Index: Relative biovolume of order Pennales (applied type 15g) Cyano Index: Relative biovolume of class Cyanobacteria (applied type 15g) Averaged metric score	ok	ok	(fs)
Poland	Trophic index (Composition of indicator taxa) and chlorophyll concentration Averaged metric score	ok	ok	(fs)
Romania	Pantle Buck Saprobic Index, Simpson Diversity Index, Chlorophyll a, taxa number, numeric abundance (Bacillariophyceae) Weighted averaging	ok	ok	(fs)
Slovakia	1., Abundance of Cyanophyta, Chromophyta, Chlorophyta and Euglenophyta per 1 ml recalculated to percentage form; 2. Total abundance (units/ml) 3. Biomass as Chlorophyll-a Worst quality class	ok	ok	(fs)

(fs) = The GIG group agreed that it is necessary to have at least 6 samples within vegetation season to cover algal blooms.

Table A-3.3.2: Information about combination rules for metrics in national methods.

Country	Average metric scores	Weighted average metric scores	Worst metric score	Mean quality class	Worst quality class	Other (specify)	Not relevant	GIG lead remark
Austria		X						see DE method
Bulgaria		X						see DE method
Czech Republic	X							
Flanders (Belgium)	X						Expert judgement	see DE method PhytoFluss 2.2
Germany	(X)	X						
Estonia*		X						see HU & see update
Croatia		X						see HU
Hungary		X						
Latvia	X							see update (6.10.2015)
Lithuania	X							see DE method PhytoFluss 2.2
Poland	X							
Romania		X						
Slovakia	X					YES (in partial evaluation, from the taxonomic groups is considered the worst class with the lowest score)		

* EE method after update (17.04.2015) of EstPRI by including chlorophyll a-index for HU type

Explanation on the compliance of all phytoplankton methods

Concluding, all methods are considered fully indicative of anthropogenic pressure although they do not take into account frequency and intensity of the type-specific planktonic blooms. There is no common definition about this parameter. Planktonic blooms can be measured based in chlorophyll a concentrations, which are carried out in all MS more frequent than taxonomic composition analysis. MS with bi-weekly or monthly sampling for assessment of abundance (based on chlorophyll a) in the vegetation period declare that their methods are sufficiently indicative of the phytoplankton status as a whole, while reflecting indirectly also blooms.

A.3.4 Compliance criterion 4 "type coverage"

"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." (EC 2011)

Compliance statement of the XGIG IC group

The table below specifies the national types relevant for the assessment methods of each Member State.
Informatively, the type of another country is provided, when its method is applied.
All national types were attached to one of 2 intercalibration types (IC type) for the phytoplankton exercise of large rivers (see Annex III).
Each country covers one or both IC types.

Compliance criterion 4 is considered to be fully met by all national methods.

Table A-3.4.1: Information about national river types covered by national methods.

Member State	Relevant national type(s)
Austria	Large Alpine rivers; Very large rivers of >10,000 km^2 catchment size (Danube) dominated by cobbles and gravel *For phytoplankton assessment applied following type from other country:* *Very large rivers with high specific run-off (10.1 + 20.1; DE)*
Belgium (Flanders)	Only one relevant river >10,000 km^2 *For phytoplankton assessment applied following type from other country:* *Very large rivers with low specific run-off (10.2 + 20.2; DE)*
Bulgaria	Very large rivers of >800,000 km^2 catchment size dominated by fine substrata (sand, clay, loess) *For phytoplankton assessment applied following type from other country:* *Very large rivers with high specific run-off (10.1 + 20.1; DE)*
Croatia	Very large lowland rivers on siliceous and calcareous bedrocks (Lower Mura, Middle Drava and Sava); Very large lowland rivers on siliceous bedrock (Lower Drava and Sava); Very large lowland rivers on siliceous bedrock (Danube) *For phytoplankton assessment applied following type from other country:* *River type group 3 (Middle-sized and large colline and lowland rivers with coarse -fine substrate; HU)* *River type group 5 (Lower Danube; HU)*
Czech Republic	Large non-wadeable rivers of 8th and 9th order of Strahler´s system, altitude < 500 m a.s.l.
Estonia	Very large rivers of >10,000 km^2 catchment size *For phytoplankton assessment applied following type from other country:* *River type group 5 (Lower Danube; HU)*
Germany	Very large rivers of >10,000 km^2 catchment size dominated by sandy channel substrate; Very large rivers of >10,000 km^2 catchment size with channel substrates dominated by cobbles and gravels Large sand and loam-dominated lowland rivers Large highland rivers *For phytoplankton assessment grouped or merged in following types:* *Large lowland rivers with sandy or gravel bedrock (15.2+17.2)*

	Large rivers of the low mountain region (9.2) *Very large rivers with high specific run-off (10.1 + 20.1)* *Very large rivers with low specific run-off (10.2 + 20.2)*
Hungary	Large lowland rivers (0-200 m altitude, large to very large catchment size) dominated by fine substrate National types: 6, 7, 14, 14, 19, 20, 23, 24 *For phytoplankton assessment grouped or merged in following types:* *River type group 3 (Middle-sized and large colline and lowland rivers with coarse -fine substrate)* *River type group 4 (Upper Danube; HU)* *River type group 5 (Lower Danube; HU)*
Latvia	Very large lowland rivers of >10,000 km^2 catchment size *For phytoplankton assessment applied following type from other country:* *River type group 3 (Middle-sized and large colline and lowland rivers with coarse -fine substrate; HU)*
Lithuania	Baltic lowland rivers *For phytoplankton assessment applied following type from other country:* *Large lowland rivers with sandy or gravel bedrock (DE 15.2+17.2)*
Poland	Very large rivers of >10,000 km^2 catchment size dominated by sandy and gravel channel substrate (with different size fractions); Very large rivers of >10,000 km^2 catchment size dominated by sandy substrate, with high organic matter retention and influence of brackish water
Romania	Water stream sector with floodplains in plain area, ecoregion 11,12,16; Danube River- Cazane area, ecoregion 12; Danube River– lower sector between Cazane and Calarasi; ecoregion 12; Danube River between Calarasi and Isaccea, ecoregion 12; Danube Delta, ecoregion 12
Slovakia	very large Panonian lowland river >10,000 km2 type up to 200 m above see level

A.3.5 Compliance criterion 5 "reference conditions"

"The water body is assessed against type-specific near-natural reference conditions." (EC 2011)

Taken from the conceptual paper on large river bioassessment (Schöll et al. 20122) on 'reference conditions' "Compared to smaller streams large rivers are relatively rare and exposed to substantial human influence for centuries. This is why none of the large rivers, at least in most of Europe, meet near-natural reference conditions anymore. Due to intensive anthropogenic use (e.g., discharge of industrial and municipal waste water and/or cooling water, power generation, navigation, commercial fishery, water extraction, reclamation of agricultural land, flood protection works) biological reference communities cannot be described satisfactorily."

Compliance statement of the XGIG IC group

Despite the huge challenges to establish appropriate reference conditions for large rivers, the Member States demonstrated considerable creativity in defining sound assessment baselines.
Compliance criterion 5 is considered to be fully met by all national methods.

Countries which use the German method do not change its underlying reference conditions reconstructed in Mischke et al. (2011), but Lithuania use the German river type 15.2 which has the same reference conditions but more stringent class boundaries.

Table A3.5.1: Overview on the national definitions of reference conditions.

Member State	Existing near-natural sites	Modelling	Expert knowledge	Historical data	Least disturbed conditions
Austria (adopted from DE)		X			
Belgium (Flanders) (adopted from DE)		X	X		
Bulgaria (adopted from DE)		X			
Croatia (adopted from HU)			X		X
Czech Republic			X		X
Estonia (adopted from HU)			X		
Germany		X			
Hungary			X		X
Latvia (adopted from HU)			X		
Lithuania (adopted from DE)	X	X			X
Poland			X		X
Romania			X	X	X
Slovakia			X		
SUM	***1***	***5***	***9***	***1***	***6***

[2] Schöll, F. et al. (2012) Conceptual paper on large river bioassessment. Annex II of Schöll, F., Birk, S. & Böhmer, J. (eds.): XGIG Large
River Intercalibration Exercise – WFD Intercalibration Phase 2: Milestone 6 report. Joint Research Centre, Ispra (IT): 73 pp.

Table A_3.5.2: Details on the national definitions of reference conditions.

Member State	Explanation
Austria	• *Modelling (extrapolating model results)* *No explanations yet*
Belgium (Flanders)	No site in reference or LDS conditions. Key source(s) to derive reference conditions: • *Expert knowledge*
Bulgaria	• *Modelling (extrapolating model results) [adopted from DE]* *No explanations yet*
Croatia	No site in reference or LDS conditions. Key source(s) to derive reference conditions: • *Expert knowledge* • *Least Disturbed Conditions*
Czech Republic	Reference communities in selected sites on the Labe river. Key source(s) to derive reference conditions: • *Expert knowledge* • *Least Disturbed Conditions*
Estonia	There is only one large river in Estonia which has proper reference sites nowhere. Hydrochemical very good class: Dissolved oxygen >70%, N_{total} <0.5 mg N/l, P_{total} <0.04 mg P/l, NH_4^+ <0.10 mg N/l (90% of cases), pH 6.0–9.0. More than 50% of landuse is not natural in the catchments is allowed. Two sites: Vasknarva (outflow from lake Peipsi), Narva (downstream the Narve reservoir dam) Key source(s) to derive reference conditions: • *Expert knowledge*
Germany	Germany used the MONERIS nutrient emission model (BEHRENDT et al., 2003) to estimate zero anthropogenic nutrient conditions for phytoplankton. They were extrapolated from the statistical relationship between anthropogenic influences and in-stream nutrient concentrations within the MONERIS model (BEHRENDT et al., 2003) for 170 sites. Potentially natural background conditions were calculated by switching off all direct and indirect anthropogenic inputs. In result of this modelling approach, total phosphorus concentrations below 0.05 mg/L were assumed as background conditions for all different large German rivers and streams. LDS: Rhein, Karlsruhe, Rhein, Reckingen, Rhein, Breisach and modelling Key source(s) to derive reference conditions: • *Modelling (extrapolating model results)* • *Least Disturbed Conditions (only for validation)*
Hungary	Reference conditions are described using least disturbed sites according to land use and BOD_5 <3.0 mg for phytoplankton. No downstream dam effect Key source(s) to derive reference conditions: • *Expert knowledge* • *Least Disturbed Conditions (Danube, Göd village)*
Latvia	Reference sites (Daugava, stretch from border Latvia-Belarus to upstream Jekabpils) were selected using hydro-chemical status (BOD5<2 mg/L, Ptot <0,1 mg/L) morphological quality (no dams or HPP) and riparian land-use . Key source(s) to derive reference conditions: • *Expert knowledge*
Lithuania	Reference monitoring sites were selected according surrounding areas are not dominated by agricultural land use; high hydrochemical status (total P < 0.1 mg/L, PO_4-P < 0.05 mg/L, total N < 2.0 mg/L, NO_3-N < 1.3 mg/L, NH_4-N < 0.1 mg/L, BOD_7 < 2.3 mg/L). Key source(s) to derive reference conditions: • *Existing near-natural reference sites* • *Least Disturbed Conditions* In LT stations at river Nemunas and Neris these conditions are met in at least some of the years of investigation, except the BOD5 values (2.5 – 4.7mg/L).
Poland	*PL comment: will be verified/completely developed by end of 2015.* Large and very large lowland rivers in the 'V biocoenotic river type'. For this biocoenotic type, several sites semi reference rivers were analysed for phytoplankton: Bobr (PLRW6000201695: $a_{\_e}$: 5831km^2); Biebrza (PLRW200024262999; $a_{\_e}$: 7057km^2); San (PLRW20002122999; $a_{\_e}$: 16870 km^2) Key source(s) to derive reference conditions: • *Expert knowledge*

Member State	Explanation
	• *Least Disturbed Conditions*
Romania	For large rivers PP relevant reference conditions *in situ* are no longer available, thus best available sites were identified and used (Danube: Gruia section). These were characterised by biological elements. Key source(s) to derive reference conditions: • *Expert knowledge* • *Historical data* • *Least Disturbed Conditions* Boundaries for GPC elements for H/G were set by 90th percentile from less impacted sites (< 2.5 mg/L TN; =<1.5 mg/l N-NO_3; <0.3 mg/l N-HH_4; <0.15 mg/L TP; < 0.08 mg/L P-$P0_4$; <3 mg/L O_2 for CBO5).
Slovakia	PP relevant reference values were based on expert judgement according to results of TP in chemical status, because there are no real reference sites for large lowland rivers in Slovakia (experts at meetings of Slovak Algological Society and Slovak Limnological Society) there was no presence of whole scale of water quality in this relevant water type (high–bad) The undisturbed (reference) sites for very large rivers do not exist in the Slovak territory. Additionally the obtained monitoring data did not cover the whole pollution gradient of the very large rivers.

A.3.6 Compliance criterion 6 "EQR"

"Assessment results are expressed as EQRs." (EC 2011)

Compliance statement of the XGIG IC group

All national methods express their assessment results as EQRs (that are used in the analytical procedure for the boundary comparison and harmonisation).
Compliance criterion 6 is thus considered to be fully met.

A.3.7 Compliance criterion 7 "sampling in space and time"

"Sampling procedure allows for representative information about water body quality/ecological status in space and time." (EC 2011)

Compliance statement of the XGIG IC group

All national methods apply sampling procedures allowing for representative information about water body quality/ecological status in time and space. See table below for detailed sampling frequency.
For taking into account the frequency and intensity of the planktonic blooms chlorophyll a concentrations is measured more frequent than taxonomic composition analysis in some countries. MS with bi-weekly or monthly sampling for assessment of abundance (based on chlorophyll a) in the vegetation period declare that their methods provide representative information.
The GIG group agreed that it is necessary to have at least 6 samples within vegetation season to cover algal blooms.
Compliance criterion 7 is considered to be fully met by all national methods.

During the intercalibration exercise the countries LV and EE agree to increase their sampling frequency in future to have at least 6 samples within the vegetation season.

Table A_3.7.1: Details on the national sampling frequency for methods based on phytoplankton

	samples per year	for abundance chl_a
Austria	**6-12**	**6-12**
Belgium (FL)	**3-5**	**6-7**
Bulgaria	**4-6**	**6**
Czech Republic	**6-7**	**6-7**
Croatia	**6**	**6**
Estonia	**3**	**6**
Germany	**6-7**	**6-7**
Hungary	**6**	**6**
Latvia	**2-4**	**6**
Lithuania	**6**	**6-7**
Poland	**5-7**	**6-7**
Romania	**2-3**	**6-7**
Slovakia	**6-7**	**6-7**

A.3.8 Compliance criterion 8 "sampling procedure"

"All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure." (EC 2011)

All countries use buckets with a rope or the vertical Ruttner sampler and some MS use additionally a planktonic net. Sampling from bridges is mainly done, except for additional samples from the shore side (RO, DE) and special monitoring programs by ship. The main channel is sampled, but Romania carry out additional shoreline sampling.

Counting technique follow the EN 15204, 2006: Water quality. Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermoehl technique). European Committee for Standardization, Brussels in all MS, except of CZ and SK, which use for abundance Cyrus I counting chamber according to National Standard STN 75 7715) and ISO 10260:1992 standard is followed for chlorophyll a.

Compliance statement of the XGIG IC group

All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure.
Compliance criterion 8 is thus considered to be fully met by all national methods.

A.3.9 Compliance criterion 9 "taxonomic level"

"Selected taxonomic level achieves adequate confidence and precision in classification." (EC 2011)

Compliance statement of the XGIG IC group

The Member States use various taxonomic levels to assess the ecological status using phytoplankton, ranging from species- to genus-level, except of Slovakian method using algal class level. The taxonomic level selected by each Member State is regarded to achieve adequate confidence and precision in classification, except of Slovakian method proving data on algal class level.
Compliance criterion 9 is thus considered to be met by all national methods, but Slovak method has to demonstrate their precision by an option 3 exercise.

Table A_3.9.1: Taxonomic level selected to assess the ecological status using phytoplankton

Member State	Taxonomic level
Austria	Species and Genus
Belgium (Flanders)	Species to family
Bulgaria	Species and Genus
Croatia	Species
Czech Republic	Species
Estonia	Species and Genus
Germany	Species and Genus
Hungary	Species
Latvia	Species
Lithuania	Species and Genus
Poland	Species and Genus
Romania	Species and Genus
Slovakia	Family to Class level

A.4. Methods' intercalibration feasibility check

Typology

Very large rivers were generally defined as running waters exceeding a total catchment area of 10,000 km^2. For the intercalibration exercise, no typological differentiation was made for the intercalibration of national methods using phytoplankton. Due to the benchmark standardization applied to the common metrics prior to boundary comparison (see explanations in part B) typological differences were minimized. For this, all national types were attached to one of 2 intercalibration types (IC type) for the phytoplankton exercise of large rivers (see Annex III). The combination of IC type and country was used for benchmark standardization applied to the common metrics.

Pressures

The national methods using phytoplankton mainly indicate the effects of pollution/eutrophication. This was demonstrated by all participating Member States using empirical pressure-impact analyses (see chapter A.2). Effect of morphological degradation was only conceptually integrated (functional group response) for some countries using the Hungarian metric Q. The combined stressor (parameters of diffuse pressures) used in the intercalibration analysis was significantly correlated with the Intercalibration Common Multimetric index (CM12b; see Section B.1). The correlation of national methods and the CM12b can be regarded as an indirect empirical testing of their pressure-impact relationship.

Assessment concept

The existing national assessment methods acquire their biological data from the main river channel and are based on concepts similar to the assessment of smaller rivers. The intercalibration exercise deals with the harmonization of the assessment methods that are currently used by the Member States.

Part B

Part B of the report presents the data basis used for intercalibration, describes the development of the pressure index and the common metric selection including the multimetric index, specifies the benchmark standardisation applied to the data, and documents the comparison of national class boundaries of ecological status according to EC (2011).

The phytoplankton exercise follow continuous benchmarking according Annex 2: Approaches for metric standardisation in intercalibration: Reference benchmarking, alternative benchmarking and continuous benchmarking in comparison compiled by Böhmer et al. 2016[3].

B.1. Global intercalibration exercise (option 2)

Where there are sufficient sites to produce statistically relationships option 2, with a biological common metric based on Chlorophyll a, the German trophic-index- potamoplankton (TIP) and the Hungarian functional trait index Q, was used.

Common metric could not be applied on SK data, so the national metric was applied to other MS data after benchmark standardisation (Option 3).

In case of IC Option 2, please explain the differences in data acquisition:

- Some MS (CZ, SK, RO) use taxa abundance instead of biovolumes (all others) and abundance information could not provide by some other countries. For the application of a common metric CZ and RO provide taxa biovolumes, which were calculated with standard cell volumes for each taxa for a sub-set of their data.
- Some MS (eg EE & LV) may have insufficient chlorophyll_a samples from spring and early summer to enable other MS to apply their methods.
- Some methods may be found to be insufficiently comparable in concept, for example Pantle Buck Saprobic Index and Simpson Diversity Index used by RO is not included in other MS methods and additional information such as size categories and heterotrophic taxa are required.
- There remain significant issues with respect to level of determination of taxonomy and the application of MS methods to the common database.

Option 2 is thus considered to be the best approach.

B.1.1: Data basis

Data provided in intercalibration were delivered by 13 countries participating in the exercise cover 762 annual mean at 275 sampling sites. These data were sampled within WFD monitoring programmes, and in case of Czech Republic sampling von 4 river Labe-sites in the frame of the international basin program (IKSE; e.g. with taxa biovolume data) in parallel to WFD monitoring.

Data sets with Ecological Quality Ratios (natEQRs) for country methods, and required stressor values, cover 459 annual mean based on 5219 phytoplankton samples and supporting information at 196 sampling sites of very large rivers (Table B1; Figure B1; Annex IX). All data are stored in a MS Access database (XGIG_LR_PP_DB.accdb; IGB Berlin,).

Data used in intercalibration were complete data sets (448 annual averages) for which additionally the calculation of the common metric was applicable and valid.

[3] Böhmer, Jürgen Sebastian Birk, Nigel Willby, Geoff Phillips & Sandra Poikane (2016: Annex 2: Approaches for metric standardisation in intercalibration: Reference benchmarking, alternative benchmarking and continuous benchmarking in comparison In: Milestone report 6 – BQE: Benthic Invertebrates. Version 1.0 – August , 2016

The total number of IC used data were further reduced to 425 cases (annual means), because of the decision of AT and DE to use the updated version of German method (PhytoFluss 4.0) which requires a more exact taxa determination level.

Table B1: Overview of national data used in the intercalibration exercise

Country	**Number of sampling sites**	***Number of annual averages***
Austria	7	*19*
Belgium (Flanders)	1	*11*
Bulgaria	15	*15*
Croatia	7	*13*
Czech Republic	4 (+35*)	*15 (+35*)*
Estonia	1	*8*
Germany	39	*136*
Hungary	38	*93*
Latvia	7	*11*
Lithuania	3	*5*
Poland	35	*40*
Romania	36	*93***
Slovakia	(7***)	*(35***)*

* Czech biological data were delivered as abundances (cells/ml) and a small set of data as taxa biovolumes (4 sites with 15 years) from an internal national monitoring (IKSE) for applying common metric

** Romanian biological data were delivered as abundances (cells/ml) and most data as calculated taxa biovolumes for applying common metric

*** Slovak biological data without any taxa biovolumes so common metric could not be applied on Slovak data (see option 3)

B.1.2: Biological data

The biological data included the taxonomic composition and abundance of phytoplankton communities sampled and processed according to national standards. Abundance of total phytoplankton was measured as concentration of chlorophyll a by photometric extinction method (ISO norm). Samples were taken between 1996 and 2014. Depending on national assessment method to be intercalibrated The countries assessed each biological sample they provided or deliver the annual assessment result, delivering an Ecological Quality Ratio score (EQR) according to. The EQRs were all normalized to 0-1 with 0.2 class steps.

We used the sampling data on chlorophyll a (chl_a) and of taxonomic composition of phytoplankton to calculate more than 10 biological assessment metrics, including biomass metric (chl_a), percentage of different algal classes (e.g. chlorophytes, cyanobacteria, bacillariophytes), functional group metric (e.g. Borics et al. 2007, Q index) trophic taxa indicator metrics (DE_TIP Mischke et al. 2011; PL_IT).

Metric calculation was done in an internal database (IGB 2016) and followed the algorithms and taxonomic information: a) HU Q-metric programmed by the Hungarian XGIG-experts b) all other metrics by GIG-group lead, while metric TIP calculated external in the Phytofluss software (Version 2.2; 3.0, 4.0; IGB) after taxa translation to German taxa code.

The following figure provides an overview of used station except of 5 further Slovak stations provided for option 3.

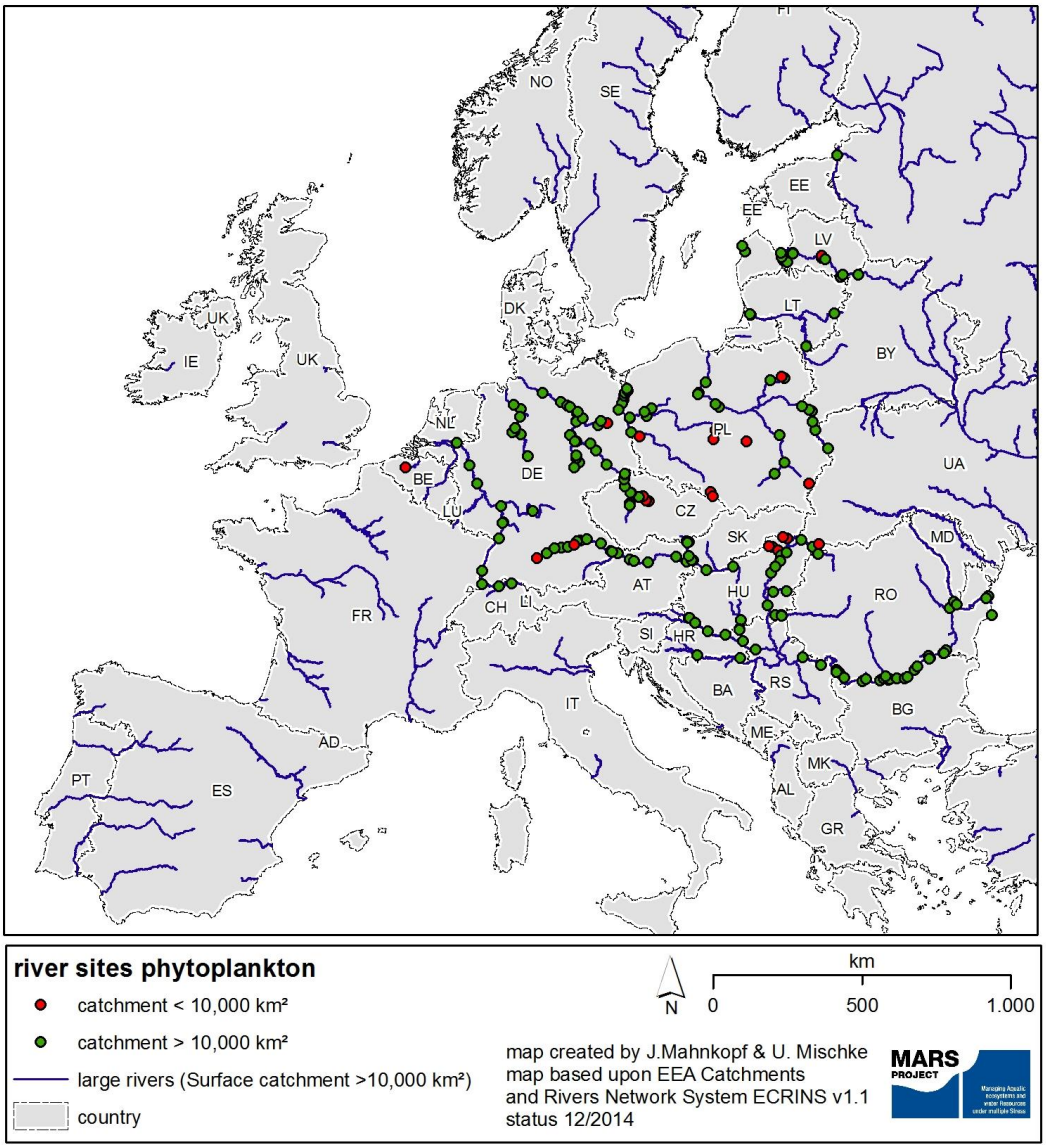


Figure B1: Location of the 196 sites from which the phytoplankton data used in the intercalibration exercise were acquired (red dots: sites with catchment area smaller than 10,000km2; green dots: sites with catchment area smaller than 10,000km2).

B.1.3: Supporting data

Environmental data

Environmental data provided by the countries included river name, national river type, name of water body and sampling site, altitude, upstream catchment area and location (latitude, longitude) of sampling site, ecoregion, alkalinity type, flow regime and discharge.

River sub-types for phytoplankton

The XGIG expert group found out that the response of chlorophyll a is different in the sub-type "high run-off" and "low run-off" when analyzing with the pooled XGIG data (see figure Annex_III_1) depending on that the catchment area specific run-off is below or higher than 10 l s^{-1} km^{-2}.

The sub-type "low run-off" and "high run-off" were defined as IC river types, which were used for benchmarking the single metrics for the common metric as random effect combined with country. The

various national river types (see chapter A.3.4) were agreed to be best summarized by this two IC river types.

Pressure data

Pressure data used to quantify the anthropogenic stressors acting at the sampling site as pollution by nutrients (P, N) and by organic substances causing secondary effects (oxygen) and information about hydromorphological parameters (dams upstream etc.) for checking abiotic selected benchmark sites.

Physio-chemical parameter values were derived by common spectrophotometer methods and hydro-morphological parameters from national expert judgement.

A list of physico-chemical pressures was provided as annual average water concentrations of nitrate-N, ammonia-N, total N, total P, orthophosphate-P, chloride and oxygen for selection of a pressure scale.

B.1.4: Data analysis

Definitions

The Intercalibration Common Metric (CM12b, Buffagni et al. 2005[4]) is a combination of single biological common metrics widely applicable across large rivers in Europe, which can be used to derive comparable information among different countries.

The Combined stressor index quantifies the level of various anthropogenic stressors acting at the sampling site/water body across different countries.

Benchmark standardisation (Birk et al. 2013[5], Böhmer et al. 2016, Poikane et al. 2015[6]) identifies and removes differences among national biological data that are not caused by anthropogenic pressure but by systematic discrepancies due to different methodology, biogeography, typology etc. If such differences are ignored they may have an overriding effect on the comparability exercise. In this exercise, we applied "continuous benchmarking" to (1) the single common metrics with the pressure index as covariate and country- river sub-type as random factor, and to (2) the national EQRs with CM12b as covariate and country as random factor (see Annex IV, VI & VII).

Normalisation transforms each benchmark standardized metric in order to get values between 0 and 1 for averaging into a multimetric index. In order to calculate normalized metrics, anchor points are defined for each metric. They are defined as minimum-maximum of the whole range of all countries (metric TIP, metric Q, national EWRs) or as 10th and 90th percentile (chlorophyll a).

Example:

- The benchmarked values of a metric range from 15.4 (worst condition) to 39.1 (best condition).
- The 10th percentile = 19.0; it corresponds to the value 0 for the standardized metric value.
- The 90th percentile = 33.4; it corresponds to the value 1 for the standardized metric value.

If a metric reacts in the opposite way (high values = bad, low values = good), the percentiles must be set the other way round.

4 Buffagni, A., Erba, S., Birk, S., Cazzola, M., Feld, C. (2005) Towards European Inter-calibration for the Water Framework Directive: procedures and examples for different river types from the E.C. project STAR. Quad. Ist. Ric. Acque 123: 1-467.

5 Birk, S., Willby, N.J., Kelly, M., Bonne, W., Borja, A., Poikane, S. & W. v. d. Bund (2013) Intercalibrating classifications of ecological status: Europe's quest for common management objectives for aquatic ecosystems. Science of The Total Environment, 454-455: 490– 499.

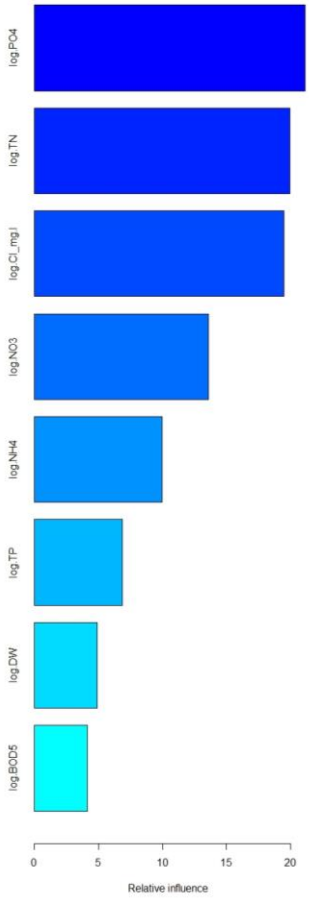
6 Poikane, S., Birk, S., Böhmer, J., Carvalho, L., Hoyos, C. De, Gassner, H., Hellsten, S., Kelly, M., Lyche Solheim, A., Olin, M., Pall, K., Phillips, G., Portielje, R. Ritterbusch, D., Sandin, L., Schartau, A.-K., Solimini, A.G., v. d. Berg, M., Wolfram, G. & W. v. d. Bund (2015) A hitchhiker's guide to European lake ecological assessment and intercalibration. Ecological Indicators, 52: 533–544.

B.1.5: Selection of a common pressure scale for IC

At a first step the single stressor parameter total phosphor (TP; mg/L) was tested. TP is the main pressure for phytoplankton in lakes, and therefore IC exercises in all GIG-lake-groups were carried out with TP as the only pressure.

In contrast, in river IC exercise the relationship of TP to most of the national EQRs are weak or not significant correlated when restricting to very large river sites (see chapter A.2).

A common pressure scale is used for benchmarking the common metrics, but its sensitivity to pressure is the pre-requisite. To derive a common metric, a series of single biological metric candidates were tested for TP response. Single metric candidates were chlorophyll a and taxonomic compositions metrics applicable to most of the XGIG data, such as proportion of algal classes, the functional metric Q from the Hungarian method and trophic indicator indices as the Polish and German single metric. Again all metrics are weak or not significant correlated to TP.



In a second step the most influencing factors on the responding variable chlorophyll a were detected with the optimized linear mixed model, which was established by performing cross-validation optimisation of a boosted regression tree model according library packages gbm and dismo in R; for further explaintions see Feld et al. (2016[7]).

1650 iterations with learning rate 0.03 were chosen to train the model with all chemical pressure variables available as annual means at 196 sites in the XGIG data and with chlorophyll a as the response variable. Log-transformed values were used for parameters TP, PO_4-P, NO_3-N (nitrate), NH_4-N (ammonia), TN (total nitrogen), BOD_5 (biological oxygen demand in 5 days), CL (chloride) and DW (dry matter).

Figure B2: Relative influence of chemical stressor parameters on responding chlorophyll a in very large rivers in the glm- model.

The ranking of relative influence (cv correlation = 0.814 ; se = 0.017*)*

var	rel.inf
log.PO4	21.148707
log.TN	19.930494
log.Cl_mg.l	19.489512
log.NO3	13.586792
log.NH4	9.972984
log.TP	6.877583
log.DW	4.887213
log.BOD5	4.106713

It was concluded, that beside TP also nitrogen components and chloride are acting as important explanatory parameters, when predicting chl_a with model. Taking into account completeness of parameter values in the XGIG data base and parameters used by countries to derive the assessment methods, TP, TN and chloride were finally chosen to create the combined stressor (Lelystad meeting March 2016). Each chemical parameter was normalized to Min-Max value within the global XGIG data and chloride was log-transformed before normalisation.

Normalized values of TP, TN and logCl resulting into 3 single stressor indices (each operating 0-1) which were additively merged to combined stressor index used for phytoplankton intercalibration.

[7] Feld, C.K., Segurado, P., Gutiérrez-Cánovas C, 2016: Analysing the impact of multiple stressors in aquatic biomonitoring data: A 'cookbook' with applications in R, Sci Total Environ. http://dx.doi.org/10.1016/j.scitotenv.2016.06.243

B.1.6: IC common metric phytoplankton for very large rivers

Describe the IC Common metric:

The IC common metric for phytoplankton CM12b) was developed and used for continuous benchmarking in option 2 following the report of the benthic invertebrates IC exercise for Very Large Rivers (Birk et al. 2016).

The IC common metric (CM12b) is the average of 3 benchmarked and normalised metrics: Chlorophyll a, the metric TIP and the metric Q. Each single metric was benchmark standardized to remove country and river type differences using linear regressions derived from linear mixed models with country – type (country combined with one of the IC river types) as a random effect (see Annex IV). The performance of the common metric was checked by linear regression with the combined stressor, a surrogate for pressure (Annex VIII; $r^2 = 0.567$).

Selection of single metrics for common metric

Metric chlorophyll a (chl_a) and 6 biological assessment metrics for taxonomic composition were tested for significant correlation to combined stressor scale: algal classes (% chlorophytes, % cyanobacteria, % bacillariophytes), functional group metric (e.g. Borics et al. 2007[8], Q index) trophic taxa indicator metrics (DE_TIP Mischke et al. 2011[9]; Polish PL_IT). Each of the single metrics were averaged for vegetation period (April-October) and were normalized to the minimum-maximum-range of the global XGIG data, except of chlorophyll a, which was normalized to the 10%-90%-percentiles (norm_chla).

Arithmetic averaged indices of norm_chl_a, norm_TIP and norm_Q index correlated the most to combined stressor (see Annex VIII).

Main preconditions/criteria for selecting the final combined stressor and CM12b were:

- -a correlation of r≥0.5 with the national EQRs for all countries
- a sufficient coverage of the status gradient (spanning at least 50% of the full ecological status gradient);
- best whole dataset correlation with single national EQRs amongst the CM variants
- best whole dataset correlation with the combined stressor amongst the CM variants

The national delegates (Annex V) were involved in all process steps, partly by providing dynamic data spreadsheets allowing them to individually test stressor and common metric variants.

The partial regressions of combinations between combined stressor, CM12b and national EQR are shown in Annex VIII.

When splitting the data in the two IC river sub-types the coverage of the status gradient was not sufficient anymore, and do not spanning at least 50% of the full ecological status gradient. Therefore, the XGIG group decided to pool all data and remove random effect of IC sub-type-country by offset correction (see Annex VI) of each single metric for common metric (CM12b).

Reflection of indicative parameters by common metric

Normalized chlorophyll a concentration (norm_chla) reflects abundance of phytoplankton and also algal blooms, if measured at least 6 times in vegetation period (see table B3).

The normalized index TIP r(norm_TIP) reflects the taxonomic composition scores for trophic gradient with TP.

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8 Borics, G., Várbiró, G., Grigorsky, I., Krasznai, E., Szabo, S. & Kiss, K. T. 2007. A new evaluation technique of potamo-plankton for the assessment of the ecological status of rivers. Arch. Hydrobiol. Suppl., 161(3.4), 465.486

9 Mischke, U., Venohr, M. and H. Behrendt (2011): Using Phytoplankton to Assess the Trophic Status of German Rivers. International Revue of Hydrobiology 96 (5): 578-598

The normalized annually averaged Q-metric (norm_Q_avg) reflects the taxonomic composition grouped in functional groups and also indicates secondary effects by sensitive functional groups for organic pollution.

Table B3: Single common metrics composing the CM12b, including the assignment of indicative parameters

(Annex V, WFD)
ABD: Abundance; BLOOM: algal bloom; TAX: Taxonomic composition; SecE: secondary effects

Metric name	*ABD*	*BLOOM*	*TAX*	*SecE*
Norm_chl_a	*X*	*(X)*		
Norm_TIP			*X*	
Norm_Q_avg			*X*	*X*

B.1.7: Benchmark standardisation of national EQRs

Using the same approach to benchmark standardise the single common metrics of the CM12b, we benchmark standardised the national EQRs per country against the final CM12b (see Annex VII for the resulting offsets). This allowed us to combine all national datasets and EQR scores into a global regression analysis without the influence of national differences in EQR-CM12b relationships or class boundary setting. This approach follows the IC exercise for benthic invertebrates outlined in Böhmer et al. 2016.

The global regression enabled the integration of national classifications that do not fulfil the data quality criteria due to lacking gradients in ecological status or low number of relevant water bodies. In case of the phytoplankton exercise here, the following countries had narrow stressor gradients and a low number of sites (Belgium-Flanders, Bulgaria, Estonia, Latvia, Lithuania, Romania, and Czech Republic for sub-data set with taxa biovolumes).

Boundary comparison

Intercalibration Excel Template Sheet for IC Option 2 (version 1.24) was used to compare the national class boundaries of ecological status (see Birk et al. 2011 for documentation). A spreadsheet with extended capacity for data import was used provided by the benthic invertebrates group in LR-XGIG.

Partial regressions within type- and pressure-groups

We analysed the relationships between phytoplankton CM12b, national EQRs and pressures separately within different groups of IC river types and pressures. The typological groups were devised on the basis of preliminary analyses done with German very large rivers (Mischke et al. 2011[9]) and with XGIG data (see Annex III) and covered two very large river types. All stressor parameters belong to pressure group pollution/nutrients and were checked for single and combined effect on CM12b and on national EQR. Annex VIII summarises the outcomes of these analysis.

The data of the German rivers “Neckar” and “Saale” were excluded from the regressions between combined stressor to common metric because they were identified as extreme outliers: CM12b results were much too relaxed compared to the high stressure level acting as these sites. Both rivers are characterized by cascades of upstream- dams.

B.1.8: Results of IC exercise option 2

In option 2 all countries listed in A.1 were included, except of Slovak method because the common metric was not applicable to Slovak phytoplankton data.

Austria and Bulgaria use the updated German method PhytoFluss version 4.0 in this exercise, which is indicated with "_2" in countries abbreviation.

Germany will eventually decide officially also to use the updated German method PhytoFluss version 4.0 until the end of January 2017, therefore the offset correction was alternatively done for resulting EQRs with DE_2 (see Annex VII) and a separate option 2 sheet was run to carry out the boundary comparison. If Germany will use alternatively DE_2 in future, the results of option 2 would be almost the same (shown in alternative table B4_b listed in Annex VII).

Boundaries of Estonian method were adjusted during the option 2 exercise, because its G/M boundary turns out to be much more stringent than all other countries. Their original G/M boundary with EQR 0.75 would lead to an Estonian method G/M bias of class width of 2.6. For harmonisation Estonia decided to contribute an adjusted Estonian method (EE_adj) into the intercalibration exercise with H/G 0.8, G/M 0.65, M/P 0.45 and P/B 0.25.

Boundary comparison

Figure B3 presents the linear regression of the benchmark standardised national EQRs against the CM12b.

Figures B4 and B5 show the national boundary biases resulting from the boundary comparisons.

In Table B.4 all bias and boundaries are listed for each country and for high/good (H/G) and good/moderate (G/M) boundaries.

In Annex VIII the performance of the common metric (CM12b) against the combined pressure and also against the original EQRs of each countries are illustrated.

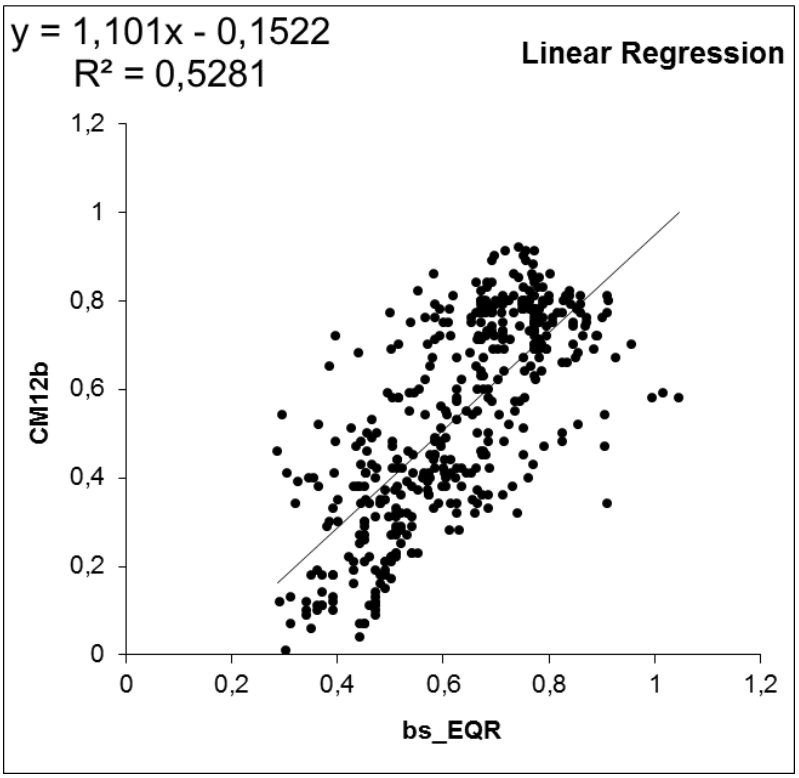

Figure B3: Linear regression of benchmark standardised national EQRs (bsEQR) against the Intercalibration Common Multimetric index (CM12b) including 457 annual averages for phytoplankton of 12 countries.

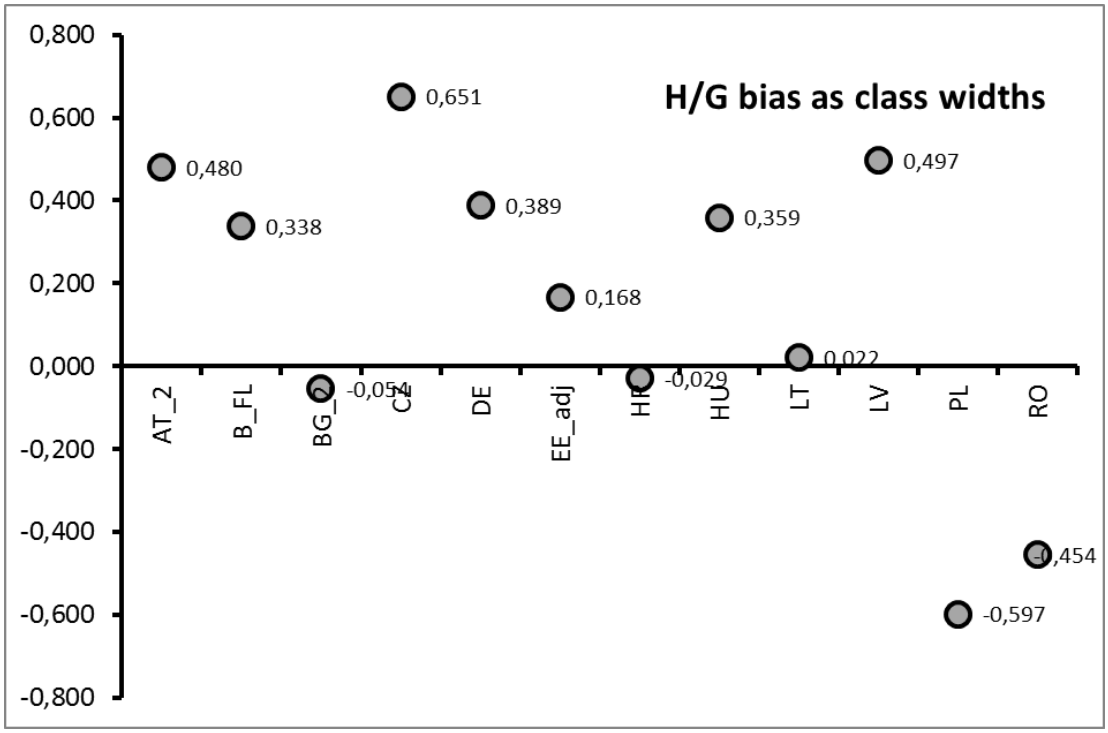

Figure B4: High-good boundary bias as class width (with Poland and Romania being too relaxed, thus requiring boundary adjustment).

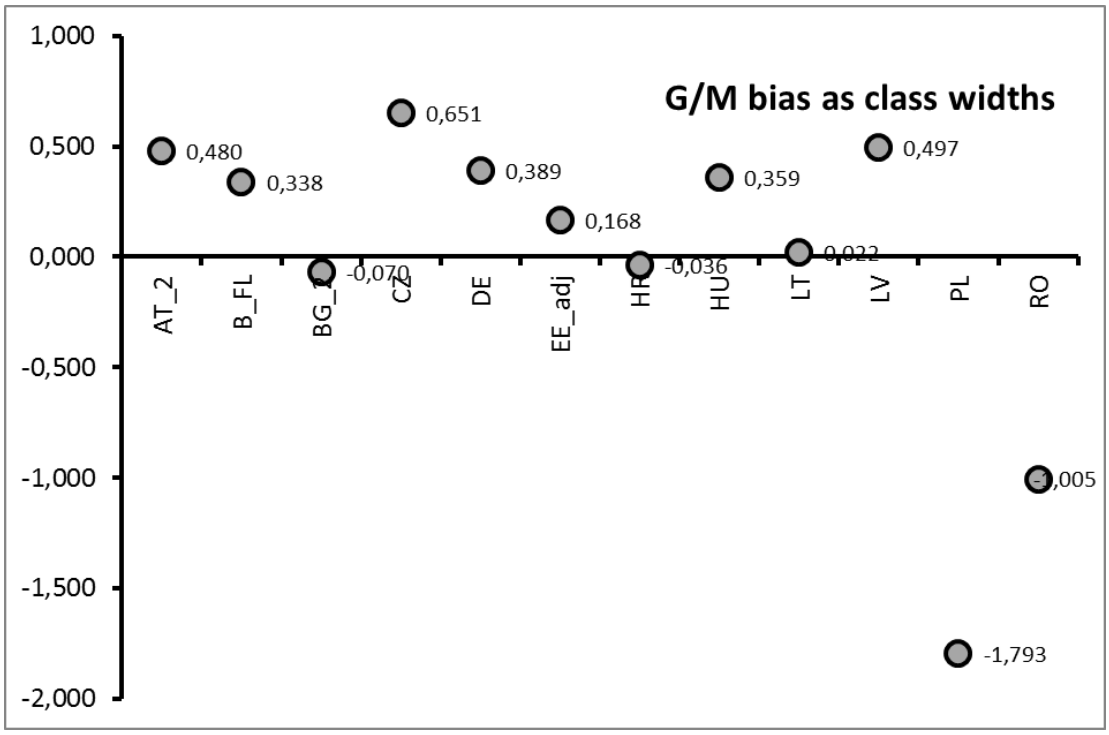

Figure B4: Good-moderate boundary bias as class width (with Poland and Romania being too relaxed, thus requiring boundary adjustment).

National boundary adjustments

Table B4 lists the national class boundaries, boundary bias and the proposed adjusted boundary values, if bias falls below -0.25.

Table B4: National class boundaries, boundary biases and adjusted boundary values if bias falls below -0.25.

		original		adjusted	
country		H/G	G/M	H/G	G/M
AT_2	boundary	0,80	0,60		
AT_2	bias	0,480	0,480		
B_FL	boundary	0,80	0,60		
B_FL	bias	0,338	0,338		
BG_2	boundary	0,80	0,60		
BG_2	bias	-0,054	-0,070		
CZ	boundary	0,80	0,60		
CZ	bias	0,651	0,651		
DE	boundary	0,80	0,60		
DE	bias	0,389	0,389		
EE_adj	boundary	0,85	0,65		
EE_adj	bias	0,168	0,168		
HR	boundary	0,80	0,60		
HR	bias	-0,029	-0,036		
HU	boundary	0,80	0,60		
HU	bias	0,359	0,359		
LT	boundary	0,80	0,60		
LT	bias	0,022	0,022		
LV	boundary	0,80	0,60		
LV	bias	0,497	0,497		
PL*	boundary	0,80	0,60	1,08	0,92
PL*	bias	-0,597	-1,793	-0,249	-0,246
RO**	boundary	0,80	0,60	0,92	0,76
RO**	bias	-0,454	-1,005	-0,249	-0,246

*Poland agreed with this proposal to adjust their national class boundaries and adjust also the reference value, because the adjusted H/G boundary already surpasses 1.

**Romania agreed with this proposal to adjust their national class boundaries.

B.1.9: Characterisation of biological community

The biological community was characterized by increasing biomass (chlorophyll a and total biovolume) and a change of indicator taxa (TIP; single metric of common multi-metric CM12b) and functional traits (Q metric; single metric of multi-common metric CM12b) when comparing high, good and moderate status.

The taxa with the lowest trophic score according the metric TIP (Trophic Indicator taxa Potamoplankton; Mischke et al. 2011) for river group 20.1+20.1 (high run-off type) and 10.2+20.2 (low run-off type) are listed subsequently with increasing score to moderate status (Index value 2.5-3).

The functional trait groups with highest value for HU river type 4 and 5 are listed according Hungarian functional trait metric Q (Borics et al. 2007) and HU method modifications.

Additionally an analysis of the XGIG data were made, by classifying samples to status classes based on the common metric value and combined stressor level of this year and site benchmarked according the Global Metric View derived in option 2.

status	CM12b	combined stressor level
high	>0.7	<0.75
good	0.48-0.7	0.75-1.2
moderate	0.25-0.48	1.2-1.7

High status taxa characterisation

According the metric TIP (Trophic Indicator taxa Potamoplankton; Mischke et al. 2011) following species occur in high status rivers: Small chryso- & haptophytes including Kephyrion and Pseudokephyrion, Dinoflagellates such as Ceratium and Gymnodinium, various Fragilaria species including F. crotonensis Ulnaria, ulna var. acus, Amphora (excl. A. pediculus, ovalis), Cymatopleura elliptica, Cymatopleura solea.

According Hungarian functional trait metric Q (Borics et al. 2007) the high status is indicated by the functional group TIB with Nitzschia spp., Navicula, Gomphonema, Didymosphaenia, Fragilaria, Achnanthes, Surirella and can be accompanied by taxa mesotrophic conditions trait “B” such as Aulacoseira subarctica, A. islandica, Stephanodiscus neoastraea, S. rotula, Cyclotella comta.

In samples (N = 184) with a CM12b value higher than 0.7 and combined stressor index less than 0.75 benchmarked for high status class, the mean chlorophyll a concentration is 5,9µg/L. In total 478 taxa are found, from which 29 taxa are at least 25% of all samples (see table B5).

Good status taxa characterisation

According the metric TIP (Trophic Indicator taxa Potamoplankton; Mischke et al. 2011) following species occur in good status rivers besides those also found in high status: Diatoma vulgaris, Trachelomonas, Asterionella Formosa, Cryptomonas, Plagioselmis (Rhodomonas), Melosira varians, Oocystis, Staurastrum, Fragilaria ulna angustissima Fragilaria ulna, Rhoicosphenia.

According Hungarian functional trait metric Q (Borics et al. 2007) the good status is indicated by the functional groups “C” (Asterionella formosa, Aulacoseira ambigua, Stephanodiscus rotula, Cyclotella meneghiniana, C. stelligera), “A” (Urosolenia (Rhizosolenia), Cyclotella comensis, C..glomerata) and more seldom “E” (Dinobryon, Mallomonas, Synura), while eutropic taxa become more common (taxa of groups “T”, “P”, “Z”, “N”, Closterium, Staurastrum, Pediastrum, Coelastrum, Synechoccus, Tabellaria).

In samples (N = 288) with a CM12b values between 0.48 -0.7 and combined stressor index 0.75-1.2 benchmarked for good status class, the mean chlorophyll a concentration is 12,5µg/L. In total 447 taxa are found, from which 26 taxa are at least in 25% of all samples (table B5).

Moderate Status taxa characterisation

According the metric TIP (Trophic Indicator taxa Potamoplankton; Mischke et al. 2011) following species occur additionally in moderate status rivers: Euglena, Crucigenia, Crucigeniella, Skeletonema potamos, and various cyanobacteria such as Aphanizomenon, Microcystis, Planktothrix.

According Hungarian functional trait metric Q (Borics et al. 2007) the moderate status is indicated by the increased proportion of functional groups “X2” (Plagioselmis (Rhodomonas), Chrysochromulima), “W0” (Chlamydomonas, Spermatozopsis, Pyrobotrys, Chlorella, Polytoma, Oscillatoria chlorina), “W1” (Euglena, Phacus, Lepocinclis, Gonium pectorale, G. sociale(Pandorina morum), but also various chlorococcales in trait “J” and “F” occur (Scenedesmus, Tetrastrum, Crucigenia, Actinastrum, Botryococcus, Pseudosphaerocystis, Coenpchlorys, Oocystis, Elakatothrix), and higher contribution by diatom taxa belonging to trait “D” (Ulnaria (Synedra) acus, Nitzschia, Stephanodiscus hanztschii, C. ocellata and C. pseudostelligera).

In samples (N = 571) with CM12b values between 0.25-0.48 -0.7 and combined stressor index 1.2-1.7, benchmarked for moderate status class, the mean chlorophyll a concentration is 30,9µg/L. In total 511 taxa are found, from which 26 taxa are at least in 25% of all samples (table B5).

Table B5: Mean chlorophyll a concentration and mean and maximum taxa biovolume and its proportional frequency of only those taxa found in at least 25% of all samples belonging to corresponding status class "high (blue), good (green), of moderate (yellow) within the XGIG data.

	mean biovol in high	max biovol in high	% in high status	mean biovol in good	max biovol good	% in good status	mean biovol in moderate	max biovol moderate	% in moderate status
chlorophyll a (µg/L) mean	5.9			12.5			30.9		
Taxon name									
Nitzschia acicularis	0,02	0,3	58	0,17	4,3	78	0,07	1,96	80
Stephanodiscus hantzschii	0,15	2,4	47	1,19	19,6	44			
Diatoma vulgaris	0,16	1,7	46	0,11	1,9	29			
Cryptomonas sp.	0,04	0,5	46	0,07	0,8	36	0,11	1,63	65
Cryptomonas ovata				0,09	1,0	27			
Cryptomonas erosa							0,15	2,12	32
Nitzschia sp.	0,02	0,2	45	0,05	0,6	56	0,04	0,58	60
Navicula lanceolata	0,12	1,2	43	0,03	0,4	31	0,14	1,15	31
Navicula sp.	0,01	0,1	43	0,01	0,3	27	0,04	0,67	38
Chlamydomonas sp.	0,02	0,1	42	0,07	0,8	36			
Stephanodiscus minutulus	0,06	0,7	40	0,37	5,3	33			
Stephanodiscus hantzschii				1,19	19,6	44			
Asterionella formosa	0,01	0,1	39	0,12	2,74	34	0,05	0,78	60
Melosira varians	0,12	2,2	39	0,20	8,1	29	0,12	1,06	38
Fragilaria crotonensis	0,03	0,6	35						
Ulnaria ulna	0,09	0,8	34	0,53	26,5	40	0,15	3,09	31
Ulnaria acus	0,04	0,4	34	0,10	2,1	42	0,07	1,57	49
Cocconeis placentula	0,06	0,8	30						
Plagioselmis lacustris	0,01	0,1	30						
Monoraphidium contortum	0,00	0,0	29	0,04	0,5	68	0,02	1,10	44
Monoraphidium griffithii				0,01	0,1	30			
Monoraphidium arcuatum				0,01	0,1	28			
Monoraphidium sp.							0,02	0,68	44
Scenedesmus quadricauda	0,02	0,1	28	0,07	1,0	56	0,04	2,45	54
Scenedesmus sp.	0,01	0,1	28				0,13	1,89	60
Scenedesmus acuminatus				0,04	0,4	37	0,06	1,46	38
Scenedesmus intermedius				0,01	0,1	33			
Cyclotella meneghiniana	0,12	0,9	27	2,23	48,3	72	1,14	46,11	42
Plagioselmis nannoplanctica	0,02	0,3	27						
Nitzschia palea	0,01	0,1	27	0,04	1,3	33			
Rhodomonas sp.	0,10	1,0	26				0,04	0,70	38
Skeletonema potamos	0,09	1,6	25	0,46	8,3	33	0,31	5,15	32
Navicula tripunctata	0,04	0,3	25						
Cryptomonas marssonii	0,02	0,1	25						
Gomphonema sp.	0,00	0,1	25						
Chlamydomonas sp.				0,12	1,5	29	0,07	0,88	36
Chrysococcus rufescens				0,02	0,21	32			
Aulacoseira granulata							0,20	5,67	57
Aulacoseira sp.							0,25	16,20	39
Planktothrix agardhii							0,15	2,72	44
Pseudanabaena sp.							0,03	0,74	35
Tetrastrum staurogeniaeforme							0,03	0,24	43
Chlorococcales sp.							0,33	13,79	38
Chrysophyceae sp.							0,03	1,48	36
Centrales sp.							4,20	64,16	43
Cyclostephanos dubius							0,32	10,60	39

B.2. Intercalibration exercise (option 3)

B.2.1. Option 3 exercise to compare Slovak phytoplankton method

Comparison of methods and boundaries

IC Option and Common Metrics

Explanation for the choice of the IC option:

Common metric could not be applied on SK data, so the national metric was applied to other MS data after benchmark standardisation (Option 3).

B.2.1.1. Strategy of option 3

In case of the Slovak data the common metric was not applicable (missing taxa biovolumes and missing taxa determination on a least genus level), so option 3 was used.

Option 3 was carried in the following steps:

1) Check of the sensitivity of method against pressure as a pre-exquisite.
2) Supporting information: Direct class comparison of SK-EQR to other intercalibrated methods (HU, AT) of nearby Danube sites with abundance data.
3) Supporting information: Direct class comparison of sites evaluated with SK-EQR and with a metric using anthropogenic pressure (CM_abiotic).
4) Comparison of class boundaries of SK-EQR to levels of anthropogenic pressure (CM_abiotic) corresponding to H/G and G/M (established in option 2)

The option 3 exercise uses a pressure index as the common metric (CM_abiotic), as was done for intercalibrating fish assessments in transitional waters (see Lepage et al. 2016)[10]. The pressure index was developed and agreed in the option_2 procedure for phytoplankton methods, named “combined stressor”, and includes the stressor parameters P-, N-concentrations and chloride (see chapter B.1.5). Ecological class boundaries values were established according the Global Mean View (GMV) taken over from option 2 results including 12 countries with continuous benchmarking (regressions to stressor scale).

B.2.1.2. Check of sensitivity of SK method against combined pressure

The sensitivity of the SK method was demonstrated within the country report (chapter A.2) by linear regression of national EQR to pressure, Total Phosphorus (TP; N=28 annual averages; $r^2 = 0.33$).

In addition to the pressure parameter TP, the XGIG decided to include normalized values of total nitrogen (TN) and chloride (log CL) to produce a combined stressor (chapter B.1.5) which reflect more complete potential anthropogenic pressures. This combined stressor was used to select and judge the performance of a common metric (chapter B.1.6). Therefore, it was needed to check the sensitivity of the SK method also against the combined stressor.

For Slovak data provided in the XGIG data base (figure B_2-1), the EQR of Slovak method correlates to combined stressor with a regression coefficient of $r^2 = 0.30$ and Pearson R of 0,552.

In conclusion: SK method is sufficient sensitive according common view based on combined stressor.

[10] Lepage M., Harrison T., Breine J., Cabral H., Coates S., Galván C., García P., Jager Z., Kelly F., Mosch E. C., Pasquaud S., Scholle J., Uriarte A., Borja A.(2016): An approach to intercalibrate ecological classification tools using fish in transitional water of the North East Atlantic. Ecological Indicators 67: 318–327

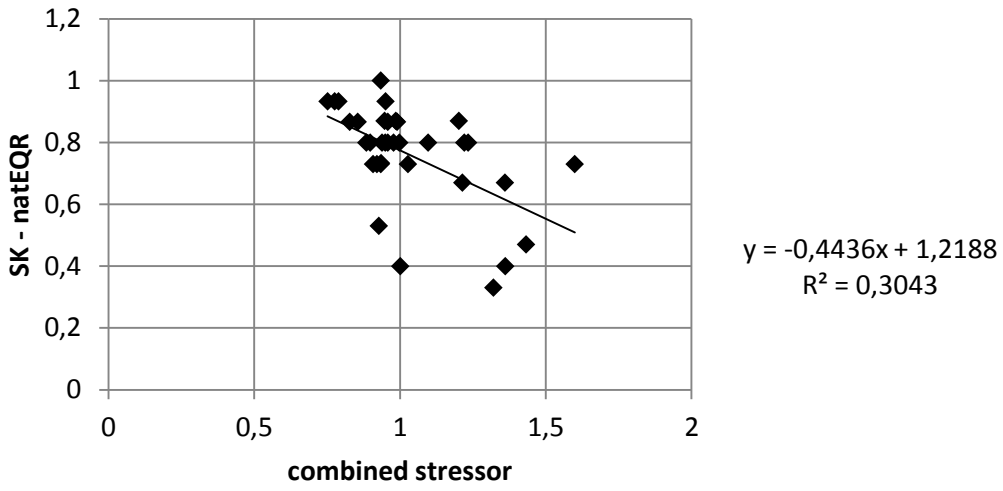

Figure B_2-1: National EQRs for Slovak method on their own data against combined stressor of TP, TN and logCl.

B.2.1.3. Direct class comparison of SK-EQR to other methods

Introduction

Direct class comparison of SK-EQR was carried out to other successful intercalibrated methods (HU, AT) of nearby Danube sites with abundance data as a supporting information.

There are two reasons for that the direct class comparison to other methods are used only as a supporting information to the second approach with continuous benchmarking (SK-EQR to common abiotic metric with class boundaries according Global Metric View; see next chapter).

There is only a small number of available data pairs to other countries.

The range of the combined stressor is very narrow in this Danube sub-data set (pressure index range is 0.64-1.1), while the total XGIG phytoplankton data span a much wider range in stressor index (0.26-2.2).

Neighbouring Danube sites were used, because SK method was developed for sites from this region, so less country random effect is expected. Taxa abundance data were needed to apply the SK-method, and were not available for all countries data.

SK to HU class comparison

To compare classification between SK and HU, 28 annual averages from Hungarian Danube sites were available. In conclusion, the boundaries for H/G of SK method are stricter, and for G/M the same as HU-method (figure B_2-2).

For IC exercise the classes above G/M are not directly relevant. M/P and P/B of SK method appear to be more relaxed, because out of the 10 as “moderate” classified values were classified as “poor” (N=4) or classified as “bad” (N=2) with the HU methods.

It is notable that all 5 status classes are covered with HU method although the corresponding combined stressor range is very narrow (0.64-1.1). While the combined stressor is based on nutrient and chemical status (P, TN and Chloride) the HU metrics are developed also to react on hydro morphological pressures (e.g. dams) by the increased share of functional groups belonging to lake plankton. A functional trait metric is not included in the SK-method.

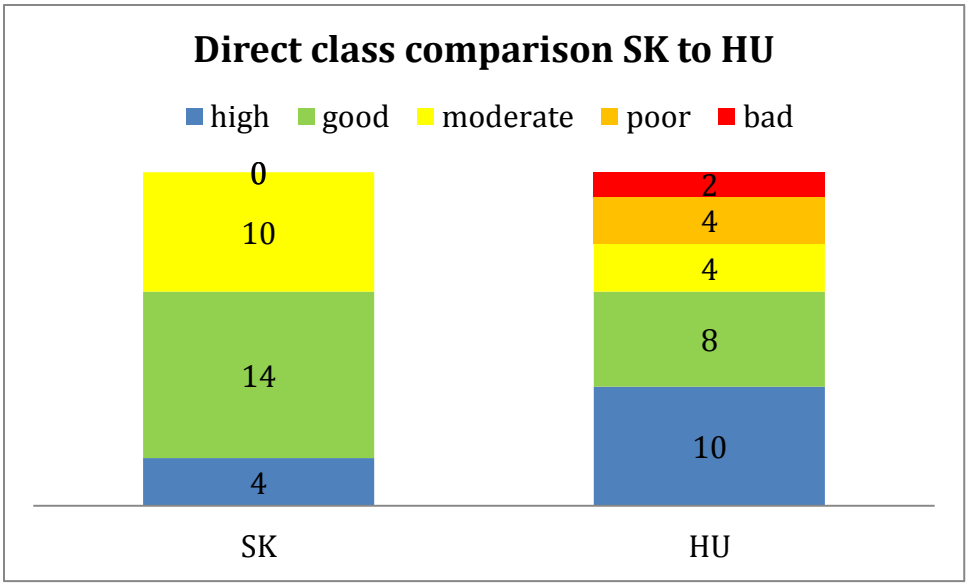

Figure B_2-2: Classification of Slovak method applied on data of HU sites compared to classification of HU method.

SK to AT_2 boundaries

To compare classification between SK and AT_2, 26 annual averages from Austrian Danube sites were available (figure B_2-3).

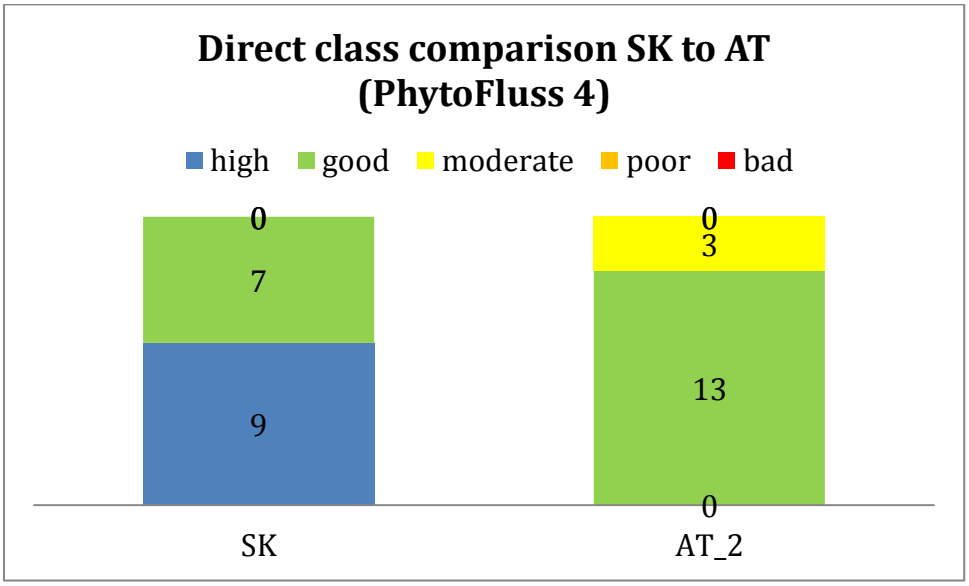

Figure B_2-3: Classification of Slovak method applied on data of AT sites compared to classification of AT_2-method.

In conclusion, the boundary for H/G of SK method is much more relaxed, and for G/M it is slightly more relaxed as the AT_2-method. Still, in accordance to each other, most of the site-year-averages were classified better than moderate with both methods.

SK to RO boundaries

The ECO-FITO (RO method before adjustment) was not successfully intercalibrated, because boundaries were too relaxed. Direct status class comparison to Phytoplankton-SK of 78 samples of RO from Danube River revealed that SK method is more stringent at least for H/G boundary (not shown here).

B.2.1.4. Comparison of class boundaries of SK-EQR to CM_abiotic supported with direct class comparison

The combined stressor index is used as the abiotic common metric to compare the position of the class boundaries to those of the SK method.

In the first step of "direct class comparison", all sites assessed by the SK-method were also classified by the abiotic common metric (CM_abiotic). Note that stressor range is too narrow in the Danube data sub-set to cover all status classes with direct class comparison, and CM_biotic (CM12b) scatter strongly in this stressor range.

With the aim to cover the full range of stress found in global XGIG data and to compare with the Global Metric View for class boundary position gained in option 2 exercises, continuous benchmarking to common metric is used.

Class bias at H/G and G/M-boundaries can be derived by using continuous benchmarking, when compared EQRs are both normalized with equal distant class width (e.g. H/G 0.8, G/M 0.6, 0.4, 0.2) and the same level of stress is regarded at each class boundaries. Data transformations are described in the following chapter.

Data base and CM-EQR benchmarking and normalisation

Data set: Indices for common metric and for combined stressor were available for 105 Danube sites from countries AT, HU, RO, which were assessed also with the SK-method, using abundance data and chlorophyll_a.

Additionally there are 35 years of investigation with SK-EQR for 7 SK-sites, for which the stress level can be calculated according the combined stressor index.

Indices for common metric based on chlorophyll a and biovolume data at station and year were classified according the Global Metric View (GMV) gained by the averaged view of all other 12 countries participating to the option 2. This CM classification was used for direct class comparison with SK-method.

Indices for common metric based on combined stressor indices at station and year were calculated using the global XGIG equation between CM12b to stressor gained in the option 2 exercises:

CM12b = -0.4557 *comb. Stressor + 1.0419.

Position of H/G and G/M-boundaries in biological common metric: The biological common metric (CM12b) was benchmark standardized by the Global Metric View (GMV) gained by the averaged view of all other 12 countries participating to the option 2. A piece-wise normalization was not necessary because of almost equal distance between Max to H/G (diff = 0.266) and between H/G to G/M (diff = 0.22) of the GMV boundaries.

Stressor level in abiotic common metric at GMV: To establish class boundaries in the abiotic common metric (combined stressor) the reciprocal global XGIG equation between CM12b to stressor is used to find the corresponding stress level.

Combined stressor = -2.1944 * CM12b + 2.2864

Results at class boundaries are shown in table B_2-1.

Predicting SK-EQR at same stressor level: Index values of combined stressor at the class boundaries were linked to the corresponding SK-EQR values. The SK-EQR at same level of stress were gained by using the linear regression equation between combined stressor and SK-EQR (y = -0.4235 x + 1.1877) based on the 105 cases for Danube sites of other countries plus 35 cases for Slovak sites (see figure B_2-4).

Please note that only few data of SK sites fall into the range of moderate abiotic status.

An illustration of the calculation steps described above are shown in figure B_1.9-5.

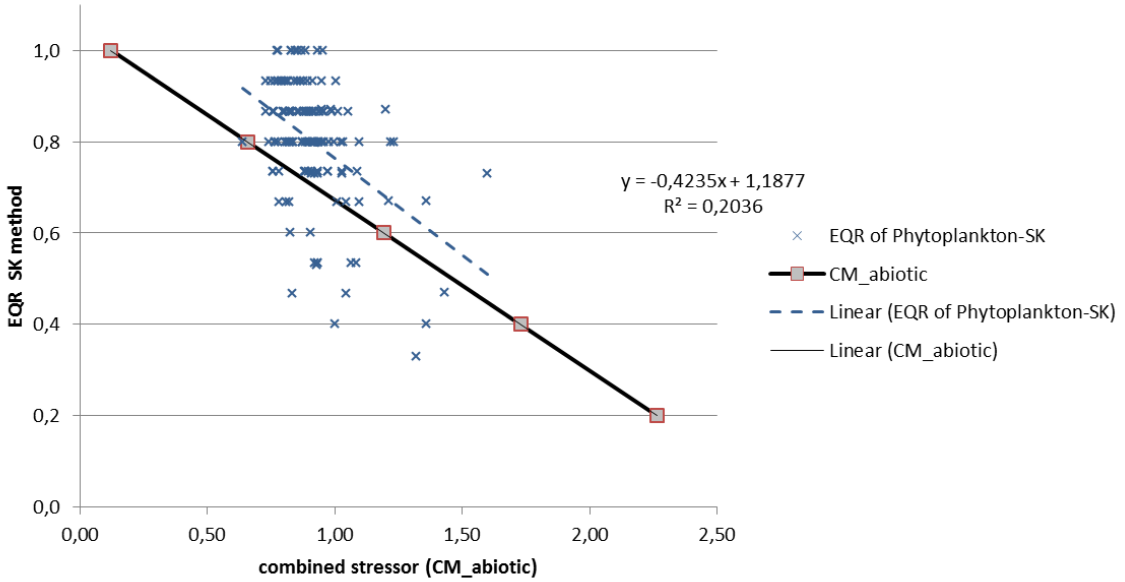

Figure B_2-4: Position of class boundaries (red box symbols) of abiotic common metric (CM_abiotic) against combined stressor according the Global Mean View compared to Slovak EQR for all evaluated sites (including data of SK sites) and its linear regression. A linear regression line (CM_abiotic) can be fitted to the position of H/G is at EQR of 0.8 and G/M boundary at 0.6 to which the SK-bias is redrawn from direct EQR difference (see table B_2-1).

Result of direct class comparison SK-EQR to CM

To compare classification between SK and biological CM (CM12b), 105 classified years from sites of RO, AT and HU were available (figure B_2-5).

The common abiotic metric (CM_abiotic) classify the same 105 years form Danube sites of other countries as "good" status.

Note that stressor range is narrow in this data sub-set (all good abiotic range). Additionally the Danube data sub-set may differ systematically form the Global XGIG data set.

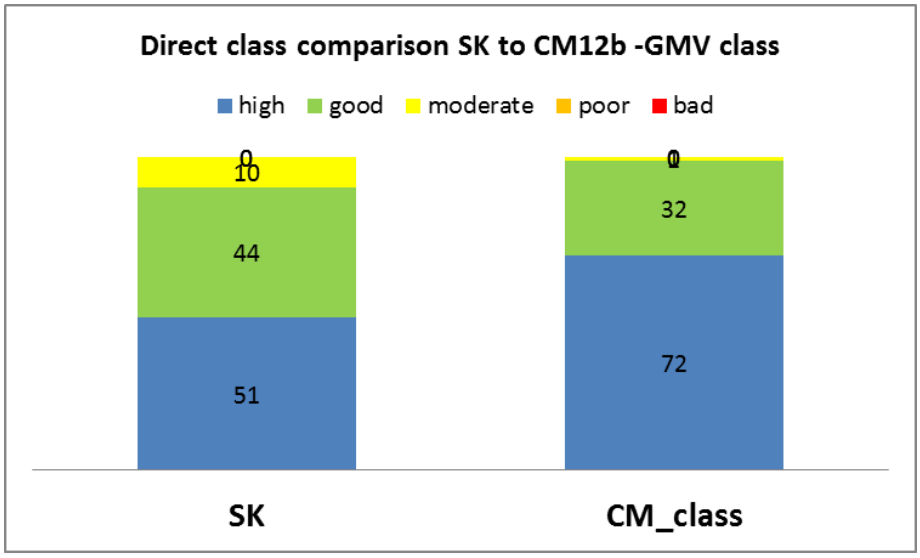

Figure B_2-5: Distribution of status classes according Slovak method compared to CM12b according the Global Mean View for class boundaries gained in option 2 exercises.

In conclusion, the classification with SK method is slightly stricter than the classification according the common metric (see also table B_2-2).

Boundary bias between SK-EQR to CM12b by continuous benchmarking

Comparison of class boundaries of SK-EQR to CM12b boundary according Global Metric View (GMV) were carried out with continuous benchmarking (regressions to stressor scale), spanning the full global stressor range in the XGIG data base. An illustration is shown in figure B_2-4 and data transformation is described before. EQRs are both normalized with equal distant class width (e.g. H/G 0.8, G/M 0.6, 0.4, 0.2) and compared at the same level of stressor. All calculation results are in table B_2-1.

The SK method is sensitive to the pressure with a similar slope as the abiotic common metric but with a different offset (less stringent).

As a final step the distance (SK bias) were calculated between normalized common metric (normCM) to SK-EQR. This was done by subtraction (SK bias = normCM – SK_EQR) at the certain stressor level corresponding to H/G (normCM = 0.8) and G/M (normCM = 0.6) of the Global Metric View class boundaries.

The SK-EQR differs with a bias of -0.10 from normalized common metric at H/G boundary.

The SK-EQR differs with a bias of -0.10 from normalized common metric for G/M boundary.

In conclusion of option 3 exercise for Slovak method, the method is intercalibrated. The bias is in the range of +-0.25 bias band.

Table B_2-1: Corresponding indices of combined stressor and Slovak EQR to the fixed boundary positions of Common metric (CM12b) gained in the option 2 exercise as the Global Mean View (GMV). These boundaries equal to a certain stress level (CM_abiotic) and to SK-EQRs which are derived by linear regression (figure B_2.4). The SK bias is provided for each class boundary, and is result of SK_EQR subtracted from normCM at same stressor level.

Status boundaries	combined stressor level at GMV = CM_abiotic	Biological CM12b acc. GMV	normCM	SK-EQR at same combined stressor level	SK bias
Ref	0,093	1,000	1,00	1,148	-0,148
H/G	**0,677**	**0,733**	**0,80**	**0,901**	**-0,101**
G/M	**1,160**	**0,513**	**0,60**	**0,696**	**-0,096**
M/P	1,644	0,293	0,40	0,492	-0,092
P/B	2,127	0,073	0,20	0,287	-0,087

Remark on uncertainty of prediction:

The correlation between combined stressor (CM_abiotic) and biological common metric (CM12b) is highly significant (r^2 = 0.567; p-value = 0.001; see Annex VIII), therefore the class boundaries in the scale of the CM12b, which are established in the option 2 exercise, can be transferred to the scale of the CM_abiotic by regression. Still, the relationship, fitted to a linear model, has itself a high global standard deviation of +- 0.25. Therefore the ecological status (biological CM) can be predicted from the combined stressor level only with high uncertainty, and so the position of class boundaries.

For illustration of the uncertainty, the CM12b normalized with boundaries of GMV (EQR_CM12b) and the EQR results for all years assessed with EQR-SK in parallel were plotted in boxplots (figure B_2-6). Both biological assessment methods assess the data set mainly on the border between high and good states, while the abiotic CM assessed all as “good”.

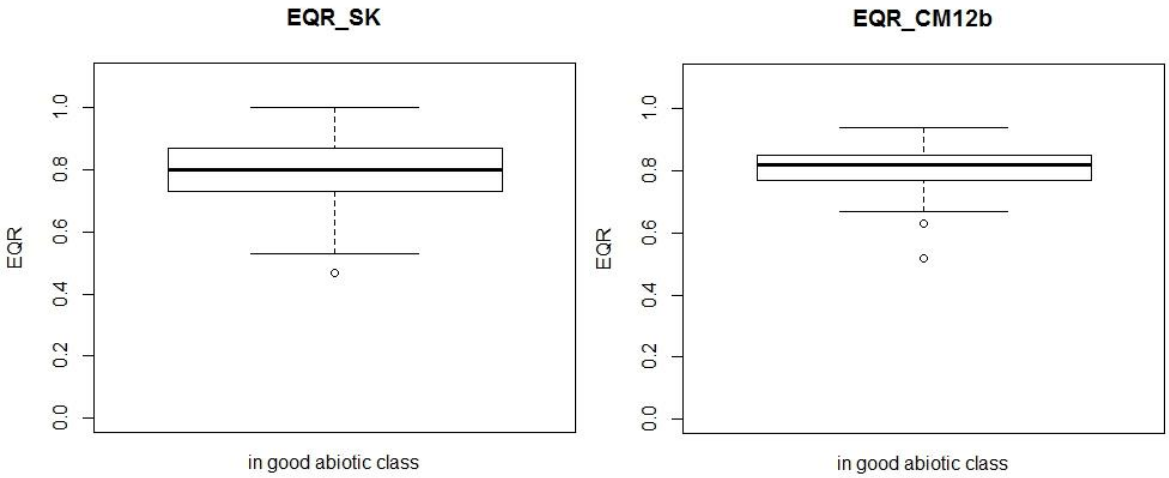

Figure B_2-6: Distribution of metric values of SK method (left boxplot) and of biotic common metric (right boxplot; normalized CM12b) for 105 years of investigation which are all in the combined stressor range enable "good" status (abiotic class status)

Table B_2-2: Statistic of values distribution for EQR and biological status class of the metrics SK_method and common metric (CM12b)

	EQR_SK	EQR_CM12b	class_SK	class_CM12b
Min.	0,470	0,520	1	1
1stQu.	0,730	0,770	1	1
Median	0,800	0,820	2	2
Mean	0,814	0,806	1,6	1,7
3rd Qu.	0,870	0,850	2	2
Max.	1,000	0,940	3	3

Annexes

Annex I: Completed questionnaires methods - phytoplankton

Annex I. 1: Austria, Bulgaria and Lithuania– method description in questionnaire

No questionnaire was filled-in by Austria, Bulgaria and Lithuania.

These MS apply the Phyto Fluss Index.

- see questionnaire from Germany and Annex II – D for a complete method description

Annex I. 2: Belgium (FL) – method description in questionnaire

Questions	Phytoplankton - very large rivers
A. General information	
A-01. Name of person completing this questionnaire	Jeroen Van Wichelen
A-02. E-mail adress	jeroen.vanwichelen@UGent.be
A-03. Institution	Ghent University
A-04. Full method name (dutch)	
A-05. Full method name (english)	German phytoplankton assessment method for rivers
A-06. Abbreviation of method	Phytofluss
A-07. EU member state	Flanders (Belgium)
A-08. BQE	Phytoplankton
A-09. Name and description of very large river type(s)	-
A-10. Has the pressure-impact relationship of the assessment method been tested at very large rivers?	not for this type separately (only one river in Flanders of this type, no pressure gradient available)
A-10. If yes, please specify	-
A-11. If no	Eutrophication
A-12. Status of assessment method: By when is the method fully intercalibrate-able (give month and year)?	At present
A-13. Pertinent literature of mandatory character (e.g. official note, national standard):	VMM (2009). Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive. September 2009. Available in Dutch and English. Vlaamse Milieumaatschappij, Erembodegem, Belgium.
A-14. Scientific literature (preferably quote references written in English):	
A-15. Comments:	
B. Data acquisition	
B-01. Which guidelines	EN 15204:2006
B-02. Specify sampling/survey device	Surface water sample taken with a bucket
B-03. Sampled/surveyed habitat (all available (multi-habitat) / single habitat)	single habitat
Main channel	X
Shorelines	
Secondary and side-channels	
Connected backwaters	
Isolated backwaters	
Alluvial wetlands	
Other (specify)	

B-04. How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	at least one occasion per month during the growing season
B-05. Sampling/survey months Please specify, if answer differs between sampled habitats (see B-03)!	april-september
B-06. How many spatial	3 to 5
B-07. Total sampled area or volume	Total volume sampled (prior to subsampling) is (bucket volume) x (3-5 samples per occasion) x (6 months) x (number of monthly samples; at least one)
B-08. Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!	Water, ideally from the middle of the stream, is collected in a large container using a large plastic bucket and a rope. After the sample is taken, subsamples are taken from the large container for microscopic and pigment analysis. The water should be thoroughly stirred in advance in order to homogenize floating organisms.
B-09. Record of biological data: taxonomic level (X if applicable):	
Species/species groups	X
Genus	X
Family	
Other	
B-10. If level of taxonomical identification differs	To the species level where possible, otherwise genus
B-11. Record of biological data: How is the biota's abundance within the sample/survey measured?	
Individual counts	
Percent coverage	
Abundance classes (ordinal scale)	
Relative abundance	
Other (specify):	Counts of individuals or, where applicable, colonies
B-12. Record of biological data: abundance is related to...	
Area	
Volume	X
Time	
Other (specify):	
B-13. Please specify unit in which the biota's abundance is expressed	Biomass per volume
B-14. If biomass is measured, please specify how it is quantified	Determination of chlorophyll-a concentration by spectrophotometric analysis; Determination of fresh weight by microscopic counting, cell size measurement and cell volume calculation (Utermöhl technique)
B-15. Other records	-
B-16. Comments	
C. Data evaluation	

C-01. Complete list of biological metric(s) used in assessment	Biomass (chlorophyll a); relative proportion of pennate diatoms; relative proportion of green algae; relative proportion of cyanobacteria; Typspezifischen Indexwertes Potamoplankton
C-02. If habitats other than the main channel	-
C-03. How are alien species considered in the assessment?	Included
C-04. Combination rule for multi-metrics	
Average metric scores	X
Weighted average metric scores	
Worst metric score	
Mean quality class	
Worst quality class	
Other (specify):	
Not relevant	
C-05. Describe the definition of reference conditions	Expert judgement
C-06. Key source(s) to derive reference conditions	
Existing near-natural reference sites	
Modelling (extrapolating model results)	
Expert knowledge	X
Historical data	
Least Disturbed Conditions	
Other (specify):	
C-07. Location of sites used to derive reference/least disturbed conditions (if applicable)	-
C-08. Setting of ecological status boundaries (X if applicable):	
Using discontinuities.	
Using paired metrics that respond in different ways	
High-good boundary derived from metric variability at near-natural reference sites	
Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).	
Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).	
Other (specify):	Expert judgement
C-09. Please describe the boundary setting procedure in relation to the pressure.	EQR gradient is assumed to represent a continuous trend with general degradation
C-10. Comments	

Annex I. 3: Czech Republic – method description in questionnaire

A - General information

A-01	Name of person completing this questionnaire
	Pavla Wildova, Libuse Opatrilova
A-02	Email address of person completing this questionnaire
	libuse_opatrilova@vuv.cz
A-03	Institution of person completing this questionnaire
	Ministry of the Environment, TGM Water Research Institute, p.r.i.
A-04	Name of assessment method (original full name)
	Metodika hodnocení ekologického stavu útvarů povrchových vod tekoucích pomocí biologické složky fytoplankton
A-05	Name of assessment method (translated into English)
	Assessment method for ecological status of rivers based on phytoplankton
A-06	Abbreviation of assessment method
	no abbreviation is available
A-07	EU Member State
	Czech Republic
A-08	Biological Quality Element
	phytoplankton
A-09	Name and description of very large river type(s) relevant for bioassessment of this BQE in your country
	Phytoplankton is evaluated only in rivers of category 3 (according to the Czech national river typology), which includes rivers of 7th - 9th Strahler stream order. This category is for evaluation divided into three groups (subtypes) according to a specific stream order. For large rivers can be approximately regarded rivers of 9th Strahler stream order.
A-10	Has the pressure-impact relationship of the assessment method been tested **at very large rivers**?
	Yes
	If yes, please specify pressure and impact metrics, the amount of data used, statistical significance of pressure etc.
	Testing the relationship between values of biological metrics and nutrients in sampling sites was carried out on a dataset that included 131 samples, however, only 24 samples were taken at 9th Strahler stream order. Data was tested in three ways. Statistical factor analysis for the search and testing of relationships between datasets (biological metrics and nutrient values) was used already at the stage of selecting metrics appropriate for inclusion in the multimetric index. Furthermore, differences between values of selected metrics on the best available sites and impacted sites were tested. Finally relationship between values of multimetric index and selected nutrients (total phosphorus P-PO4, N-NO3, N-NH4, N-NO2) was examined. Significant relationship was statistically validated mainly for total phosphorus. Here, the Spearman correlation coefficient using the whole dataset (rivers of 7th – 9th Strahler order) was around 0.6, but for large rivers (9th Strahler order) the correlation coefficient was significantly lower especially due to the small number of data (24 samples) and short gradient.
A-11	If no pressure-impact relationship was tested **at very large rivers**, which pressures does the assessment method detect, and why do you think that the method is capable of detecting these pressures?
A-12	Status of assessment method: By when is the method fully Intercalibrate-able (give month and year)?
	Method was completed in 2011 and is ready for intercalibration.
A-13	Pertinent literature of mandatory character (e.g. official note, national standard)
	Opatrilova, L. et al., 2011. Metodika hodnoceni ekologickeho stavu utvaru povrchovych vod tekoucich pomoci biologicke slozky fytoplankton. Ministerstvo zivotniho prostredi Ceske republiky. Praha. http://www.mzp.cz/cz/metodiky_normy [In Czech].
A-14	Scientific literature (preferably quote references written in English)

A-15	Comments

B - Data acquisition

B-01	Which guidelines are followed for the sampling/surveying and sample processing?	
	Hetesa, J. & Marvan P., 2006. Metodika odberu a zpracovani vzorku fytoplanktonu tekoucich vod. http://www.mzp.cz/cz/metodiky_normy. [In Czech].	
B-02	Please specify sampling/survey device	
	Water sampler	
B-03	Sampled/surveyed habitat	
	Main channel	yes
	Shorelines	
	Secondary and side-channels	
	Connected backwaters[11]	
	Isolated backwaters[12]	
	Alluvial wetlands[13]	
	Other (specify)	
B-04	How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	6 – 7 occasion per sampling season	
B-05	Sampling/survey month(s) Please specify, if answer differs between sampled habitats (see B-03)!	
	April to October	
B-06	How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	1	
B-07	Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based Please specify, if answer differs between sampled habitats (see B-03)!	
	n.a.	
B-08	Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!	
	Sampling by water sampler (not specified) preferably in the mid of the river flow. The sampling should be carried out monthly between April and October. The samples have to be processed (determination, abundance) not later than 48 hours. If this requirement is not possible to meet samples are preserved with Lugol solution.	
B-09	Record of biological data: Level of taxonomical identification	
	Species/species groups level	yes
	Genus level	
	Family level	
	Other level	

[11] Lacking upstream connection, but with downstream connection at mean water level (i.e. parapotamon)
[12] Lacking upstream and downstream connection at mean water level (i.e. plesiopotamon)
[13] Including strongly disconnected water bodies (i.e. palaeopotamon)

B-10	If level of taxonomical identification differs (multiple answers on B-09), please specify what groups are mainly identified to which level.

B-11	Record of biological data: How is the biota's abundance within the sample/survey measured?	
	Individual counts	yes
	Percent coverage	
	Abundance classes (ordinal scale)	
	Relative abundance (i.e. one species relatively to other species)	
	Other (specify)	
B-12	Record of biological data: Abundance is related to …	
	Area	
	Volume	yes
	Time	
	Other (specify)	
B-13	Please specify unit in which the biota's abundance is expressed	
	Number of cells/individuals per millilitre	
B-14	If biomass is measured, please specify how it is quantified.	
	Chlorophyll_a	
B-15	Other records of biological data (e.g. organism length, plant growth form, shoot density)	
	Vitality of cells (mobility, presence of deformed cells), empty frustules, length of individual specimens etc.	
B-16	Comments	

C - Data evaluation

C-01	Complete list of biological metric(s) used in the assessment	
	Relative proportion of Bacillariophyceae (%Bacillariophyceae) Relative proportion of Cyanophyceae (%Cyanophyceae) Relative proportion of Chlorophyceae (%Chlorophyceae) Chlorophyll_a concentration	
C-02	If habitats other than the main channel are considered differently in the assessment, please describe how this is done.	
C-03	How are alien species considered in the assessment?	
	Alien species are not considered in the assessment.	
C-04	Combination rule for multi-metrics	
	Average metric scores	yes
	Weighted average metric scores	
	Worst metric score	
	Mean quality class	
	Worst quality class	
	Other (specify)	
	Not relevant	
C-05	Describe the definition of reference conditions Please specify, if answer differs between sampled habitats (see B-03)!	
	Best available sites – selection based on low nutrient concentration and expert judgement	
C-06	Key source(s) to derive reference conditions	
	Existing near-natural reference sites	No
	Modelling (extrapolating model	No

	results)	
	Expert knowledge	yes
	Historical data	No
	Least Disturbed Conditions	yes
	Other (specify)	
C-07	Location of sites used to derive reference / least disturbed conditions (if applicable)	
	Selected sites on the Labe river.	
C-08	Setting of ecological status boundaries	
	Using discontinuities in the relationship of anthropogenic pressure and the biological response.	
	Using paired metrics that respond in different ways to the influence of the pressure (e.g. % sensitive taxa compared to % of impact taxa for benthic invertebrates in rivers and lakes).	
	High-good boundary derived from metric variability at near-natural reference sites (e.g. 5th percentile value).	
	Yes – best available sites were used instead of near-natural reference sites	
	Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).	
	yes	
	Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).	
	Other (specify)	
C-09	Please describe the boundary setting procedure in relation to the pressure.	
	The reference value was defined as 25% (or 75%) quantile of metric values at the best available sites for each stream order separately and in some cases expertly adjusted. The lower limit (for EQR calculation) was determined as the average of 99% (or 1%) quantile of metric values at all sites of each stream order. The EQR range 0-1 was divided into five categories in the same range (= ecological status classes). Final assessment results from the tested dataset were subjected to expert judgement and subsequently limits for the EQR calculation were revised so as to achieve a compromise between statistical calculations and expert opinion. For more detailed analyses the sufficient dataset was not available.	
C-10	Comments	

Annex I. 4: Croatia – method description in questionnaire

A - General information

A-01	Name of person completing this questionnaire
	Igor Stanković
A-02	Email address of person completing this questionnaire
	igor.stankovic@voda.hr
A-03	Institution of person completing this questionnaire
	Hrvatske vode (Croatian Waters)
A-04	Name of assessment method (original full name)
	Mađarski riječni potamoplanktonski indeks
A-05	Name of assessment method (translated into English)
	Hungarian River Potamoplankton Index
A-06	Abbreviation of assessment method
	HRPI
A-07	EU Member State
	Croatia
A-08	Biological Quality Element
	Phytoplankton
A-09	Name and description of very large river type(s) relevant for bioassessment of this BQE in your country
	Very large lowland rivers 5b Very large lowland rivers on siliceous and calcareous bedrocks – the Lower Mura course and the Middle Drava and Sava courses 5c Very large lowland rivers on siliceous bedrock – the Lower Drava and Sava courses 5d Very large lowland rivers on siliceous bedrock – the Danube
A-10	Has the pressure-impact relationship of the assessment method been tested **at very large rivers**?
	Yes, but more testing is necessary on larger set of data.
	If yes, please specify pressure and impact metrics, the amount of data used, statistical significance of pressure etc.
	Data used for this analysis were from 2010 in PhD thesis Stanković, I. 2013. PHYTOPLANKTON AS INDICATOR OF ECOLOGICAL STATUS OF LARGE LOWLAND RIVERS IN CROATIA. University of Zagreb, Croatia. There were 6 samples per sampling site (April-September) on 9 sampling sites (1 in Mura River, 4 in Drava River, 2 in Danube River and 2 in Sava River). Kendal tau and Spearman Correlation Coefficients were calculated for relationship of HRPI and BOD, COD, NO3, TN, DOP, TP and Qs (average monthly discharge) and they are presented in the following table:
A-11	If no pressure-impact relationship was tested **at very large rivers**, which pressures does the assessment method detect, and why do you think that the method is capable of detecting these pressures?
A-12	Status of assessment method: By when is the method fully Intercalibrate-able (give month and year)?
	January 2014
A-13	Pertinent literature of mandatory character (e.g. official note, national standard)
	Regulation on water quality standards (OG 73/13)

	Kendall's tau				Spearman's rho			
	Mura River	Drava River	Danube River	Sava River	Mura River	Drava River	Danube River	Sava River
	τ	τ	τ	τ	ρ	ρ	ρ	ρ
BOD	0,138	-0,121	**-,636****	-0,254	0,232	-0,152	**-0,805****	-0,348
COD	0,333	-0,146	-0,419	**-0,708****	0,486	-0,176	**-0,608***	**-0,821****
NO3	0,600	**0,550****	0,394	-0,242	0,771	**0,682****	0,441	-0,350
TN	0,600	**0,454****	0,121	**-0,455***	0,771	**0,580***	0,140	**-0,622***
DOP	0,067	-0,132	**0,606****	**-0,727****	-0,029	-0,225	**0,678***	**-0,874****
TP	-0,200	-0,296	0,321	**-0,636****	-0,257	-0,433	0,431	**-0,797****
Qs	-0,067	-0,098	0,229	0,394	-0,029	-0,152	0,291	**0,587***

A-14	Scientific literature (preferably quote references written in English)
	Stanković, I. 2013. PHYTOPLANKTON AS INDICATOR OF ECOLOGICAL STATUS OF LARGE LOWLAND RIVERS IN CROATIA. University of Zagreb, Croatia. PhD Thesis
A-15	Comments

B - Data acquisition

B-01	Which guidelines are followed for the sampling/surveying and sample processing?	
	Kiss, K. T., A. Schmidt & E. Acs, 1996. Sampling strategies for phytoplankton investigations in a large river (River Danube, Hungary). In Whitton, B. A., E. Rott & G. Friedrich (eds), Use of Algae for Monitoring Rivers II. Universita¨t Innsbruck, Institut fu¨r Botanik: 179–185. Water quality – Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique) (EN 15204:2006) Hillebrand, H., C.-D. Dürselen, D. Kirschtel, U. Pollingher, & T. Zohary, 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35: 403–424.	
B-02	Please specify sampling/survey device	
	Plastic bucked	
B-03	Sampled/surveyed habitat	
	Main channel	yes
	Shorelines	no
	Secondary and side-channels	no
	Connected backwaters[14]	no
	Isolated backwaters[15]	no
	Alluvial wetlands[16]	no
	Other (specify)	Middle of the river (“Thalweg”)
B-04	How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	Monthly from April till September	
B-05	Sampling/survey month(s) Please specify, if answer differs between sampled habitats (see B-03)!	
	No	
B-06	How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	One direct Utermöhl sample – 250 mL of water	
B-07	Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based Please specify, if answer differs between sampled habitats (see B-03)!	
B-08	Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!	
	Water is sampled from the bridge with plastic bucked on a rope.	
B-09	Record of biological data: Level of taxonomical identification	

[14] Lacking upstream connection, but with downstream connection at mean water level (i.e. parapotamon)
[15] Lacking upstream and downstream connection at mean water level (i.e. plesiopotamon)
[16] Including strongly disconnected water bodies (i.e. palaeopotamon)

	Species/species groups level	Yes
	Genus level	Yes
	Family level	
	Other level	
B-10	If level of taxonomical identification differs (multiple answers on B-09), please specify what groups are mainly identified to which level.	
	All groups are identified to species level when possible.	

B-11	Record of biological data: How is the biota's abundance within the sample/survey measured?	
	Individual counts	Yes
	Percent coverage	
	Abundance classes (ordinal scale)	ind./L for abundance or mg/L for biomass
	Relative abundance (i.e. one species relatively to other species)	
	Other (specify)	

B-12	Record of biological data: Abundance is related to …	
	Area	no
	Volume	yes
	Time	no
	Other (specify)	

B-13	Please specify unit in which the biota's abundance is expressed
	Individuals per liter – ind./L and Biomass per volume – mg/L
B-14	If biomass is measured, please specify how it is quantified.
	Determination of fresh weight by microscopic counting, cell size measurement and cell volume calculation (Utermöhl technique)
B-15	Other records of biological data (e.g. organism length, plant growth form, shoot density)
	No
B-16	Comments
	-

C - Data evaluation

C-01	Complete list of biological metric(s) used in the assessment
	Qr index and Chl a concentration as two components of HRPI

C-02	If habitats other than the main channel are considered differently in the assessment, please describe how this is done.
	No

C-03	How are alien species considered in the assessment?
	They are not assessed for phytoplankton.

C-04	Combination rule for multi-metrics	
	Average metric scores	
	Weighted average metric scores	
	Worst metric score	
	Mean quality class	
	Worst quality class	
	Other (specify)	
	Not relevant	

C-05	Describe the definition of reference conditions Please specify, if answer differs between sampled habitats (see B-03)!
	Types of rivers and assessment method were adopted form Hungarian method.

C-06	Key source(s) to derive reference conditions	
	Existing near-natural reference sites	
	Modelling (extrapolating model results)	
	Expert knowledge	
	Historical data	
	Least Disturbed Conditions	
	Other (specify)	
C-07	Location of sites used to derive reference / least disturbed conditions (if applicable)	

C-08	Setting of ecological status boundaries
	Using discontinuities in the relationship of anthropogenic pressure and the biological response.
	Using paired metrics that respond in different ways to the influence of the pressure (e.g. % sensitive taxa compared to % of impact taxa for benthic invertebrates in rivers and lakes).
	High-good boundary derived from metric variability at near-natural reference sites (e.g. 5th percentile value).
	Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).
	Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).
	Other (specify)

C-09	Please describe the boundary setting procedure in relation to the pressure.

C-10	Comments

Annex I. 5: Germany – method description in questionnaire

A - General information

A-01	Name of person completing this questionnaire
	Ute Mischke
A-02	Email address of person completing this questionnaire
	mischke@igb-berlin.de
A-03	Institution of person completing this questionnaire
	Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) Dept. II, Müggelseedamm 310, D-12587 Berlin, Germany
A-04	Name of assessment method (original full name)
	Gesamtindex PhytoFluss
A-05	Name of assessment method (translated into English)
	PhytoFluss Index
A-06	Abbreviation of assessment method
	PhytoFluss Index
A-07	EU Member State
	Germany
A-08	Biological Quality Element
	phytoplankton
A-09	Name and description of very large river type(s) relevant for bioassessment of this BQE in your country
	10.1 Very large gravel-dominated rivers with high area specific run-off 10.2 Very large gravel-dominated rivers with low area specific run-off 20.1 Very large sand-dominated rivers in the lowlands with high area specific run-off 20.2 Very large sand-dominated rivers in the lowlands with low area specific run-off
A-10	Has the pressure-impact relationship of the assessment method been tested **at very large rivers**?
	Yes, pooled for very large rivers with low specific run-off (10.2,20.2) plus Baltic sea tributary rivers (type 23) . Yes, with qualitative data (e.g. response at reference against impacted sites).
	If yes, please specify pressure and impact metrics, the amount of data used, statistical significance of pressure etc.
	Chlorophyll concentration against total phosphorus (107 years of investigation; 82% very large rivers (low area specific run-off) in this pooled data Mischke et al. 2011, figure 5) **PhytoFluss Index version 2.2** Composition metrics all against total phosphorus combined with chlorophyll a (trophic status classes): grouped for rivers sensitive to the specific metric (see Mischke et al. 2011, figure 4) - % Chlorophytes (96 years of investigation; 80% very large rivers in this pooled data) - % Pennales (171 years of investigation; 14% very large rivers in this pooled data) - % Cyanobacteria (83 years of investigation; 29% very large rivers in this pooled data) Trophic Index potamal (TIP) with indicator taxa (314 years of investigation 37% very large rivers in this pooled data) No linear regression statistics – Obvious increasing or decreasing trends for at least 3 status classes. – boundary setting along 75% percentile of pre-classified data into 5 status classes **PhytoFluss Index version 4.0** See pressure-impact report in IC-report in A.2 to TP and to combined stressor stressor
A-11	If no pressure-impact relationship was tested **at very large rivers**, which pressures does the assessment method detect, and why do you think that the method is capable of detecting these pressures?
	The method detects eutrophication pressure (total phosphorus) because the same assessment metrics are used for detecting this pressure also at mid-sized rivers (>1000km2). For all mid-sized to very large rivers pooled (N, the pressure-impact relationship was tested empirically. See paper Mischke et al. 2011
A-12	Status of assessment method: By when is the method fully Intercalibrate-able (give month and year)?
	PhytoFluss 2.2 since April, 2007 (see handbook: Mischke & Behrendt 2007) **PhytoFluss 4.0** – national decision is expected until January 2017

A-13	Pertinent literature of mandatory character (e.g. official note, national standard)
	LAWA-AO, 2006. RaKon Monitoring Teil B. Arbeitspapier III: Untersuchungsverfahren für biologische Qualitätskomponenten. Ständiger Ausschuss "Oberflächengewässer und Küstengewässer" der Bund/Länder-Arbeitsgemeinschaft Wasser (LAWA-AO).
A-14	Scientific literature (preferably quote references written in English)
	Mischke, U., Venohr, M. and H. Behrendt (2011): Using Phytoplankton to Assess the Trophic Status of German Rivers. International Revue of Hydrobiology 96 (5): 578-598 [PhytoFluss 2.2] Mischke, U. (2016): Endbericht zum Teilvorhaben „Modul 3 Weiterentwicklung des Verfahrens „PhytoFluss“ Within main project FKZ 3714 22 211 0 supported by Federal Environ. Agency [PhytoFluss 4.0)
A-15	Comments
	Method up-date: There is an on-going project to improve the assessment method PhytoFluss Index based on 120 new years of investigation at more than 100 sites: - The boundaries of the biomass index will be adapted to the usual chlorophyll a which is corrected for phaeophytin (see ISO 10260, 1992), instead of formerly used parameter “total pigment (chl a uncorrected)”. - The former genus based indicator list in the metric “TIP” will be replaced by indicator list based on more than 220 species. - the multi-metric index will have weighted average metric scores **See German Method description in annex II – D-2**

B - Data acquisition

B-01	Which guidelines are followed for the sampling/surveying and sample processing?
	EN 15204, 2006: Water quality. Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermoehl technique). European Committee for Standardization, Brussels. ISO 10260, 1992: Water quality – Measurement of biochemical parameters – Spectrometric determination of the chlorophyll a concentration, Int. Standard., Geneva, 1st edition 1992–07–15, 6 pp Nixdorf, B., Hoehn, E., Riedmüller, U., Mischke U. & I. Schönfelder (2010): III-4.3.1 Probenahme und Analyse des Phytoplanktons in Seen und Flüssen zur ökologischen Bewertung gemäß der EU-WRRL. In: Handbuch Angewandte Limnologie – 27. Erg.Lfg. 2/10 1. S. 1- 24 (book chapter) Mischke, U. & H. Behrendt (eds. 2007): Handbuch zum Bewertungsverfahren von Fließgewässern mittels Phytoplankton zur Umsetzung der EU-Wasserrahmenrichtlinie in Deutschland – Appendix: Operational taxalist of phytoplankton – Weißensee-Verlag, Berlin, ISBN 978-3-89998-105-6. p 88. (Handbook about the German system for phytoplankton-based assessment of rivers for implementation of the EU WFD).

B-02	Please specify sampling/survey device
	A description of the sampling method is published to implement the Water Framework Directive in Germany (Mischke and Behrendt, 2007). Sampling of **original water samples from the main channel** by water samplers preferably from bridges. In cases when sampling from a bridge or a boat is not possible, a graduated jug is fixed on a 2m stick for water sampling from the main channel. The unfiltered water sample is fixed with iodine rich Lugol´s solution and stored glass bottles in the dark and at low temperatures. The counting procedure is according EN 15204, 2006 with additional taxa biovolume determination. The chlorophyll a concentration (biomass parameter) is determined according ISO 10260, 1992 **Sampling frequency per year:** at least 6 times in the period April to October.

B-03	Sampled/surveyed habitat	
	Main channel	yes
	Shorelines	no
	Secondary and side-	no

	channels	
	Connected backwaters[17]	preferably no, but in practise it is done
	Isolated backwaters[18]	no, if so than assessed according the method for lakes
	Alluvial wetlands[19]	no
	Other (specify)	
B-04	How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	Sampling frequency per year: at least 6 times in the period April to October.	
B-05	Sampling/survey month(s) Please specify, if answer differs between sampled habitats (see B-03)!	
	period April to October	
B-06	How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	no replicates, obligate sample from the 0,5m depths but see B-08	
B-07	Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based Please specify, if answer differs between sampled habitats (see B-03)!	
	100ml for microscopic analysis and 1000ml for chlorophyll a	
B-08	Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!	
	sample from the 0,5m water depths. If Secchi depth is below 1m or an algal bloom is visible, a second sample is taken from the direct sub-surface of the water body and is mixed with obligate sample from the 0,5m depths.	
B-09	Record of biological data: Level of taxonomical identification	
	Species/species groups level	yes
	Genus level	yes
	Family level	seldom
	Other level	no
B-10	If level of taxonomical identification differs (multiple answers on B-09), please specify what groups are mainly identified to which level.	
	There is an operational taxa list for phytoplankton which indicates the level of determination requested for the PhytoFluss Index. In the currently applied method, genus level is mainly sufficient.	
B-11	Record of biological data: How is the biota's abundance within the sample/survey measured?	
	Individual counts	yes
	Percent coverage	
	Abundance classes (ordinal scale)	
	Relative abundance (i.e. one species relatively to other species)	
	Other (specify)	taxa biovolumes are calculated based on cell counts multiplied bystandard cell volume
B-12	Record of biological data: Abundance is related to …	
	Area	
	Volume	water volume
	Time	

[17] Lacking upstream connection, but with downstream connection at mean water level (i.e. parapotamon)
[18] Lacking upstream and downstream connection at mean water level (i.e. plesiopotamon)
[19] Including strongly disconnected water bodies (i.e. palaeopotamon)

	Other (specify)	
B-13	Please specify unit in which the biota's abundance is expressed	
	phytoplankton or taxa biovolume in m^3/L, which is equal to mm^3/m^3	
B-14	If biomass is measured, please specify how it is quantified.	
	Determination of fresh weight by microscopic counting, cell size measurement and cell volume calculation (Utermöhl technique)	

B-15	Other records of biological data (e.g. organism length, plant growth form, shoot density)
	no
B-16	Comments

C - Data evaluation

C-01	Complete list of biological metric(s) used in the assessment
	Indices applied to all very large rivers: Total biomass of phytoplankton (parameter: chlorophyll a (uncorrected for phaeophytin a) in µg/L) TIP Index: Composition of indicator taxa scored along the gradient of trophic status Indices applied only to some of the river sub-types: Pennales Index: Relative biovolume of order Pennales (applied only for very large rivers 10.1 and 20.1) Chloro Index: Relative biovolume of class Chlorophytes (applied only for very large rivers 10.2 and 20.2) Cyano Index: Relative biovolume of class Cyanobacteria (applied only for very large rivers 10.2 and 20.2)

C-02	If habitats other than the main channel are considered differently in the assessment, please describe how this is done.
	permanent isolated former river sections larger than 0,5km2 are assessed according the German assessment method for lakes (PhytoSee)

C-03	How are alien species considered in the assessment?	
	no consideration	
C-04	Combination rule for multi-metrics	
	Average metric scores	Average metric scores
	Weighted average metric scores	
	Worst metric score	
	Mean quality class	
	Worst quality class	
	Other (specify)	
	Not relevant	
C-05	Describe the definition of reference conditions Please specify, if answer differs between sampled habitats (see B-03)!	
	No sites in reference status were available for very large rivers. Sites in least disturbed conditions: Chemistry – total phosphate: < 0.05 mg/l (mean) and excluding river sections, which are backwaters of dams. high status very large rivers for with high area specific run-off: sections of the river Rhine in some years (type 10.1) no high status very large rivers for with low area specific run-off: Sites in least disturbed conditions	
C-06	Key source(s) to derive reference conditions	
	Existing near-natural reference sites	
	Modelling (extrapolating model results)	Modelling for rivers Elbe, Odra, Weser

	Expert knowledge	
	Historical data	
	Least Disturbed Conditions	Least Disturbed Conditions for rivers Danube and Rhine
	Other (specify)	
C-07	Location of sites used to derive reference / least disturbed conditions (if applicable)	
	Rhein, Karlsruhe // Rhein, Reckingen // Rhein, Breisach and modelling	
C-08	Setting of ecological status boundaries	
	Using discontinuities in the relationship of anthropogenic pressure and the biological response.	
	yes in case of the metrics Pennales, Chloro and Cyano	
	Using paired metrics that respond in different ways to the influence of the pressure (e.g. % sensitive taxa compared to % of impact taxa for benthic invertebrates in rivers and lakes).	
	High-good boundary derived from metric variability at near-natural reference sites (e.g. 5th percentile value).	
	Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).	
	yes	
	Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).	
	yes	
	Other (specify)	
C-09	Please describe the boundary setting procedure in relation to the pressure.	
	River types were placed into three nutrient response groups using empirical analysis (low, high and very high response in phytoplankton biomass). To set the "high/good" status class boundary (H/G), in the first step, a TP background level of 0.05 mg L^{-1} was applied to all river types (background modelling see Behrendt et al., 2003) . The boundaries for the biomass metric approximately fit the different nutrient response curves of the phytoplankton biomass, measured as chlorophyll a uncorrected for phaeophytin a (chla_uncorr), along the 75% percentiles within the five TP classes. Regression curves were mathematically fitted to cross the boundaries along the parameter responses, and were transformed to index "calculation functions" operating between 0.5 to 5.5. To set the "poor/bad" status class boundary (P/B), we used the point of "no further response of biomass to TP". When TP concentrations exceeded 0.25 mg L^{-1}, the 75% percentile of chla_uncorr concentration did not further increase in nutrient sensitive rivers, but was assumed to be more influenced by saproby. The "poor/bad" boundary of TP was set higher (at 0.30 mg L^{-1}) for those river types with an overall low slope of the response curve between phytoplankton biomass and TP concentration. To set the remaining two boundaries the range of the TP scale between H/G and P/B was fitted to a linear or an exponential curve, and divided into 3 equal parts, and the resulting TP values were finally rounded. Metrics taxa composition: The boundaries for the taxonomic composition metrics Pennales, Chlorophytes and Cyanobacteria were derived also from the 75% percentile values, when the parameter distribution was plotted in box-plots and grouped by a pre-classification of all sites in five eutrophication classes, based on the biomass boundaries combined with those for TP according to pre-set boundaries (trophic status assessment).	
C-10	Comments	

Annex I. 6: Estonia – method description in questionnaire

Please note, the information provided here were not up-dated.
The Estonian method was modified in 2015: metric for abundance was added and period of sampling is restricted to summer months (see Annex II - C).

A - General information

A-01	Name of person completing this questionnaire
	Irja Truumaa
A-02	Email address of person completing this questionnaire
	Irja.Truumaa@envir.ee
A-03	Institution of person completing this questionnaire
	Ministry of the Environment of Estonia, water department
A-04	Name of assessment method (original full name)
	Vooluveekogumi seisundi hindamine fütoplanktoni koosluse alusel
A-05	Name of assessment method (translated into English)
	Estonian national method for river ecological status assessment based on phytoplankton
A-06	Abbreviation of assessment method
	EST_PHYPLA_R
A-07	EU Member State
	Estonia
A-08	Biological Quality Element
	phytoplankton
A-09	Name and description of very large river type(s) relevant for bioassessment of this BQE in your country
	Narva river national type 4B, intercalibration type R-L2 Very large medium to high alkalinity rivers, alkalinity > 0.5 meq /L, mixed substrate
A-10	Has the pressure-impact relationship of the assessment method been tested **at very large rivers**?
	Yes, small number of samples have been tested
	If yes, please specify pressure and impact metrics, the amount of data used, statistical significance of pressure etc.
	The share of X2 group (representative species *Rhodomonas lacustris*) was negatively correlated with TP r=-0,58, P<0,05, 15 samples
A-11	If no pressure-impact relationship was tested **at very large rivers**, which pressures does the assessment method detect, and why do you think that the method is capable of detecting these pressures?
	-
A-12	Status of assessment method: By when is the method fully Intercalibrate-able (give month and year)?
	We are ready to intercalibrate the method in the second half of 2013 . the method will be legally binding in 2015

Example: *May 2013*

A-13	Pertinent literature of mandatory character (e.g. official note, national standard)
	For sampling , sample conservation and preparation, counting the relevant EN standards are used; sampling method is not legally binding yet
A-14	Scientific literature (preferably quote references written in English)
	1) Padisák, J., Borics, G., Grigorszky, I. & Soróczki-Pintér, É. 2006. Use of phytoplankton assemblages for monitoring ecological status of lakes within the Water Framework Directive: the assemblage index. *Hydrobiologia*, **553**, 1.14. 2) Borics, G., Várbiró, G., Grigorsky, I., Krasznai, E., Szabo, S. & Kiss, K. T. 2007. A new evaluation technique of potamo-plankton for the assessment of the ecological status of rivers. *Arch. Hydrobiol. Suppl.*, **161**(3.4), 465.486. 3) Piirsoo, K., Pall, P., Tuvikene,A., Viik, M., Vilbaste, S. Assessment of water quality in a large lowland river (Narva, Estonia/Russia) using a new Humńgarian potamoplanctic Method.; *Estonian Journal of Ecology, 2010, **59**, 4, 243.258*
A-15	Comments
	-

B - Data acquisition

B-01	Which guidelines are followed for the sampling/surveying and sample processing?
	European standard EN 15204 (2006).

B-02	Please specify sampling/survey device
	Standard device EN 15204 (2006)

B-03	Sampled/surveyed habitat	
	Main channel	X
	Shorelines	
	Secondary and side-channels	
	Connected backwaters[20]	
	Isolated backwaters[21]	
	Alluvial wetlands[22]	
B-04	How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	one sampling point per water body, sampling every third year, three times equally distributed by vegetational season (July , August September); monthly measured nutrient content data are available for every year.	
B-05	Sampling/survey month(s) Please specify, if answer differs between sampled habitats (see B-03)!	
	May August September, only main channel is sampled	
B-06	How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	One sampling point – one sampling replicate, the place has to be representative to flow rate (center of the riverbed) , sampling depth 50 cm from surface.	

B-07	Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based Please specify, if answer differs between sampled habitats (see B-03)!
	One sampling point Samples taken in May, August, September
B-08	Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!
	Quantitative phytoplankton samples (100.200 mL) were taken from a depth of 0.1 m from the thalweg. Samples were preserved in dark glass bottles with Lugol.s iodine solution (1% final concentration) according to the European standard EN 15204 (2006).

B-09	Record of biological data: Level of taxonomical identification	
	Species/species groups level	yes
	Genus level	yes
	Family level	
	Other level	

20 Lacking upstream connection, but with downstream connection at mean water level (i.e. parapotamon)

21 Lacking upstream and downstream connection at mean water level (i.e. plesiopotamon)

22 Including strongly disconnected water bodies (i.e. palaeopotamon)

B-10	If level of taxonomical identification differs (multiple answers on B-09), please specify what groups are mainly identified to which level.
	-

B-11	Record of biological data: How is the biota's abundance within the sample/survey measured?	
	Individual counts	yes
	Percent coverage	
	Abundance classes (ordinal scale)	
	Relative abundance (i.e. one species relatively to other species)	
	Other (specify)	
B-12	Record of biological data: Abundance is related to …	
	Area	
	Volume	yes
	Time	
	Other (specify)	
B-13	Please specify unit in which the biota's abundance is expressed	
	Number of counting units (cells, filaments or colonies) per volume	
B-14	If biomass is measured, please specify how it is quantified.	
	The number of counting units (cells, filaments, or colonies, 10_6 L.$_{-1}$) was converted to biovolume (wet weight biomass, mg L.$_1$) using stereometric formulae after Olrik et al. (1998).	
B-15	Other records of biological data (e.g. organism length, plant growth form, shoot density)	
	-	
B-16	Comments	
	-	

C - Data evaluation

C-01	Complete list of biological metric(s) used in the assessment	
	Assemblage index Q by Padisák, J.et al. 2006.	
C-02	If habitats other than the main channel are considered differently in the assessment, please describe how this is done.	
	Only main channel	
C-03	How are alien species considered in the assessment?	
	Not considered	
C-04	Combination rule for multi-metrics	
	Average metric scores	
	Weighted average metric scores	
	Worst metric score	
	Mean quality class	
	Worst quality class	
	Other (specify)	
	Not relevant	X only one metric
C-05	Describe the definition of reference conditions Please specify, if answer differs between sampled habitats (see B-03)!	
	Refcond are defined using the expert opinion; For Narva river Reference Ptot is 0,02 mg P /L, H/G border is 0,04 mg P/L (EQR 0,8), Reference Ntot is 0,43 mgN/L, H/G border is 0,5 mgN/L (EQR 0,8)	

C-06	Key source(s) to derive reference conditions	
	Existing near-natural reference sites	
	Modelling (extrapolating model results)	
	Expert knowledge	X
	Historical data	X
	Least Disturbed Conditions	
	Other (specify)	
C-07	Location of sites used to derive reference / least disturbed conditions (if applicable)	
	Two sites: Vasknarva (outflow from lake Peipsi), Narva (downstream the Narve reservoir dam)	
C-08	Setting of ecological status boundaries	
	Using discontinuities in the relationship of anthropogenic pressure and the biological response.	
	Using paired metrics that respond in different ways to the influence of the pressure (e.g. % sensitive taxa compared to % of impact taxa for benthic invertebrates in rivers and lakes).	
	High-good boundary derived from metric variability at near-natural reference sites (e.g. 5th percentile value).	
	Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).	
	Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).	
	Other (specify)	
C-09	Please describe the boundary setting procedure in relation to the pressure.	
C-10	Comments	

Annex I. 7: Hungary – method description in questionnaire

Metric for taxonomic composition is described in Borics, G., Várbiró, G., Grigorsky, I., Krasznai, E., Szabo, S. & Kiss, K. T. 2007. A new evaluation technique of potamo-plankton for the assessment of the ecological status of rivers. Arch. Hydrobiol. Suppl., 161(3.4), 465.486
Additionally, the chlorophyll-metric was delveloped.

A - General information

A-01	Name of person completing this questionnaire
	Gábor Borics
A-02	Email address of person completing this questionnaire
	borics.gabor@okologia.mta.hu
A-03	Institution of person completing this questionnaire
	Centre for Ecological Research, Hungarian Academy of Sciences, Danube Research Institute, Department of Tisza Research
A-04	Name of assessment method (original full name)
	Folyóvízi Fitoplankton Index
A-05	Name of assessment method (translated into English)
	Hungarian River Phytoplankton Index
A-06	Abbreviation of assessment method
	HRPI
A-07	EU Member State
	Hungary
A-08	Biological Quality Element
	Phytoplankton
A-09	Name and description of very large river type(s) relevant for bioassessment of this BQE in your country
	The method is used for large and small rivers as well.
A-10	Has the pressure-impact relationship of the assessment method been tested **at very large rivers**?
	The relationship was tested for TP and TN.
	If yes, please specify pressure and impact metrics, the amount of data used, statistical significance of pressure etc.
	Significant relationship was not found, because of the narrow windows of pressures (both TP and TN values were in the eutrophic range). The other reason for the lack of relationship is that the amount of suspended particles can occasionally be high, and this causes light limitation.
A-11	If no pressure-impact relationship was tested **at very large rivers**, which pressures does the assessment method detect, and why do you think that the method is capable of detecting these pressures?
	When the hydrometeorological situation makes it possible, the phytoplankton biomass and composition reflect the nutrient load of the river and the impact of impoundments.
A-12	Status of assessment method: By when is the method fully Intercalibrate-able (give month and year)?
	May 2013
A-13	Pertinent literature of mandatory character (e.g. official note, national standard)
	KEOP-2.5.0/A Vízgazdálkodási Tervek Készítése, Zárójelentés 2009.
A-14	Scientific literature (preferably quote references written in English)
	Borics G, Várbiró G, Grigorszky I, Krasznai E, Szabó S, Kiss K T., 2007. A new evaluation technique of potamoplankton for the assessment of the ecological status of rivers. ARCHIV FÜR HYDROBIOLOGIE SUPPLEMENTBAND LARGE RIVERS 17:(3-4) 465-486. Szilágyi F, Ács É, Borics G, Halasi-Kovács B, Juhász P, Kiss B, Kovács T, Müller Z, Lakatos G, Padisák J, Pomogyi P, Stenger-Kovács C, Szabó KÉ, Szalma E, Tóthmérész B 2008 Application of Water Framework Directive in Hungary: Development of biological classification systems, WATER SCIENCE AND TECHNOLOGY 58:(11) 2117-2125. Várbíró G, Ács É, Borics G, Érces K, Fehér G, Grigorszky I, Japoport T, Kocsi G, Krasznai E, Nagy K,

	Nagy-László Zs, Pilinszky Zs, Kiss K T., 2007. Use of Self-Organizing Maps (SOM) for characterization of riverrine phytoplankton associations in Hungary ARCHIV FÜR HYDROBIOLOGIE SUPPLEMENTBAND LARGE RIVERS 17:(3-4) 383-394.
A-15	Comments
	The method has been used in Hungary for river quality assessment since 2009.

B - Data acquisition

B-01	Which guidelines are followed for the sampling/surveying and sample processing?	
	Folyók, patakok. Mintavétel fizikai, kémiai és mikrobiológiai vizsgálatok céljára. *MSZ ISO 5667-6:1995*	
B-02	Please specify sampling/survey device	
	Bucket	
B-03	Sampled/surveyed habitat	
	Main channel	Main channel is sampled in the thalweg.
	Shorelines	NO
	Secondary and side-channels	NO
	Connected backwaters[23]	NO
	Isolated backwaters[24]	If they are fully isolated (are outside the embankments), than these belong to lakes category.
	Alluvial wetlands[25]	NO
	Other (specify)	
B-04	How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	Six occasions in the growing season.	
B-05	Sampling/survey month(s) Please specify, if answer differs between sampled habitats (see B-03)!	
	April-October	
B-06	How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	Three buckets of water are taken	
B-07	Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based Please specify, if answer differs between sampled habitats (see B-03)!	
	Volume of the sample is 0.5 litres.	
B-08	Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!	
	Three buckets of water are taken. A couple of minutes should be between the samplings. Water of the three buckets is averaged.	
B-09	Record of biological data: Level of taxonomical identification	
	Species/species groups level	YES
	Genus level	
	Family level	

[23] Lacking upstream connection, but with downstream connection at mean water level (i.e. parapotamon)
[24] Lacking upstream and downstream connection at mean water level (i.e. plesiopotamon)
[25] Including strongly disconnected water bodies (i.e. palaeopotamon)

	Other level	
B-10	If level of taxonomical identification differs (multiple answers on B-09), please specify what groups are mainly identified to which level.	
	–	
B-11	Record of biological data: How is the biota's abundance within the sample/survey measured?	
	Individual counts	Algae are counted (Utermohl's method))
	Percent coverage	
	Abundance classes (ordinal scale)	
	Relative abundance (i.e. one species relatively to other species)	
	Other (specify)	Phytoplankton biomass is estimated
B-12	Record of biological data: Abundance is related to …	
	Area	
	Volume	Biovolume
	Time	
	Other (specify)	Biovolume expressed as Chlorophyll-a ug/l
B-13	Please specify unit in which the biota's abundance is expressed	
	Chl-a ug/l Biovolume mg/l	
B-14	If biomass is measured, please specify how it is quantified.	
	Determination of fresh weight by microscopic counting, cell size measurement and cell volume calculation (Utermöhl technique)	
B-15	Other records of biological data (e.g. organism length, plant growth form, shoot density)	
	–	
B-16	Comments	
	–	

C - Data evaluation

C-01	Complete list of biological metric(s) used in the assessment	
	Chl-a as biomass metric. Composition metric; based on relative abundance of various functional groups of algae. (The functional groups are evaluated, based on their tolerances and preferences)	
C-02	If habitats other than the main channel are considered differently in the assessment, please describe how this is done.	
	–	
C-03	How are alien species considered in the assessment?	
	Aliens are not considered.	
C-04	Combination rule for multi-metrics	
	Average metric scores	
	Weighted average metric scores	Weighted average metric scores
	Worst metric score	
	Mean quality class	
	Worst quality class	
	Other (specify)	
	Not relevant	
C-05	Describe the definition of reference conditions Please specify, if answer differs between sampled habitats (see B-03)!	
	Expert knowledge: Reference sites are not available, therefore least disturbed sites were applied. Based on the growing season data 25 th percentiles were used as reference values. As to the composition metric, the relative abundance of the sensitive taxa should be >90%	
C-06	Key source(s) to derive reference conditions	

	Existing near-natural reference sites	
	Modelling (extrapolating model results)	
	Expert knowledge	Yes (for setting boundaries of the composition metric)
	Historical data	
	Least Disturbed Conditions	Yes (for setting chl-a boundaries)
	Other (specify)	
C-07	Location of sites used to derive reference / least disturbed conditions (if applicable)	
	Upper sections of the Körös Tisza and Dráva rivers.	
C-08	Setting of ecological status boundaries	
	High-good boundary derived from metric variability at least disturbed sites (e.g. 25th percentile value).	
	Other boundaries were set by equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).	
	Other (specify)	
	–	
C-09	Please describe the boundary setting procedure in relation to the pressure.	
	–	
C-10	Comments	
	–	

Annex I. 8: Latvia – method description in questionnaire

The Latvia method was modified in 2015 and questionnaire is updated (see also Annex II - A).

A-01	Name of person completing this questionnaire
	Jolanta Jekabsone
A-02	Email address of person completing this questionnaire
	jolanta.jekabs@gmail.com
A-03	Institution of person completing this questionnaire
	Institute of Biology of University of Latvia
A-04	Name of assessment method (original full name)
	Latvijas lielo upju fitoplanktona indekss
A-05	Name of assessment method (translated into English)
	Latvian Large River Phytoplankton Index
A-06	Abbreviation of assessment method
	LatRPI
A-07	EU Member State
	Latvia
A-08	Biological Quality Element
	Phytoplankton
A-09	Name and description of very large river type(s) relevant for bioassessment of this BQE in your country
	Very large lowland rivers – Very large rivers of >10,000 km^2 catchment size, including the exception of River Gauja (catchment size 9800 km^2).
A-10	Has the pressure-impact relationship of the assessment method been tested **at very large rivers**?
	Yes
	If yes, please specify pressure and impact metrics, the amount of data used, statistical significance of pressure etc.
	Pressure-impact relationship was tested on three very large rivers using yearly average Ptot and LatRPI values (R^2=0.3116). If chlorophyll a for each sample are >18 mg/l, Ptot shows significant correlation (R^2=0.7925; 10 samples). BOD_5 also shows significant correlation with LatRPI.
A-11	If no pressure-impact relationship was tested **at very large rivers**, which pressures does the assessment method detect, and why do you think that the method is capable of detecting these pressures?
	Eutrophication, pollution by organic matter.
A-12	Status of assessment method: By when is the method fully Intercalibrate-able (give month and year)?
	Finished method, June 2015.
A-13	Pertinent literature of mandatory character (e.g. official note, national standard)
	No
A-14	Scientific literature (preferably quote references written in English)
	Borics, G., G. Varbiro, I. Grigorszky, E. Krasznai, S. Szabo & K.T. Kiss, 2007. A new evaluation technique of potamo-plankton for the assessment of the ecological status of rivers. Large Rivers Vol. 17, No. 3-4 Arch. Hydrobiol. Suppl. 161 (3-4): 465-486.
A-15	Comments
	Latvian River Phytoplankton Index is adapted Hungarian River Phytoplankton index. Asamblage index formula is used: $Q = \sum_{i=1}^{n} p_i F_i$ where *pi* is the relative share of the *i*-th functional group in biomass, and *F* is the value of the factor estimated from the following components. LatRPI=(Q_EQR+NChl a)/2, where LatRPI : Latvian River Phytoplankton Index

	NChla: Normalised Chl-a metric Q_EQR: Composition metric The EQR values are calculated as average of normalized Chl a metric and normalized Q metric. H/G=0.8, G/M=0.6, M/P=0.4, P/B=0.2.

B - Data acquisition

B-01	Which guidelines are followed for the sampling/surveying and sample processing?	
	ISO 10260:1992 for chlorophyll a SM 10200 (C1; E3,5; F1,2; I2): 2012, Utermöhl's technique for phytoplankton	
B-02	Please specify sampling/survey device	
	Ruthner type Water sampler	
B-03	Sampled/surveyed habitat	
	Main channel	Yes
	Shorelines	No
	Secondary and side-channels	No
	Connected backwaters[26]	No
	Isolated backwaters[27]	No
	Alluvial wetlands[28]	No
	Other (specify)	No
B-04	How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	2-4 samples per vegetation season	
B-05	Sampling/survey month(s) Please specify, if answer differs between sampled habitats (see B-03)!	
	July, August, September	
B-06	How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	n.a.	
B-07	Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based Please specify, if answer differs between sampled habitats (see B-03)!	
	1 l	
B-08	Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!	
	Samples at 0.5 m deep in the middle of river, fixed by Lugole solution.	
B-09	Record of biological data: Level of taxonomical identification	
	Species/species groups level	Yes
	Genus level	Yes
	Family level	Yes
	Other level	n.a.

[26] Lacking upstream connection, but with downstream connection at mean water level (i.e. parapotamon)
[27] Lacking upstream and downstream connection at mean water level (i.e. plesiopotamon)
[28] Including strongly disconnected water bodies (i.e. palaeopotamon)

B-10	If level of taxonomical identification differs (multiple answers on B-09), please specify what groups are mainly identified to which level.	
	Most organisms to species/species groups level.	
B-11	Record of biological data: How is the biota's abundance within the sample/survey measured?	
	Individual counts	Yes
	Percent coverage	Yes
	Abundance classes (ordinal scale)	Yes
	Relative abundance (i.e. one species relatively to other species)	No
	Other (specify)	No
B-12	Record of biological data: Abundance is related to …	
	Area	No
	Volume	Yes
	Time	No
	Other (specify)	No
B-13	Please specify unit in which the biota's abundance is expressed	
	Number of individuals (thousand/ ml)	
B-14	If biomass is measured, please specify how it is quantified.	
	Chlorophyll-a concentration, Utermöhl technique. Total biomass of sample.	
B-15	Other records of biological data (e.g. organism length, plant growth form, shoot density)	
	None	
B-16	Comments	
	None	

C - Data evaluation

C-01	Complete list of biological metric(s) used in the assessment	
	Relative abundance of taxa	
C-02	If habitats other than the main channel are considered differently in the assessment, please describe how this is done.	
	none	
C-03	How are alien species considered in the assessment?	
	n.a.	
C-04	Combination rule for multi-metrics	
	Average metric scores	Yes
	Weighted average metric scores	No
	Worst metric score	No
	Mean quality class	No
	Worst quality class	No
	Other (specify)	No
	Not relevant	No
C-05	Describe the definition of reference conditions Please specify, if answer differs between sampled habitats (see B-03)!	
	Expert knowledge: BOD5 <2 mg/L, Ptot <0.1 mg/L (yearly mean), no impoundments and hydro power plants, riparian vegetation dominated by forests and shrubs.	
C-06	Key source(s) to derive reference conditions	
	Existing near-natural reference sites	No
	Modelling (extrapolating model results)	No

	Expert knowledge	Yes
	Historical data	No
	Least Disturbed Conditions	No
	Other (specify)	No
C-07	Location of sites used to derive reference / least disturbed conditions (if applicable)	
	River Daugava from border Latvia-Belarus to downstream Jekabpils, upstream Aiviekste river mouth.	
C-08	Setting of ecological status boundaries	
	Using discontinuities in the relationship of anthropogenic pressure and the biological response.	
	Yes	
	Using paired metrics that respond in different ways to the influence of the pressure (e.g. % sensitive taxa compared to % of impact taxa for benthic invertebrates in rivers and lakes).	
	n.a.	
	High-good boundary derived from metric variability at near-natural reference sites (e.g. 5th percentile value).	
	n.a.	
	Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).	
	Yes	
	Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).	
	Yes	
	Other (specify)	
	n.a.	
C-09	Please describe the boundary setting procedure in relation to the pressure.	
	The functional groups of algae were evaluated on basis of their ecological characteristics. Nutrient status, tolerance of turbulent conditions, time sufficient for development of the given assemblage and general risk. All the groups were given a factor number (1-5). All the boundaries were set by the relative abundance of the reference (F=5) and good (F=4) taxa.	
C-10	Comments	
	None	

Annex I. 9: Poland – method description in questionnaire

A - General information

A-01	Name of person completing this questionnaire
	Joanna Picińska-Fałtynowicz
A-02	Email address of person completing this questionnaire
	Joanna.faltynowicz@imgw.pl
A-03	Institution of person completing this questionnaire
	Department of Ecology, Institute of Meteorology and Water Management
A-04	Name of assessment method (original full name)
	Metoda oceny dużych rzek na podstawie fitoplanktonu
A-05	Name of assessment method (translated into English)
	Method for large rivers assessment using phytoplankton
A-06	Abbreviation of assessment method
	IFPL
A-07	EU Member State
	Poland
A-08	Biological Quality Element
	Phytoplankton
A-09	Name and description of very large river type(s) relevant for bioassessment of this BQE in your country
	Type 19 Sandy-cleyel lowland river (river of > 5000 km^2 catchment sized dominated by sandy channel substrate) Type 20 Gravely lowland river (large river of > 5000 km^2 catchment sized dominated by gravely channel substrate) Type 21 Large lowland river (very large river of > 10000 km^2 catchment sized dominated by sandy channel substrate) Type 24 Organic lowland river (river of > 5000 km^2 catchment sized dominated by sandy and organic channel substrate) Type 25 River connecting lakes (river of > 5000 km^2 catchment sized dominated by sandy channel substrate)
A-10	Has the pressure-impact relationship of the assessment method been tested **at very large rivers**?
	Yes
	If yes, please specify pressure and impact metrics, the amount of data used, statistical significance of pressure etc.
	Ecological data from 102 sites (12 sites type 19, 3 sites type 20, 77 sites type 21, 9 sites type 24 and 1 type 25) were examined to establish pressure-impact relationship between phytoplankton metric and eutrophication gradient. The relationship between phytoplankton metric and TP (average from vegetation season) showed significant correlation (R^2=0,451); between phytoplankton metric and PO_4; TN and nitrate correlation was weak (R^2 respected 0,206; 0,288 and 0,146).
A-11	If no pressure-impact relationship was tested **at very large rivers**, which pressures does the assessment method detect, and why do you think that the method is capable of detecting these pressures?
	See above
A-12	Status of assessment method: By when is the method fully Intercalibrate-able (give month and year)?
	Not intercalibrated yet
A-13	Pertinent literature of mandatory character (e.g. official note, national standard)
	National standard: Rozporządzenie Ministra Środowiska z dnia 9 listopada 2011 r. w sprawie sposobu klasyfikacji stanu jednolitych części wód powierzchniowych oraz środowiskowych norm jakości dla substancji priorytetowych. Dz.U.11.257.1545.
A-14	Scientific literature (preferably quote references written in English)
	none
A-15	Comments
	None

B - Data acquisition

B-01	Which guidelines are followed for the sampling/surveying and sample processing?	
	J. Picińska-Fałtynowicz, J. Błachuta, 2012: Wytyczne metodyczne do przeprowadzenia badań fitoplanktonu I oceny stanu ekologicznego rzek na jego podstawie. GIOŚ Warszawa.	
B-02	Please specify sampling/survey device	
	Vertical Ruthner (preffered) or bucket	
B-03	Sampled/surveyed habitat	
	Main channel	YES
	Shorelines	no
	Secondary and side-channels	no
	Connected backwaters[29]	no
	Isolated backwaters[30]	no
	Alluvial wetlands[31]	no
	Other (specify)	no
B-04	How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	5-7 samples during vegetation season	
B-05	Sampling/survey month(s) Please specify, if answer differs between sampled habitats (see B-03)!	
	March to October	
B-06	How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	One sample per month	
B-07	Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based Please specify, if answer differs between sampled habitats (see B-03)!	
	1 l for chlorophyll examination; 1 l for diatom fraction examination; 1 l for phytoplankton examination.	
B-08	Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!	
	Samples was taken from main channel of river, from depth of 0,5 m. If algal blooms are visible on the surface additional samples of it is taken and mixt with the samples from depth of 0,5 m.	
B-09	Record of biological data: Level of taxonomical identification	
	Species/species groups level	Yes (part of indicator taxa)
	Genus level	Yes (part of indicator taxa)
	Family level	Yes (part of indicator taxa)
	Other level	no
B-10	If level of taxonomical identification differs (multiple answers on B-09), please specify what groups are mainly identified to which level.	

	TAKSON
	Achnanthidium minutissimum [komplex]

[29] Lacking upstream connection, but with downstream connection at mean water level (i.e. parapotamon)
[30] Lacking upstream and downstream connection at mean water level (i.e. plesiopotamon)
[31] Including strongly disconnected water bodies (i.e. palaeopotamon)

Actinastrum, Ankistrodesmus, Kirchneriella, Monoraphidium
Actinocyclus normanii
Amphora (without A. pediculus i A. ovalis)
Anabaena (circinalis, compacta, crassa, flos-aquae; lemmermanaii, planctonica, solitaria, spiroides)
Aphanizomenon
Asterionella formosa
Aulacoseira (ambigua, islandica, muzzanensis, subarctica)
Aulacoseira (other)
Aulacoseira granulata
Botryococcus
Ceratium
Chlamydomonales/Volvocales
Chrysophyceae ≥10μm
Chrysophyceae <10μm
Cocconeis placentula
Coelastrum
Crucigenia/Crucigeniella
Cryptomonas
Cyclostephanos (other)
Cyclostephanos dubius
Cyclostephanos invisitatus
Cyclotella [other]
Cyclotella glomerata
Cyclotella meneghiniana
Cyclotella ocellata
Cyclotella striata
Cymatopleura elliptica
Cymatopleura solea
Desmodesmus armatus
Desmodesmus communis/D. opoliensis
Diatoma tenuis
Diatoma vulgaris
Dictyosphaerium
Discostella pseudostelligera
Discostella stelligera
Euglena/Lepocinclis
Fragilaria [other] + Staurosira construens
Fragilaria crotonensis
Gomphonema/Rhoicosphenia
Kephyrion/Pseudokephyrion
Melosira varians
Microcystis
Navicula [other] + Craticula/Hippodonta/Luticola/Sellaphora
Navicula gregaria
Navicula lanceolata
Navicula menisculus
Nitzschia [pozostałe]
Nitzschia acicularis
Nitzschia fonticola
Nitzschia sigmoidea
Centrices <20 μm
Centrices ≥20 μm
Oocystis
Oscillatoriales [without Planktothrix]
Pediastrum
Peridiniales
Planktothrix [other]
Planktothrix rubescens
Planktotrhix agardhii
Planothidium frequentissimum + lanceolatum
Rhodomonas
Scenedesmus acuminatus
Scenedesmus/Desmodesmus (other)
Skeletonema
Sphaerocystis/Planktosphaeria/Eutetramorus
Staurastrum
Stephanodiscus (other)
Stephanodiscus hantzschii
Stephanodiscus minutulus
Stephanodiscus neoastraea
Surirella
Tabellaria
Tetraedron/Tetrastrum
Thalassiosira pseudonana
Thalassiosira weissflogii
Trachelomonas
Ulnaria acus
Ulnaria delicatissima var. angustissima
Ulnaria ulna

B-11	Record of biological data: How is the biota's abundance within the sample/survey measured?	
	Individual counts	no
	Percent coverage	no
	Abundance classes (ordinal scale)	no
	Relative abundance (i.e. one species relatively to other	yes

	species)	
	Other (specify)	no

B-12	Record of biological data: Abundance is related to …	
	Area	no
	Volume	yes
	Time	no
	Other (specify)	no

B-13 Please specify unit in which the biota's abundance is expressed
biomass
B-14 If biomass is measured, please specify how it is quantified.
Cell volume (Utermohl technique)

B-15 Other records of biological data (e.g. organism length, plant growth form, shoot density)
no
B-16 Comments
none

C - Data evaluation

C-01	Complete list of biological metric(s) used in the assessment	
	Trophic index and chlorophyll concentarion	
C-02	If habitats other than the main channel are considered differently in the assessment, please describe how this is done.	
	–	
C-03	How are alien species considered in the assessment?	
	no	
C-04	Combination rule for multi-metrics	
	Average metric scores	yes
	Weighted average metric scores	no
	Worst metric score	no
	Mean quality class	no
	Worst quality class	no
	Other (specify)	no
	Not relevant	no
C-05	Describe the definition of reference conditions Please specify, if answer differs between sampled habitats (see B-03)!	
	Expert knowledge: rivers with least disturbed conditions were selected.	
C-06	Key source(s) to derive reference conditions	
	Existing near-natural reference sites	no
	Modelling (extrapolating model results)	no
	Expert knowledge	yes
	Historical data	no
	Least Disturbed Conditions	yes
	Other (specify)	o
C-07	Location of sites used to derive reference / least disturbed conditions (if applicable)	
	Biebrzański National Park; Narwiański Landscape Park; and Protected Birds Area The Lower San Valley	

C-08 Setting of ecological status boundaries
Using discontinuities in the relationship of anthropogenic pressure and the biological response.
no

	Using paired metrics that respond in different ways to the influence of the pressure (e.g. % sensitive taxa compared to % of impact taxa for benthic invertebrates in rivers and lakes).
	no
	High-good boundary derived from metric variability at near-natural reference sites (e.g. 5th percentile value).
	no
	Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).
	yes
	Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).
	yes
	Other (specify)
	no
C-09	Please describe the boundary setting procedure in relation to the pressure.
	In the beginning, equidistant intervals of class boundaries were applied. In the years 2009-2011, more than 80 localities on large rivers were examined. Those with average values (from vegetation season) of TP concentration not higher than 0.13 mgP/l were selected and 95 percentile of IFPL index data set was calculated to obtain the boundary value between high and good status classes. The 95 percentile equalled 0.812 and therefore the value 0.8 of H/G boundary was accepted. The rG/M, M/P and P/B boundaries were indicated by dividing the remaining value on equidistant intervals..
C-10	Comments
	999

Annex I. 10: Romania – method description in questionnaire

A - General information

A-01	Name of person completing this questionnaire
	Nicoleta Rotaru & Ruxandra Gîrbea
A-02	Email address of person completing this questionnaire
	nicoleta.rotaru@rowater.ro & ruxandra.garbea@rowater.ro
A-03	Institution of person completing this questionnaire
	Romanian Water Authority Administratia Nationala „ Apele Române“
A-04	Name of assessment method (original full name)
	Metoda de evaluare a starii ecologice a corpurilor de apa pe baza fitoplanctonului
A-05	Name of assessment method (translated into English)
	Assessment Method for Ecological Status of the Water Bodies based on Phytoplankton
A-06	Abbreviation of assessment method
	ECO-FITO
A-07	EU Member State
	Romania
A-08	Biological Quality Element
	Phytoplankton

A-09 Name and description of very large river type(s) relevant for bioassessment of this BQE in your country

Type	Symbol	Parameters		
		Catchment area km^2	Geology	Dominant Substrate
Water sector in plain area F>3000 km^2 - ECO 11 F>5000 km^2 - ECO 12,16	RO10 RO10*	>3000 >5000	a-siliceous b- calcareous c-organic	sand, mud, clay
Water sector with floodplains in plain area F>3000 km^2 - ECO 11 F>5000 km^2 - ECO 12,16	RO11 RO11*	>3000 >5000	a-siliceous b- calcareous c-organic	sand, mud, clay
Danube-Cazane	RO12	570.900-574.850	calcareous	sand, gravel, stones
Danube- Lower sector Cazane-Calarasi	RO13	574.000-698.000	siliceous	sand, clay, gravel
Danube-Calarasi-Isaccea	RO14	698.000-780.650	siliceous	sand, clay
Danube Delta	RO15	805.300	organic	sand, mud

A-10	Has the pressure-impact relationship of the assessment method been tested **at very large rivers**?
	Not yet
	If yes, please specify pressure and impact metrics, the amount of data used, statistical significance of pressure etc.
A-11	If no pressure-impact relationship was tested **at very large rivers**, which pressures does the assessment method detect, and why do you think that the method is capable of detecting these pressures?
	The method was tested for Nutrient pollution, Organic pollution and general degradation. The indices used were Pantle Buck Saprobic Index, Simpson Diversity Index, Chlorophyll a, taxa no, numeric and biomass abundance of different algal groups.These were tested on smaller river types.

A-12	Status of assessment method: By when is the method fully Intercalibrate-able (give month and year)?
	Second RBMP (2015)
A-13	Pertinent literature of mandatory character (e.g. official note, national standard)
	Governmental Decision no 80/2011 (published in Official Journal no 265/14.04 2011)
A-14	Scientific literature (preferably quote references written in English)
	- Chiriac, G., Vintilă, Florentina, Galasiu, Luminiţa, Lungu, Aurica, Ureche D. (2007): *Assessment of the ecological status of various lotic ecosystems from the H.B. Jiu using biotic communities according to the WFD requirements*, « Oltenia. Studii şi comunicări. Ştiintele naturii », Craiova - Preda, Elena, Chiriac, G., Gălie, Andreea, Cristofor, S., Vădineanu, A. (2007): *Aspecte teoretice şi practice ale abordării multimetrice în evaluarea stării ecologice a ecosistemelor acvatice lotice din România*, Conferinţa Naţională de Ecologie, 11-14 octombrie 2007, Mamaia - Chiriac, G., Vintilă, Florentina (2005): *Inventarierea comunităţilor biotice acvatice din b.h. Mureş în conformitate cu cerinţele Directivei Cadru a apelor*, vol. « Oltenia. Studii şi comunicări. Ştiinţele naturii », XXI/2005, Craiova

A-15	Comments
	Method will be tested and validated until RBMP 2015.

B - Data acquisition

B-01	Which guidelines are followed for the sampling/surveying and sample processing?
	Draft „Water Quality - Guidance on quantitative and qualitative sampling of phytoplankton from inland waters“ Standard SR EN 15204:2007

B-02	Please specify sampling/survey device
	Suitable sampler

B-03	Sampled/surveyed habitat	
	Main channel	Yes
	Shorelines	Yes
	Secondary and side-channels	No
	Connected backwaters[32]	No
	Isolated backwaters[33]	No
	Alluvial wetlands[34]	No
	Other (specify)	No
B-04	How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	2 – 3 Times / year based on monitoring type	

B-05	Sampling/survey month(s) Please specify, if answer differs between sampled habitats (see B-03)!
	April /May; July / August; September / October.

B-06	How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!
	Large rivers are usually sampled on left bank, middle and right bank.

[32] Lacking upstream connection, but with downstream connection at mean water level (i.e. parapotamon)
[33] Lacking upstream and downstream connection at mean water level (i.e. plesiopotamon)
[34] Including strongly disconnected water bodies (i.e. palaeopotamon)

B-07	Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based Please specify, if answer differs between sampled habitats (see B-03)!
	250-500 ml for Phytoplankton analysis and 1000 ml for chlorophyll analysis.

B-08	Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!
	The samples are usualy taken from the river banks and from boat. 250-500ml of water are sampled for Phytoplankton analysis and 1000 ml for chlorophyll analysis from different depth profiles using Ruttner, Vandorn sampler devices or bucket. When turbidity is high, sampling is avoided. Samplings are made in spring, summer and autumn and more frequent in summer. Alkaline Lugol's solution is used for preservation.

B-09	Record of biological data: Level of taxonomical identification	
	Species/species groups level	Yes
	Genus level	Yes
	Family level	No
	Other level	No

B-10	If level of taxonomical identification differs (multiple answers on B-09), please specify what groups are mainly identified to which level.	
	Cyanobacteria – genus / species levels; Bacillariophyta - species levels; Chryptophyta, Dinophyta, Euglenopyta, Chlorophyta - genus / species levels;	
B-11	Record of biological data: How is the biota's abundance within the sample/survey measured?	
	Individual counts	Yes
	Percent coverage	No
	Abundance classes (ordinal scale)	No
	Relative abundance (i.e. one species relatively to other species)	No
	Other (specify)	No
B-12	Record of biological data: Abundance is related to …	
	Area	No
	Volume	Yes
	Time	No
	Other (specify)	No
B-13	Please specify unit in which the biota's abundance is expressed	
	Number of algal objects /ml	
B-14	If biomass is measured, please specify how it is quantified.	
	Determination of fresh weight by microscopic counting, cell size measurement and cell volume calculation (Utermöhl technique)	
B-15	Other records of biological data (e.g. organism length, plant growth form, shoot density)	
	No	

B-16	Comments

C - Data evaluation

C-01	Complete list of biological metric(s) used in the assessment
	Saprobic index, chlorophyll a concentration, Simpson's diversity index, taxa number, numeric abundance (Bacillariophyceae)
C-02	If habitats other than the main channel are considered differently in the assessment, please describe how this is done.
	Not applicable
C-03	How are alien species considered in the assessment?
	not applicable

C-04	Combination rule for multi-metrics	
	Average metric scores	No
	Weighted average metric scores	Yes
	Worst metric score	No
	Mean quality class	No
	Worst quality class	No
	Other (specify)	No
	Not relevant	No
C-05	Describe the definition of reference conditions Please specify, if answer differs between sampled habitats (see B-03)!	
	For large rivers there are no longer available reference conditions *in situ*. Best available sites were identified and data used. These were characterised by representative biological elements. Thanks to historical data, an image most similar to reference conditions was created, for the biological communities from sites. Also statistical analyse was used.	
C-06	Key source(s) to derive reference conditions	
	Existing near-natural reference sites	Yes
	Modelling (extrapolating model results)	No
	Expert knowledge	Yes
	Historical data	Yes
	Least Disturbed Conditions	No
	Other (specify)	No
C-07	Location of sites used to derive reference / least disturbed conditions (if applicable)	
	Danube: Cozla – Orsova sector and Gruia	

C-08	Setting of ecological status boundaries
	Using discontinuities in the relationship of anthropogenic pressure and the biological response.
	No
	Using paired metrics that respond in different ways to the influence of the pressure (e.g. % sensitive taxa compared to % of impact taxa for benthic invertebrates in rivers and lakes).
	No
	High-good boundary derived from metric variability at near-natural reference sites (e.g. 5th percentile value).
	Percentile (10%) using data from less impacted sites.
	Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).
	No
	Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).
	No
	Other (specify)
	No
C-09	Please describe the boundary setting procedure in relation to the pressure.
	Nutrient and organic pollution are reflected by Pantle Buck Saprobic Index (modified). Boundary between High/Good status for large rivers (real value = 2,3) represents 10% from existing data from less impacted sites. Boundary between good/moderate for large rivers (real value = 2,5) represents 30% from existing data from less impacted sites

C-10	Comments

Annex I. 11: Slovakia – method description in questionnaire

A - General information

A-01	Name of person completing this questionnaire
	Mária Plachá
A-02	Email address of person completing this questionnaire
	placha@vuvh.sk
A-03	Institution of person completing this questionnaire
	Water Research Institute
A-04	Name of assessment method (original full name)
	Metodika pre odvodenie referenčných podmienok a klasifikačných schém pre hodnotenie ekologického stavu vôd-vodná flora-fytoplanktón
A-05	Name of assessment method (translated into English)
	Assessment method for rivers using phytoplankton
A-06	Abbreviation of assessment method
	Phytoplankton-SK
A-07	EU Member State
	Slovakia
A-08	Biological Quality Element
	Phytoplankton
A-09	Name and description of very large river type(s) relevant for bioassessment of this BQE in your country
	The Danube. Gravel-dominated very large rivers – Very large rivers of >10,000 km^2 catchment size with channel substrates dominated by cobbles and gravels.
A-10	Has the pressure-impact relationship of the assessment method been tested **at very large rivers**?
	YES
	If yes, please specify pressure and impact metrics, the amount of data used, statistical significance of pressure etc.
	It was tested onTP on 28 annual average of EQR from 7 sites from 4 years
A-11	If no pressure-impact relationship was tested **at very large rivers**, which pressures does the assessment method detect, and why do you think that the method is capable of detecting these pressures?
A-12	Status of assessment method: By when is the method fully Intercalibrate-able (give month and year)?
	The assessment method is intercalibrate-able from December 2007.
A-13	Pertinent literature of mandatory character (e.g. official note, national standard)
	NV SR č. 398/2012 Z.z., ktorým sa mení a dopĺňa Nariadenie vlády Slovenskej republiky č. 269/2012 Z.z., ktorým sa ustanovujú požiadavky na dosiahnutie dobrého stavu vôd. (Decree of the Government).
A-14	Scientific literature (preferably quote references written in English)
	Šporka F., Makovinská J., Hlúbiková D., Tóthová L., Mužík V. Magulová R., Kučárová K., Pekárová P., Mrafková L. 2007. Metodika pre odvodenie referenčných podmienok a klasifikačných schém pre hodnotenie ekologického stavu vôd. VÚVH Bratislava, SHMU Bratislava, ÚZ SAV Bratislava, SAŽP Banská Bystrica, 288 pp. We do not have references in English. It is published only as a methodical, not as a scientific paper.
A-15	Comments
	-

B - Data acquisition

B-01	Which guidelines are followed for the sampling/surveying and sample processing?
	STN 75 7715 2008: Kvalita vody-Biologický rozbor povrchovej vody. (Slovak national standard 757715. 2008. Water quality- Biological analysis of surface water)
B-02	Please specify sampling/survey device

	Surface water sampler (bucket with rope) and planktonic net.	
B-03	Sampled/surveyed habitat	
	Main channel	YES
	Shorelines	NO
	Secondary and side-channels	NO
	Connected backwaters[35]	NO
	Isolated backwaters[36]	NO
	Alluvial wetlands[37]	NO
	Other (specify)	NO
B-04	How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	The samples are sampled every month during the vegetation season from april to october (7 times per year). If the weather condition are bad, it is possible evaluate the sampling area according to smaller number of sampling occasions. According to WFD the minimum for assessment is 6 samples per year.	
B-05	Sampling/survey month(s) Please specify, if answer differs between sampled habitats (see B-03)!	
	From April to October (7 times). There are no differences between sampled habitats	
B-06	How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? Please specify, if answer differs between sampled habitats (see B-03)!	
	There is only 1 sample per sampling occasion without replication. There are no differences between sampled habitats.	
B-07	Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based Please specify, if answer differs between sampled habitats (see B-03)!	
	It is sampled free water in 10 l bucket followed by homogenization and put in the sampling bottles with volume of 250 ml for phytoplankton analyses and 1000 ml for chlorophyll-a analyses.	
B-08	Short description of field sampling/survey procedure Please specify, if answer differs between sampled habitats (see B-03)!	
	The water for phytoplankton analyses is sampled by surface water sampler (bucket on the rope). There are no differences between sampled habitats.	
B-09	Record of biological data: Level of taxonomical identification	
	Species/species groups level	NO
	Genus level	NO
	Family level	YES
	Other level	YES
B-10	If level of taxonomical identification differs (multiple answers on B-09), please specify what groups are mainly identified to which level.	
	There are no differences in identification level	
B-11	Record of biological data: How is the biota's abundance within the sample/survey measured?	
	Individual counts	NO
	Percent coverage	NO
	Abundance classes (ordinal scale)	NO
	Relative abundance (i.e. one species relatively to other	NO

[35] Lacking upstream connection, but with downstream connection at mean water level (i.e. parapotamon)
[36] Lacking upstream and downstream connection at mean water level (i.e. plesiopotamon)
[37] Including strongly disconnected water bodies (i.e. palaeopotamon)

	species)	
	Other (specify)	YES (cells count in every group (Cyanophyta, Chromophyta, Chlorophyta, Euglenophyta) and then they are recalculated to percentage form)

Note: We have also records of species and their abundance in ordinal scale, but for the methodology is used real cells count of each taxonomic group (as I wrote above) per 1 ml transformed to the percentage form.

B-12	Record of biological data: Abundance is related to …	
	Area	NO
	Volume	Yes
	Time	NO
	Other (specify)	NO
B-13	Please specify unit in which the biota's abundance is expressed	
	Count of cells of specific taxonomic groups per 1 ml	
B-14	If biomass is measured, please specify how it is quantified.	
	Biomass is quantified as a chlorophyll_a in µg. l^{-1} (ISO 10 260: 1992)	
B-15	Other records of biological data (e.g. organism length, plant growth form, shoot density)	
	We have recorded each species with ordinal scale (percentual coverage of microscope viewing field in 9 level scale (**1**: under 1%; **2**: 1-3%; **3**: 3 – 10%; **5**: 10 – 20; **7**: 20-40; **9**:40 – 100%)	
B-16	Comments	
	-	

C - Data evaluation

C-01	Complete list of biological metric(s) used in the assessment	
	1., Abundance of Cyanophyta, Chromophyta, Chlorophyta and Euglenophyta per 1 ml recalculated to percentage form; 2., Total abundance per 1 ml; 3., biomas as Chlorophyll-a in µg. l^{-1}	
C-02	If habitats other than the main channel are considered differently in the assessment, please describe how this is done.	
	No, there are no differences between habitats.	
C-03	How are alien species considered in the assessment?	
	In phytoplankton is very hard to estimate which species is alien and which is native, therefore the alien species are not considered in the assessment.	
C-04	Combination rule for multi-metrics	
	Average metric scores	NO
	Weighted average metric scores	NO
	Worst metric score	NO
	Mean quality class	YES (From the worst taxonomic group, abundance and chlorophyll_a)
	Worst quality class	YES (in partial evaluation, from the taxonomic groups is considered the worst class)
	Other (specify)	NO
	Not relevant	NO
C-05	Describe the definition of reference conditions Please specify, if answer differs between sampled habitats (see B-03)!	
	There are no reference conditions for very large rivers in Slovakia. Therefore there were estimated by expert judgement.	
C-06	Key source(s) to derive reference conditions	
	Existing near-natural reference sites	NO
	Modelling (extrapolating model	NO

	results)	
	Expert knowledge	YES
	Historical data	NO
	Least Disturbed Conditions	NO
	Other (specify)	NO
C-07	Location of sites used to derive reference / least disturbed conditions (if applicable)	
	-	

C-08	Setting of ecological status boundaries
	Using discontinuities in the relationship of anthropogenic pressure and the biological response.
	no
	Using paired metrics that respond in different ways to the influence of the pressure (e.g. % sensitive taxa compared to % of impact taxa for benthic invertebrates in rivers and lakes).
	no
	High-good boundary derived from metric variability at near-natural reference sites (e.g. 5th percentile value).
	no
	Equidistant division of the EQR gradient (e.g. boundary setting at 0.8, 0.6, 0.4, 0.2).
	YES
	Calibrated against pre-classified sampling sites (e.g. pre-classification based on expert judgement).
	YES
	Other (specify)
	no
C-09	Please describe the boundary setting procedure in relation to the pressure.
	The classification of phytoplankton evaluation was carried out on total phosphorus (TP). According to Slovak method for ecological status assessment 90 percentile of TP is used for evaluation. Boundaries between first and second class of ecological status are set for large/ very large rivers as 0.1 or 0.2 mg/l depending on the type, and for boundaries between second and third class as 0.3 or 0.4 mg/l. We have one common method for phytoplankton in large and very large rivers, therefore we used the mean values between above mentioned boundaries (0.15 and 0.35 mg/l respectively).
C-10	Comments
	-

Annex II: Description of national assessment methods in detail

Annex II - A
Latvian method description for phytoplankton
Provided by Jolanta Jēkabsone (6.10.2015)

Annex II - B
Romanian method description for phytoplankton
METHODOLOGICAL ASPECTS AND LIMIT VALUES
used for the evaluation of Ecological Potential/Ecological Status of water bodies locateded on natural / heavily modified water bodies, based on Phytoplankton communities

Annex II - C
Estonian method description for phytoplankton (similar to Hungarian method)
provided by Kai Pirsoo

Annex II - D
German method description for phytoplankton
1) description is redrawn from publication Mischke et al (2011) for PhytoFluss 2.2

2) description for updated German method for PhytoFluss 4.0

Annex II - E
Slovak method description for phytoplankton
Provided by Mária Plachá

Annex II - A
Latvian method description for phytoplankton

Provided by Jolanta Jēkabsone (6.10.2015)

Phytoplankton in very large rivers is surveyed in Latvian rivers each year. The historical frequency varies from 2 to 6 samples per vegetation season. There are two monitoring periods: 2000-2001 and from 2009. The abundance of phytoplankton is expressed in terms of biovolume. All very large rivers belong to one river type: very large rivers of >9,000 km2, catchment size dominated by sandy channel substrate.

Table 1. The Latvian approach of phytoplankton monitoring.

Item	Description
Frequency per year	2-4 samples per vegetation season (in July, August, September).
Sampling	ISO 10260:1992 for chlorophyll a (spectrophotometry). SM 10200: 2012 for phytoplankton, Utermöhl's technique; counting, using inverted light microscope.
Sampling methods	Ruthner type Water sampler, samples at 0.5 m deep in the middle of river, fixed by Lugole solution.
Level of identification	Species level if possible, but large taxa (class, order) are also used as indicators .

Latvia uses adapted version of Hungarian Large River Phytoplankton Index that uses two parameters to assess the ecological quality of the phytoplankton: chlorophyll a and species composition metric Q.

We used 50:50 weighting factor to combine both metrics. Formula for calculation: LatRPI = (Norm Chla+Q) /2.

We used 50th, 75th 90th and 95th percentiles to set chlorophyll a boundaries. Chlorophyll a boundaries were set using River Daugava (stretch from Latvian-Belarusian border to upstream Aiviekste river mouth), which was selected as Least Disturbed Site.

For data normalization, we followed guidelines suggested by G. Borics (unpublished).

Normalized Chla (y) for large rivers are y=1.0728*EXP(-0.0584).

According to Hungarian river typology, River Daugava belongs to type 4, but rivers Venta and Lielupe belong to type 3. As the usable data set is too low to divide Latvian rivers in different types, we used equations, which describe Hungarian type 3 rivers, for normalization.

Latvia decided not to normalize composition metric Q prior to combination with chlorophyll a metric. The metric EQRs already are on the same scale, and after normalisation H/G boundary was too strict in comparison with other XGIG member countries.

We modified combination of metrics, because Latvian rivers naturally have relatively low chlorophyll a values. Use of original formula (HRPI= (2Chla+Q)/3) might lead to overestimating state of Latvian rivers. Using original formula, we got weak correlation between LatRPI index and composition metric Q (R^2=0.1876), which indicates that Chl a causes large input in the total index. When we use changed formula, correlation between Q and overall metric is better and LatRPI still has good correlation with chlorophyll a (R^2=0.6081). We used chlorophyll a values more than 18 µg/l as threshold to demonstrate that the LatRPI is sensitive against the pressures TP, P-PO_4.

Latvia used LatPRI of 27 samples to demonstrate sensitivity against the pressure TP (see figure B). The linear correlation was high (r^2 = 0.4377). In the updated version of the Latvian method chlorophyll a values are obligatory required and therefore the pressure-impact relationship was updated accordingly (19.02.2016).

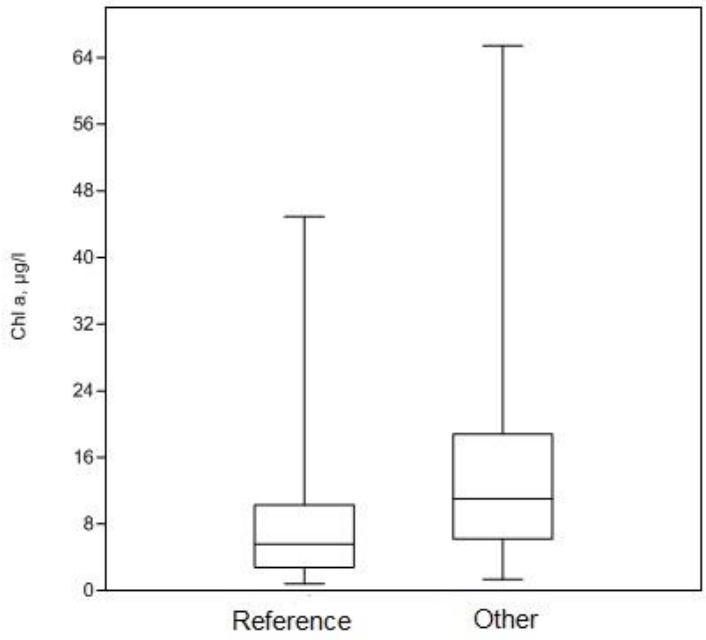

Figure 1. Chlorophyll a values for Latvian LDS and all other sites.

Table 2. Proposed boundaries for Latvian phytoplankton index.

Boundary	Chl a, mg/l	Norm Chla	Q_EQR(=Q/5)	LatRPI
H/G	5.9	0.76	0,8	0.78
G/M	**9.6**	**0.61**	**0,6**	**0.61**
M/P	25.6	0.24	0,4	0.32
P/B	31.5	0.17	0,2	0.19

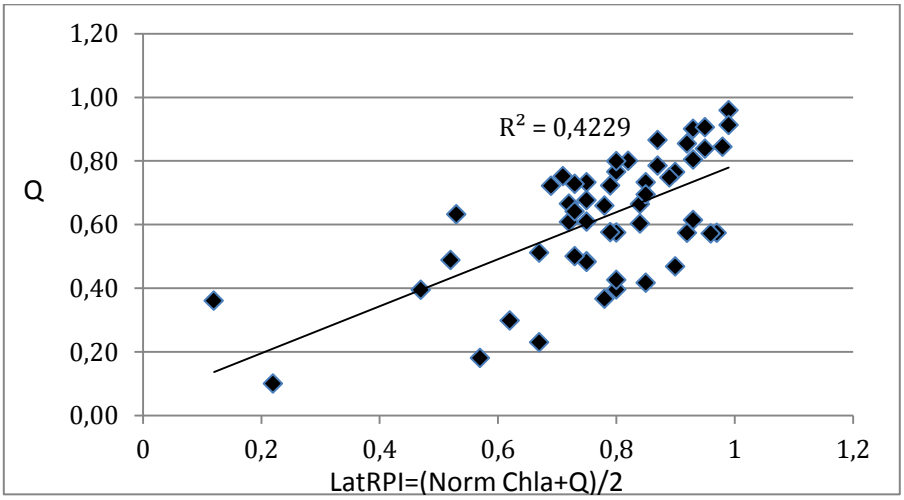

Figure 2. Latvian chlorophyll a correlation with LatRPI index.

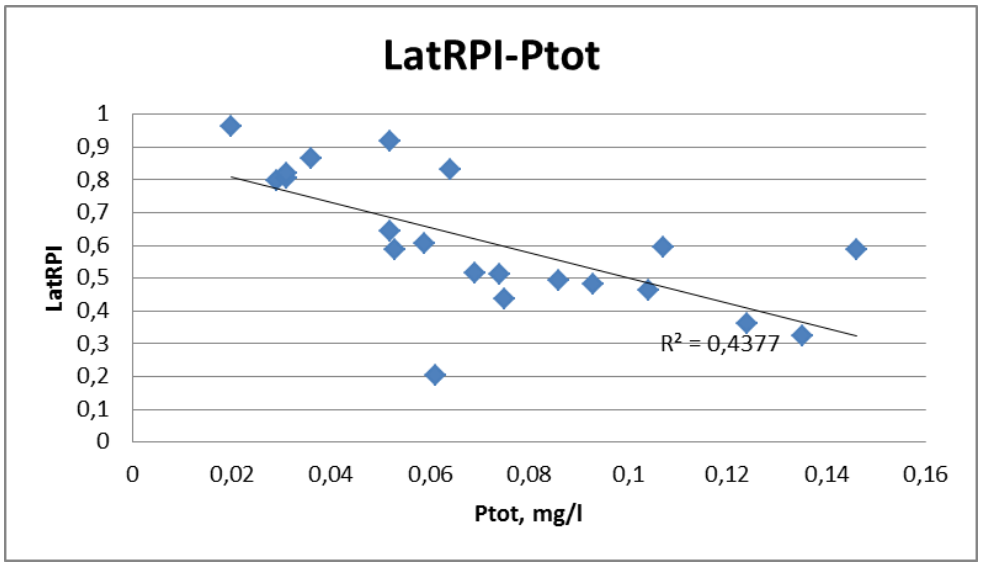

Figure 3. Relationships between Ptot and LatRPI in very large rivers.

All data set (54 samples) was tested against different pressures and impacts. Pearson correlation index showed statistically significant negative correlation between abundance of Cyanophyta and species composition metric Q (R=-0,832, p<0,01), LatRPI index (R=-0,512, p<0,05) and ecological quality assessed using national quality criteria (R=-0,328, p<0,05). This indicates successful assessment of water bodies based on functional groups. Increase in biomass of blue-green algae correlates with reduction in value of LatRPI index and quality assessment.

Annex II - B

Romanian method description for phytoplankton

METHODOLOGICAL ASPECTS AND LIMIT VALUES

used for the evaluation of Ecological Potential/Ecological Status of water bodies locateded on natural / heavily modified water bodies, based on Phytoplankton communities

Basic statements

This evaluation method based on phytoplankton communities described below is applied to all Romanian water courses and is in accordance with the principles of Water Framework Directive. The method elaboration takes into account the main pressures whom the phytoplankton/algal communities respond. The phytoplankton is sensitive at: nutrient load, organic pollution, general degradation.

The reference guidance values have been described for each typology and each metric selected. The evaluation of status is made at water body level.

Method description

For the evaluation of ecological status/potential of phytoplankton communities, based on species composition from a certain monitoring site, it is proposed the calculation of a multimetric index that includes 5 selected indices. Each metric/index was selected according to their importance for algal communities and for the evaluation of ecological status. Consequently, the calculation of Multimetric index, according to the proposed index/metric weight, is the following:

- Saprobic index (IS) 20%
- Chlorophyll “a” index a (ICL) 25%
- Simpson Diversity index (ID) 30%
- Taxa number index (INT) 15%
- Abundance index for Bacillariophycea (IAND) 10%

The calculation formula for the Multimetric Index is the following:

$$\textbf{Multimetric Index(IM)} = 0.2*EQR_{IS}+0.25*EQR_{ICL}+0.3*EQR_{ID}+0.15*EQR_{INT}+0.1*EQR_{IAND}$$

It is proposed that for the classification of ecological status/ecological potential, the multimetric index values variability domain to be divided in 5 classes as follows:

STATUS/POTENTIAL	Values
–High ecological sttaus/Maximum ecological potential	min. 0.8
–Good ecological status/Good ecological potential	min. 0.6
–Moderate ecological status /Moderate cological potential	min. 0.4
–Poor ecological status	min. 0.2
–Bad ecological status	max. 0.2

In the case (most likely) of more seasonal results for a site or a water body, an average annual multimetric index is calculated to assess the ecological status / ecological potential of the water body.

For phytoplankton, the limit values were established for the **Typologies from RO06 to RO16:**

RO06 - water stream located in plain area, ecoregion 11,12,16;

RO07 - water stream sector located in plain area, ecoregion 11;

RO08 - water stream sector located in plain area, ecoregion 12;

RO09 - water stream sector with floodplains in plain area; ecoregion 12;

RO10 - water stream sector located in plain area, ecoregion 11,12,16;

RO11 - water stream sector with floodplains in plain area, ecoregion 11,12,16;

RO12 - Danube River- Cazane area, ecoregion 12;

RO13 - Danube River– lower sector between Cazane and Calarasi; ecoregion 12;

RO14 - Danube River between Calarasi and Isaccea, ecoregion 12;

RO15 - Danube Delta, ecoregion 12;

RO16 - waterstreams affected from qualitative point of view by natural causes;

In the development of national typology, the following parameters were taken into acount: ecoregion, altitude, catchment area, geology, lithological structure, slope, annual precipitations, mean annual water temperature, water flow:q l/s/km2 and q 95% l/s and also potential ihtiofauna.

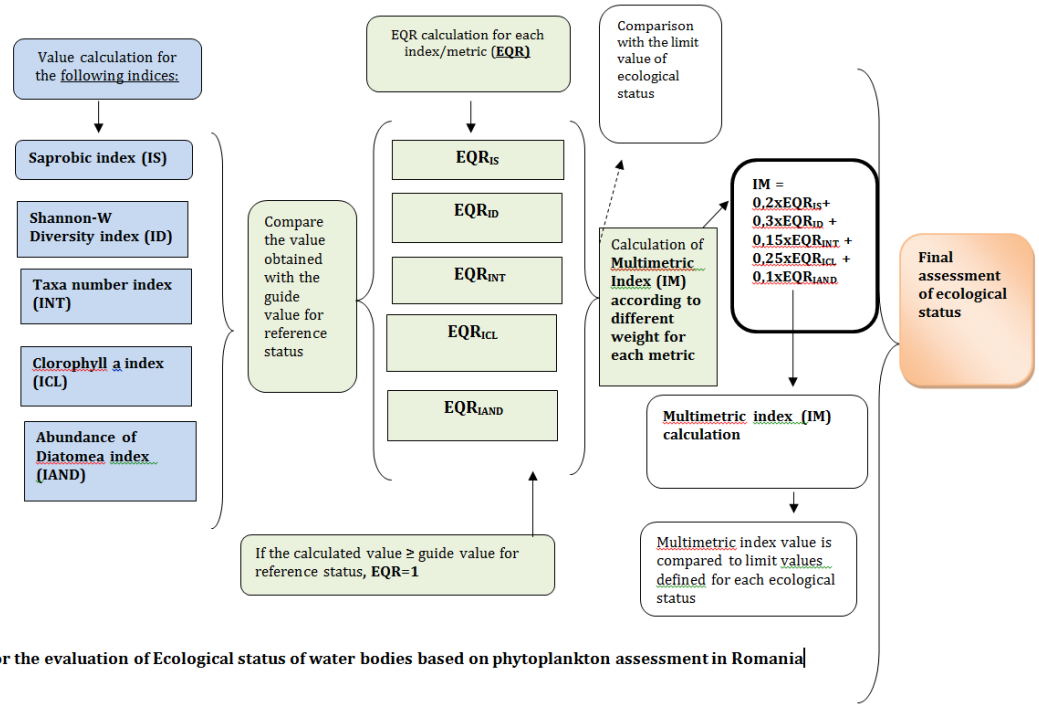

Fig. 1: Schema for the evaluation of Ecological status of water bodies based on phytoplankton assessment in Romania

Annex II - C

Estonian method description for phytoplankton

provided Kai Pirsoo

Calculation of the HRPI (=EstRPI) index step by step with examples.

Index 'EstRPI' distinguishes from Hungarian index HRPI only by final boundary setting for Estonian river Narva (see table at gthe end of this chapter).

1. Calculate biomass of each species in the phytoplankton sample using Utermöhl techique. Sum is equal to the total phytoplankton biomass in sample.

Species	Biomass (mg/L)
Urosolenia sp	1.0
Aulacoseira islandica	2.0
Cyclotella comta	3.0
Total biomass	Sum=6.0

2. Select the functional group (by Reynolds et al. 2002, Borics et al. 2007) for each species in the phytoplankton sample.

Species	Biomass (mg/L)	Functional group
Urosolenia sp	1.0	A
Aulacoseira islandica	2.0	B
Cyclotella comta	3.0	B
Total biomass	Sum=6.0	

3. Summarize biomass of the species which belonged to the same functional group.

Functional group	Biomass of functional group
A	1.0
B	5.0 (=2.0+3.0)
Total biomass	Sum =6.0

4. Calculate the relative share of each functional group to the total phytoplankton biomass.

Functional group	Relative share of the functional group to the total phytoplankton biomass
A	0.2
B	0.8
Sum	1.0

5. Select F factor value (from Borics et al. 2007).

Functional group	Relative share of the functional group to the total phytoplankton biomass	Factor F value
A	0.2	4
B	0.8	4

6. Multiply the relative share of each functional group with F factor value that is characterized for this functional group.

Functional group	Relative share of the functional group to the total phytoplankton biomass	Factor F value	Product of relative share and F value
A	0.2	4	0.8
B	0.8	4	3.2

7. Summarize these products. Sum of these scores is equal to Q index value.

Functional group	Relative share of the functional group to the total phytoplankton biomass	Factor F value	Product of relative share and F value
A	0.2	4	0.8
B	0.8	4	3.2
			Sum=Q=4.0

8. Divide Q value with number '5'; Quotient is named as EQR (Borics et al. 2007, page 478).
   Q/5= EQR=0.8

9. Calculate normalized Q value: (Formula and info that 'x=Q/5'was sent by e-mail from Gabor Borics): $Y=0.7334*x^2 + 0.3253*x – 0.0137$; x=Q/5;

   $Y=0.7334*0.8^2 + 0.3253*0.8 – 0.0137= 0.716$

10. Calculate normalized chl value; (For instance: chl a value is 2 µg/L). (HU Type 5):
    Y= - 0.01x + 1;
    Y= - 0.01*2 + 1= 0.98 (for instance, 'x' was 2 µg/L)

11. Calculated HRPI. Formula:

$$\mathrm{HRPI} = \frac{2\ \mathrm{Norm} - \mathrm{Chl} + \mathrm{Norm} - \mathrm{Qr}}{3}$$

HRPI: Hungarian phytoplankton index;

Norm - Chl: normalized chlorophyll *a* metric

Norm - Qr: normalized Q metric.

Preliminary boundaries were for H/G ≥0.8, G/M ≥0.75, M/P ≥0.7, P/B with ≥0.65.

Boundaries of Estonian method were adjusted during the option 2 exercise, because its G/M boundary turns out to be much more stringent than all other countries. Their original G/M boundary with EQR 0.75 would lead to an Estonian method G/M bias of class width of 2.6. For harmonisation Estonia decided to contribute an adjusted Estonian method (EE_adj) into the intercalibration exercise (see EE_table 1).

EE_Table 1: Adjusted boundaries for the values of EstRPI different water quality classes used intercalibration exercise , n= number of phytoplankton samples.

Very large river	HRPI				
	High n=11	Good n=4	Moderate n=2	Poor n=5	Bad n=1
Narva River	≥0.8	**<0.8 - ≥0.65**	<0.65 - ≥0.45	<0.45 - ≥0.25	<0.25

Annex II - D

German method description for phytoplankton

Provided by Ute Mischke (IGB, method developer)

The German method based on phytoplankton is called PhytoFluss.
Up to now the version PhytoFluss 2.2 is the official method.
A description for update PhytoFluss 4.0 is to find at the end of this chapter.

The PhytoFluss Index ranges from 0.5 to 5.5, where 0.5 indicates the best status and 5.5 the worst status. The values correspond to the five ecological status classes (1 to 5). To transform PhytoFluss Index to EQR the following equation can be used:

Normalized EQR = -0.2 x PhytoFluss Index +1.1

The description for PhytoFluss 2.2 is redrawn from publication
Mischke, U., Venohr, M. and H. Behrendt (2011): Using Phytoplankton to Assess the Trophic Status of German Rivers. International Revue of Hydrobiology 96 (5): 578-598

Chapter: The PhytoFluss Index assessment system (Version 2.2)
The PhytoFluss Index assessment system uses 5 parameters as metrics: "biomass", "Pennales", "Chlorophytes", "Cyanobacteria", and the indicator taxa based-index "TIP". All metrics are assessed independently, and finally averaged for the PhytoFluss Index. The values correspond to the five ecological status classes high, good, moderate, poor and bad, and can be interpreted as ecological quality (EQ) according the Water Framework Directive (EC, 2000).
For applying the PhytoFluss Index to data from a new site, information is needed for the microscopic taxa quantification (EN 15204) (2006) and photometric analyses (chla_uncorr) for at least six sampling dates per year. Taxa names must be attached to the operative German taxa code, and to the measured taxa biovolume calculated from cell counts and standard cell volumes. The determination of pelagic Centrales based on diatom slides is recommended, though not necessary (MISCHKE, 2007). PhytoFluss Index is calculated by the automatic calculation tool PhytoFluss© (BÖHMER and MISCHKE 2006 (Update May 2011 Version PhytoFluss 2.2), which calculates all vegetative means of parameters, single metrics, and the final PhytoFluss Index.

Table 1: German river types used of phytoplankton assessment

PP- river type	description	Catchment area [km²]
15.1+17.1	lowland rivers with sand, clay or gravel with small catchment size	1,000-5,000
15.2+17.2	lowland rivers with sand, clay or gravel with large catchment size	> 5,000
20.1	streams in the lowlands with high area specific run-off	>10,000
20.2	streams in the lowlands with low area specific run-off	>10,000
9.2	large, gravel rich rivers in the central mountain range	>1,000
10.1	gravel rich streams with high area specific run-off	>5,000
10.2	gravel rich streams with low area specific run-off	>5,000
23	Baltic sea tributaries	> 500

True very large rivers are in PP-type 10.1 (German section Danube, upper and middle Rhine, Neckar, Main), 20.2 (rivers: Elbe, Havel, Oder, Weser), in type 20.1 presented only by lower Rhine and in type 10.2 by upper German sections of Elbe and Weser. The lower river Saale is attached to type 15.2 although it is partly large than 10.000km^2.

Table 2: Biomass metric bases on seasonal mean concentration of chlorophyll [µg L^{-1}]. The upper boundaries for chlorophyll a plus phaeophytin a (chla_uncorr) are given for the five WFD status classes in different river type groups (heading line; for codes see Table 1). The underlying boundaries for total phosphorus concentrations (TP; µg L^{-1}) are given accordingly.

German river types:	10.1 & 20.1		9.2, 15.2 & 17.2		20.2, 10.2 & 23	
	low response		high response		very high response	
status class / parameter	TP	chl_a uncorr	TP	chl_a uncorr	TP	chl_a uncorr
High	50	10.1	54	20	54	30
Good	135	17.5	90	33	90	52
Moderate	220	30	150	55	150	90
Poor	300	51	250	90	250	155
Bad	>300	>51	>250	>90	>250	>155

A) Biomass metric

The boundaries for the biomass metric (Table 2) approximately fit the different nutrient response curves of the phytoplankton biomass, measured as chlorophyll *a* uncorrected for phaephytin *a* (chla_uncorr; Fig. 5, 6), along the 75% percentiles within the five TP classes. Regression curves were mathematically fitted to cross the boundaries along the parameter responses, and were transformed to index "calculation functions" operating between 0.5 to 5.5.

For the three groups of river types with different nutrient response (Table 2), the biomass index is calculated by the following functions for assessment:

(i) low response biomass index = 1.8527x Ln(Chla_uncorr) – 2.7981

(ii) high response biomass index = 1.9907x Ln(Chla_uncorr) – 4.4749

(iii) very high response biomass index = 1.8168x Ln(Chla_uncorr) – 4.6772

Biomass indices smaller than 0.5 are set to 0.5, and values larger than 5.5 are set to 5.5 for further PhytoFluss Index calculations. This rule is to restrict the index range to the range of empirical-based relationships.

Special rules within the PhytoFluss assessment system, and all status class boundaries including those for the following taxonomic metrics (Pennales Index (B), Chlorophyte Index (C) and Cyanobacteria Index (D) are summarized in Table 3.

B) The Pennales Index

This metric is based on the decreasing contribution of all Pennales taxa to total phytoplankton biovolume as eutrophication increases (Fig. 4A). The boundaries were derived separately for each river type (not shown). The parameter % Pennales was sensitive in all rivers, except for low-specific run-off streams (river type 10.2+20.2). Among the status worse than good (i.e. moderate, poor, and bad), the distribution of % -Pennales did not differ significantly, so only the class boundaries between "high/good" and "good to moderate" status was defined. In our proposed assessment method, only the high and good status are assessed, and below this, the Pennales Index is not applied.

C) Chlorophyte Index

This metric is based on the increasing biomass of Chlorophytes with increasing eutrophication (Fig. 4 B; see also KLOSE, 1968). It is only applicable to, and sensitive to, streams with low specific run-off (type 10.2+20.2), and the Baltic tributaries. In moderate and good trophic status, the Chlorophytes distribution did not differ significantly and no reference sites were available, thus only the class boundary between moderate and poor status was defined. In the results, only the poor and bad status are assessed. Above this status, the result of the biomass metric is set again here, instead of the Chlorophyte index.

D) Cyanobacteria Index

This metric is based on the increasing proportion of Cyanobacteria to total phytoplankton biovolume with increasing eutrophication (Fig. 4C). The metric is not applicable and sensitive in the river types with high specific run-off (type 10.1+20.1), or in type 10.2, and not when Cyanobacteria biovolume remains below 0.5 $mm^3\ L^{-1}$. For the status class high to moderate, the Cyanobacteria distribution does not differ significantly, and no reference sites are available, so only the class boundary "moderate to poor status" was defined. In the results, only the poor and bad status is assessed in some river types. Above this class status, the result of the biomass is set again, instead of the Cyanobacteria index.

Some situations are explicitly excluded from the Cyanobacteria assessment: (i) when potamoplankton biomass itself is very low, and (ii) when cyanobacteria may have derived for examples from lake outlets into

river sections; these situations are excluded by the rule > 0.5 mm^3 L^{-1} cyanobacteria biovolume, and the status is set generally as "good status" for the assessment.

D) TIP: Indicator taxa of the potamoplankton

The taxa of the Type-specific Index Potamoplankton (TIP), their trophic scores (TI), and weighting factors (WF), are listed in Table 4. The scores must be selected according to the river type to which the investigated site belongs. In preparation for index calculation, the percentage of each taxa to the total biovolume (%; DW) must be calculated based on seasonal mean values.

The index calculation for all indicator taxa found at one river site follows the equation:

$$TIP = \frac{\sum(DW_i \times TI_i \times WF_i)}{\sum(DW_i \times WF_i)}$$

The resulting TIP values (index values) smaller than 0.5 are set to 0.5, and values larger than 5.5 are set to 5.5 for further PhytoFluss Index calculations. This rule produces a classification with equidistant classes. When the TIP is applied to the whole data set of German rivers, all the status classes are covered (Fig. 4 D).

All metric results must be arithmetic averaged to calculate the final PhytoFluss Index. The resulting scores and their meanings are: < 1.5 "high", 1.5 - <2.5 "good", 2.5 - <3.5 "moderate", 3.5 - <4.5 "poor ", ≥ 4.5 "bad".

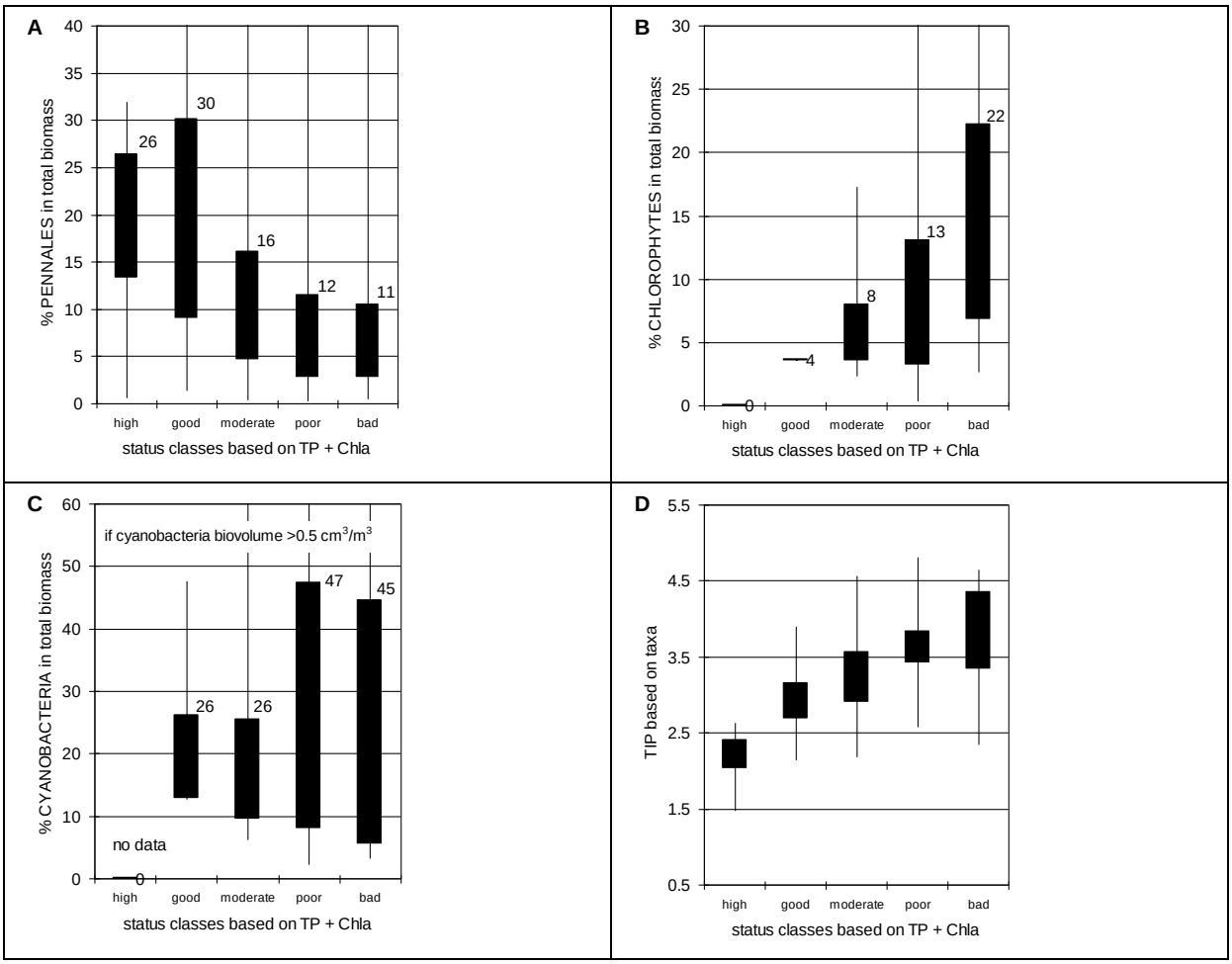

Figure 4 (redrawn from Mischke et al. 2011): Distribution of the proportion of Pennales (graph A; N = 171), Chlorophytes (graph B; N = 96) and Cyanobacteria (graph C; N = 83) to total biovolume of phytoplankton and values by Trophic Index Potamoplankton (TIP in graph D; N =314) in sensitive river types (boundary details in Table 4) grouped in the five status classes pre-assessed by TP and chlorophyll a classes (see Table 2) as box-plots (black balks presenting the range between the 25 and the 75% percentiles).

Table 3 (redrawn from Mischke et al. 2011): Overview of the status class boundaries based on the contribution of the taxonomic groups Chlorophytes (Chloro), Pennales or Cyanobacteria (Cyano) to total phytoplankton biovolume (%). For details see text. Special rules for index applications at only some of the five status classes are highlighted in the grey shaded areas. Metrics "biomass" and "TIP" (Trophic Index Potamoplankton, Table 4) are calculated according special calculation functions listed in the text. The different indices are to apply to the adapted river types listed in column 1. For characters of river types see Table 1.

river types applied	Index name	high	good	moderate	poor	bad
20.2; 10.2; 23	**Chloro**	if < 5.1%, than use again the result of the biomass metric			5.1….15%	> 15 %
9.2	**Pennales**	> 29.9 %	15….29.9 %	< 15% no application		
20.1; 15.1; 17.1; 23	**Pennales**	> 19.9 %	15….19.9 %	< 15% no application		
10.1; 15.2; 17.2	**Pennales**	> 24.9 %	20….24.9 %	< 20% no application		
all	**Cyano**	special rule: if class biovolume is < 0.5mm^3 L^{-1} = "good status", otherwise				
9.2; 15.1; 17.1; 23	**Cyano**	and if < 10%, than use again the result of the biomass metric			10.1….20%	> 20 %
15.2; 17.2	**Cyano**	and if < 20%, than use again the result of the biomass metric			20.1….40%	> 40 %
20.2	**Cyano**	and if < 2%, than use again the result of the biomass metric			2.1….5%	> 5 %
23	**Cyano**	< 0.0011 %	> 0.001..5%	>5….10 %	10.1….20%	> 20 %
	Index value for metrics above	**1**	**2**	**3**	**4**	**5**
all	**TIP and biomass metric value**	<1.51	1.51 - 2.5	2.51 - 3.5	3.51 - 4.5	> 4.5

Annex II – D-2
Description of German method PhytoFluss 4.0

The method PhytoFluss 4.0 is an update after national evaluation in two research projects with current monitoring data (Mischke & Riedmüller 2013; Mischke 2016), and is foreseen to become the official method in 2016 in Germany after decision by the German Federal States.

To achieve a stronger pressure-impact relationship to total phosphorus was the main motive to improve the method (see Figure DE_2 in corresponding chapter).

The changes of assessment with the PhytoFluss method are concerning the following metrics:

the metric "Biomass",

the metric "TIP"

the algal class metrics

the weighting factors for the final total index.

A) Metric "Biomass I"

The input parameter for metric "biomass I" is the chlorophyll a corrected for phaeophytin a (see ISO standard 1992 and specified in German version DIN 38409-60:2015-09 with ethanol). The former parameter was chlorophyll a plus phaeophytin a (chla_uncorr). Secondly, the metric "biomass I" is now based on the assessment of the components "seasonal mean" and "maximum value". The maximum value is selected from the vegetation period (April-October) and is assessed with separate boundaries in order to assess algal blooms. The biomass I index is calculated by the functions for assessment specified for the German river types (PP-type; see tables 4a and 4b).

Table PhytoFluss_4a: Upper boundaries chlorophyll a –DIN (µg/L) for seasonal mean for status classes and the functions for assessment

German PP-type:	10.1	20.1	9.2	15.1 +17.1	15.2 +17.2	10.2	20.2	23
H/G	7,9	7,9	15,6	15,6	15,6	23,4	23,4	23,4
G/M	13,5	13,5	25,7	25,7	25,7	40,6	40,6	40,6
M/P	23,2	23,2	42,7	42,7	42,7	70,2	70,2	70,2
P/B	39,78	39,78	70,3	70,3	70,3	122	122	122
Assess-ment function	Chla_DIN_function = ln(Chla_DIN)*1,8527 + (-2,322)		Chla_DIN_function = ln(Chla_DIN)*1,9907 + (-3,97)			Chla_DIN_function = ln(Chla_DIN)*1,8168 + (-4,227)		

Table PhytoFluss_4b: Upper boundaries for maximum chlorophyll a –DIN (µg/L) for status classes and the functions for assessment

German PP-type:	10.1	20.1	9.2	15.1 +17.1	15.2 +17.2	10.2	20.2	23
H_G	**15,8**	**15,8**	**31,2**	**31,2**	**31,2**	**46,8**	**46,8**	**46,8**
G_M	27,0	27,0	51,5	51,5	51,5	81,1	81,1	81,1
M_P	46,4	46,4	85,4	85,4	85,4	140,4	140,4	140,4
P_B	79,6	79,6	140,6	140,6	140,6	244	244	244
Assess-ment function	MAX DIN function = ln(MAX Chla_DIN)*1,8527 + (-3,68)		MAX DIN function = ln(MAX Chla_DIN) *1,9907 + (-5,35)			MAX DIN function = ln(MAX Chla_DIN) *1,8168 + (-5,487)		

B) Metric TIP

The metric „TIP“ is developed complete new. Three new indicators lists with trophic scores are available, which are specific for the following three eco-regions

- “Danube and its large tributaries” (TIP_Donau with 109 taxa; sites in Germany),
- “high lands” (TIP_M with 135 taxa) and
- “lowlands” TIP_T with 136 taxa).

The trophic index is specific for phytoplankton species. The trophic scores are calibrated and arranged along the scale of total phosphorus (TP). It is new, that each samples is assessed before the annual index values is calculated by averaging. The equation is the same which use trophic scores (TI), and weighting factors (WF) of all indicator species found in one sample. The annual TIP index is correlated to a certain TP value. The final assessment of TIP annual index is done by comparing with TP boundaries for each status class and which are the same for all river types.

The index calculation for all indicator taxa found at one river site follows the equation:

$$TIP\_2013 = \frac{\sum(BK_i \times TI_i \times WF_i)}{\sum(BK_i \times WF_i)}$$

Table to gain the corresponding biovolume class (AK) based on biovolume of taxon :

Biovolumes of taxa (mm³/l)	**BK**
≤ 0,0001	1
> 0,0001-0,001	2
> 0,001-0,01	3
> 0,01-0,1	4
> 0,1-1	5
> 1-10	6
> 10	7

The full list of indicator taxa and scores are listed in table Phytofluss-4d (Mischke & Riedmüller 2013). If number indicator taxa falls below 4 taxa in average, method is not applicable.

C) Algal class metrics

The Metrics Pennales-, Chloro- und Cyano-Index used in PhytoFluss version 2.2 are not used any longer. None of the former three metrics are sensitive against total phosphorus (TP) in the current German data set (2007-2013). The former algal class metrics are eliminated without any substitute in the updated PÜhytoFluss 4.0 version.

D) Weighting factors for the metrics for calculating the total index PhytoFluss 4.0

The biomasses of phytoplankton remain low in the full range of pressure (total phosphorus) in very large rivers with high run-off (PP type 10.1, 201), while the indicator taxa (metric TIP) are more sensitive to TP than in the German rivers with low run-off (PP type 10.2, 20.2), in which biomass_I response more sensitive. Therefore, the metrics TIP and biomass_I will get different weighting factors in the calculation of total index for each river type in PhytoFluss 4.0.

Table PhytoFluss_4c: Weighting factors for metric results for calculating total index (draft)

German PP-type:	**10.1**	**20.1**	**9.2**	**15.1 +17.1**	**15.2 +17.2**	**10.2**	**20.2**	**23**
Metric biomass_I	**1**	**1**	**1**	**1**	**1**	**2**	**2**	**2**
Metric TIP	3	3	1	1	1	1	1	1

Table PhytoFluss_4c: Trophic score (TI) and weighting factor (WF) for indicator taxa in TIP_2013

Indicator taxa list German method PhytoFluss 4.0	Danube and tributaries		Rhine and highland rivers		lowland rivers	
Indikatortaxon	TI	WF	TI	WF	TI	WF
Acanthoceras zachariasii					11.77	2
Achnanthes catenata			0.46	1		
Achnanthes minutissima - Sippen	1.92	1	6.20	1		
Actinastrum hantzschii	219.30	1	228.14	1	143.66	1
Actinocyclus normanii	14.06	2	27.25	2		
Amphora ovalis	1.78	1	10.52	1	32.24	1
Anabaena compacta					430.49	2
Anabaena lemmermannii			0.10	2		
Anabaena planctonica	545.23	1	587.64	1	252.78	2
Anabaena spiroides					181.19	2
Anabaena viguieri	514.91	2	406.19	1		
Ankistrodesmus falcatus			11.09	4	0.21	3
Ankistrodesmus fusiformis					12.36	3
Ankistrodesmus gracilis					2.60	1
Ankyra judayi					0.46	2
Ankyra lanceolata					21.61	1
Aphanizomenon aphanizomenoides	509.99	2	543.02	1		
Aphanizomenon gracile	277.07	1	277.40	1		
Aphanocapsa delicatissima			0.49	1		
Aphanocapsa holsatica	8.39	1			71.19	4
Aphanothece minutissima	354.01	1	361.23	1		
Asterionella formosa	29.40	1	43.87	1	151.76	2
Aulacoseira ambigua	31.66	1	46.25	1	301.86	2
Aulacoseira distans					0.15	3
Aulacoseira granulata	149.12	1	148.10	1		
Aulacoseira islandica			0.67	3		
Aulacoseira muzzanensis	398.83	1	472.24	1		
Aulacoseira pusilla			140.47	3	158.20	3
Aulacoseira subarctica			31.94	2		
Bitrichia chodatii			0.42	1		
Ceratium hirundinella	0.99	1	3.65	1		
Chlorella			410.94	2	347.85	1
Chlorogonium					102.01	2
Chlorotetraedron incus					9.64	3
Choricystis chodatii	0.02	1	0.29	1		
Chromulina					88.62	2
Chromulina					88.62	2
Chroococcus limneticus			0.27	1	7.48	3
Chroomonas nordstedtii					435.92	2
Chrysochromulina parva					13.15	2

Indicator taxa list German method PhytoFluss 4.0	Danube and tributaries		Rhine and highland rivers		lowland rivers	
Indikatortaxon	TI	WF	TI	WF	TI	WF
Chrysococcus minutus					0.02	1
Chrysolykos planctonicus	2.06	1	0.15	1		
Closteriopsis acicularis					33.38	2
Closterium aciculare	23.56	1				
Closterium acutum	0.63	4	22.05	4	1.29	3
Closterium acutum var. linea	53.07	2				
Closterium moniliferum	2.98	1			142.03	2
Closterium pronum			31.94	1		
Closterium strigosum	2.98	1				
Cocconeis pediculus					396.59	2
Cocconeis placentula	36.69	1	48.76	1		
Coelastrum pseudomicroporum	0.28	3	1.27	3		
Coelastrum reticulatum	3.21	1	1.84	1		
Cosmarium depressum	7.79	3	0.30	3		
Cosmarium humile					109.98	3
Crucigenia quadrata	0.33	2	6.53	2		
Crucigeniella apiculata	314.43	1	323.49	1	161.40	2
Crucigeniella crucifera	349.25	1				
Crucigeniella rectangularis	4.01	1	11.69	1		
Cyclostephanos delicatus	200.96	1				
Cyclostephanos dubius			221.76	1		
Cyclostephanos invisitatus	138.51	1				
Cyclotella atomus	156.35	1	148.10	1		
Cyclotella choctawhatcheeana					665.22	4
Cyclotella comensis	0.30	2	0.97	2	0.01	4
Cyclotella cyclopuncta	0.79	1	1.57	1		
Cyclotella delicatula			0.71	3		
Cyclotella distinguenda	3.59	1			0.53	4
Cyclotella kuetzingiana	0.26	4	1.65	4		
Cyclotella meneghiniana	194.74	1	202.33	1		
Cyclotella ocellata	1.06	1	4.28	1	0.83	1
Cyclotella radiosa	3.46	1	9.46	1		
Cymatopleura elliptica	16.29	2	119.86	2	165.39	4
Cymatopleura solea					138.76	2
Cymbella affinis	2.39	3	0.32	3		
Diatoma ehrenbergii	0.24	2	1.49	2		
Diatoma moniliformis					308.62	3
Diatoma tenuis	1.54	2	23.25	2		
Diatoma vulgaris	25.37	1				
Dictyosphaerium			156.15	1		
Dictyosphaerium ehrenbergianum					145.28	3

Indicator taxa list German method PhytoFluss 4.0	Danube and tributaries		Rhine and highland rivers		lowland rivers	
Indikatortaxon	TI	WF	TI	WF	TI	WF
Didymocystis bicellularis	6.24	2	28.73	2	79.46	3
Didymocystis fina					38.34	4
Didymocystis planctonica			386.70	1		
Didymogenes			162.12	3		
Dinobryon bavaricum	0.05	3	0.52	3		
Dinobryon crenulatum	4.65	3	0.38	3	0.39	4
Dinobryon divergens	0.41	1	1.94	1	15.80	1
Dinobryon sertularia	0.21	2	1.34	2		
Dinobryon sociale			1.74	1	5.78	1
Dinobryon sociale var. americana	0.28	4				
Dinobryon sociale var. stipitatum	0.38	4	0.64	4		
Discostella pseudostelligera	17.54	1				
Discostella stelligera	3.72	2	17.85	2		
Elakatothrix			87.26	3		
Entomoneis costata					0.01	1
Erkenia subaequiciliata			2.04	1	0.00	3
Euglena acus	526.31	1	563.12	1		
Euglena ehrenbergii	456.35	2	484.12	2	167.78	4
Euglena hemichromata	507.45	1	442.80	1	647.10	1
Euglena oxyuris	434.74	1	551.93	1		
Euglena tripteris	567.53	2				
Euglena variabilis					270.67	3
Euglena viridis	381.53	1	401.40	1	354.16	1
Eunotia					0.97	2
Fragilaria acus	88.96	1			122.38	1
Fragilaria arcus			54.21	2	0.62	3
Fragilaria capucina	6.24	1			60.28	1
Fragilaria construens	1.23	1	54.21	1	2.98	1
Fragilaria crotonensis	0.47	2	8.51	2	140.40	0.5
Fragilaria cyclopum	309.25	2	317.90	2		
Fragilaria nanana			0.11	4		
Fragilaria pinnata	1.65	3	0.88	3		
Fragilaria tenera					0.01	2
Fragilaria ulna	0.85	1	51.41	1	28.69	2
Fragilaria ulna angustissima - Sippen					91.55	2
Gloeotila					295.04	2
Golenkinia radiata					36.19	3
Gomphonema parvulum					20.85	3
Gomphosphaeria aponina					42.02	3
Goniochloris mutica					208.23	2
Goniochloris pulchra	427.12	4	447.16	4		

Indicator taxa list German method PhytoFluss 4.0	Danube and tributaries		Rhine and highland rivers		lowland rivers	
Indikatortaxon	TI	WF	TI	WF	TI	WF
Goniochloris sculpta					0.00	2
Gymnodinium uberrimum					0.00	2
Gyrosigma acuminatum					25.51	2
Gyrosigma nodiferum	1.14	1				
Kephyrion littorale	0.73	1	1.03	1		
Kephyrion planctonicum			0.15	4		
Kirchneriella contorta					1.71	2
Kirchneriella lunaris					48.15	3
Kirchneriella obesa	566.27	4	598.05	4		
Lagerheimia ciliata					238.18	3
Lagerheimia genevensis					155.79	1
Lagerheimia wratislawiensis	532.53	1	571.00	1		
Lepocinclis	549.90	1	589.82	1		
Lepocinclis ovum					10.92	3
Limnothrix planctonica					288.16	1
Melosira varians	119.50	1				
Merismopedia					84.84	2
Micractinium pusillum					54.51	2
Microcystis aeruginosa	445.78	1	468.19	1	147.72	2
Microcystis wesenbergii					341.47	1
Monoraphidium arcuatum			168.95	1	192.87	2
Monoraphidium circinale					223.33	3
Monoraphidium contortum	76.75	1				
Monoraphidium griffithii			246.98	1		
Mougeotia	0.55	1	2.39	1	16.79	3
Navicula antonii	1.85	1				
Navicula gregaria					45.50	2
Navicula lanceolata	111.00	1	107.82	1	94.58	2
Navicula menisculus	0.92	4	3.85	4		
Navicula radiosa					119.80	1
Navicula rhynchocephala	49.29	2	66.98	2		
Navicula slesvicensis	2.77	3	6.89	3		
Navicula tripunctata					20.12	3
Neodesmus danubialis					245.51	2
Nephrochlamys subsolitaria					17.84	2
Nitzschia acicularis var. acicularis					150.14	1
Nitzschia amphibia	2.22	1	12.32	1		
Nitzschia constricta			350.69	1		
Nitzschia frustulum	1.43	2	0.60	2		
Nitzschia fruticosa	103.10	1	102.27	1		
Nitzschia graciliformis			126.37	3		

Indicator taxa list German method PhytoFluss 4.0	Danube and tributaries		Rhine and highland rivers		lowland rivers	
Indikatortaxon	TI	WF	TI	WF	TI	WF
Oocystis borgei					183.54	3
Oocystis lacustris					223.33	2
Pandorina morum			175.72	1		
Pediastrum duplex					117.27	1
Pediastrum simplex	5.39	1	13.70	1		
Peridiniopsis cunningtonii			0.75	4		
Peridiniopsis polonicum	569.11	3				
Peridinium cinctum					63.73	2
Peridinium willei			0.21	4		
Phacotus lenticularis	1.33	1	4.76	1		
Phacus longicauda	557.83	1	583.13	1		
Phacus pleuronectes					30.77	1
Phacus pyrum					169.36	1
Phacus triqueter					3.89	4
Pinnularia					11.34	1
Planctonema lauterbornii					6.58	3
Planktosphaeria gelatinosa					4.45	2
Pseudanabaena limnetica					267.12	1
Pseudogoniochloris tripus	329.66	2	339.96	2		
Pseudokephyrion entzii	1.02	1				
Pseudopedinella erkensis	9.03	1	8.97	1	1.97	2
Pseudotetrastrum punctatum			334.52	3	177.26	3
Pteromonas	552.75	1	455.72	1		
Pteromonas aculeata					274.20	3
Pteromonas angulosa					251.33	3
Pyramimonas					0.29	3
Raphidocelis sigmoidea			148.10	2		
Rhodomonas lacustris var. nannoplanctica					113.57	2
Rhodomonas lens	2.57	1	7.26	1	1.49	1
Rhoicosphenia abbreviata					335.03	2
Romeria elegans	463.14	1	487.98	1		
Scenedesmus			215.33	1		
Scenedesmus acuminatus			300.84	1		
Scenedesmus arcuatus					13.98	1
Scenedesmus bernardii					1.12	3
Scenedesmus brasiliensis					65.89	2
Scenedesmus caudato-aculeolatus	1.59	4				
Scenedesmus costato-granulatus			113.68	1	68.11	2
Scenedesmus dimorphus			381.71	1		
Scenedesmus disciformis					34.56	3
Scenedesmus dispar	237.17	2	234.47	2		

Indicator taxa list German method PhytoFluss 4.0	Danube and tributaries		Rhine and highland rivers		lowland rivers	
Indikatortaxon	TI	WF	TI	WF	TI	WF
Scenedesmus ecornis					98.76	3
Scenedesmus ellipticus					3.41	2
Scenedesmus falcatus			70.62	3		
Scenedesmus granulatus	385.93	2	283.33	2		
Scenedesmus gutwinskii					18.94	3
Scenedesmus magnus					663.92	4
Scenedesmus obliquus	0.23	2	1.41	2	0.77	2
Scenedesmus obtusus			189.12	2		
Scenedesmus obtusus			189.12	2		
Scenedesmus opoliensis			366.42	2		
Scenedesmus praetervisus					0.34	4
Scenedesmus quadricauda	182.15	1	182.45	1		
Scenedesmus sempervirens/tenuispina					105.35	4
Scenedesmus spinosus					0.00	2
Scenedesmus subspicatus					259.98	3
Scenedesmus verrucosus	570.65	2				
Schroederia setigera					2.26	2
Schroederia spiralis					0.00	4
Skeletonema subsalsum			208.85	1		
Snowella litoralis					0.01	3
Spermatozopsis exsultans			396.55	1		
Staurastrum			57.15	2		
Staurastrum paradoxum					27.06	3
Staurastrum tetracerum					57.01	3
Stephanodiscus minutulus	4.32	1				
Stephanodiscus neoastraea	27.31	1				
Strombomonas					0.72	2
Surirella brebissonii			312.26	1	281.21	1
Synechocystis aquatilis					0.13	3
Tabellaria flocculosa	0.44	2	2.15	2		
Tetrachlorella alternans					43.48	3
Tetraedron triangulare	538.29	2	578.43	2		
Tetraedron trigonum					29.72	2
Tetrastrum komarekii					82.11	3
Tetrastrum staurogeniaeforme			162.12	1		
Thalassiosira lacustris			253.16	2		
Trachydiscus sexangulatus					40.60	4
Treubaria schmidlei	452.88	2	371.57	2		
Treubaria setigera	0.68	1				
Treubaria triappendiculata					52.70	2
Tribonema monochloron					664.34	2

Indicator taxa list German method PhytoFluss 4.0	Danube and tributaries		Rhine and highland rivers		lowland rivers	
Indikatortaxon	TI	WF	TI	WF	TI	WF
Uroglena	1.72	1	0.25	1		
Westella botryoides			312.26	4		

Literature about German PhytoFluss 4.0 method:

DIN 38409-60:2015-09: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung - Summarische Wirkungs- und Stoffkenngrößen (Gruppe H) - Teil 60: Photometrische Bestimmung der Chlorophyll-a-Konzentration in Wasser (H 60)

Mischke & Riedmüller (2013): Überarbeitung des Phytoplanktonverfahrens nach WRRL für Fließgewässer und Tool PhytoFluss 3.0. Report project part in UBA FKZ 3710 24 207; Berlin, 14.10.2013

Mischke, U (2016): Überarbeitung des Bewertungsverfahrens ‚PhytoFluss'. Report in UBA FKZ: 3714222110.

ISO 10 260(1992): Measurement of biochemical parameters. Spectrometric determination of the chlorophyll-a concentration.

Annex II - E

Slovak method description for phytoplankton

Provided by

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Status: 10.02.2014

Slovak approach for ecological status assessment based on phytoplankton in very large rivers

According to WFD is recommended to estimate ecological status based both on taxonomic composition and abundance. Slovak national method was calculated from the data of large and very large rivers (Danube and its main tributaries) sampled from 1989 to 2005.

Reference condition

Derivation of reference values were based on expert judgement, because there are no real reference sites for large lowland rivers in Slovakia. (experts from Institute of Botany, Institute of Zoology of Slovak Academy of Sciences, discussion at meetings of Slovak Algological Society and Slovak Limnological Society). The another problem was that there was no presence of whole scale of water quality in this relevant water type (high–bad).

Criteria for selection of metrics

The metrics were selected in that way, they will reflect the broad range of pressures (organic pollution, eutrophication, toxicity) based on species diversity and abundance. The requirement was also to have a simple and applicable methodology and that the metrics should reflect the impact of stressors. Therefore the candidate metric were ratio of a species number, percentage presence of individuals groups (Cyanophyta, Chromophyta, Chlorophyta, Euglenophyta), selected positive and negative indicators, abundance (cell per ml) and biomass (concentration of chlorophyll-a). Based on analysis results three metric were selected (percentage presence of individual group, phytoplankton abundance and phytoplankton biomass as concentration of chlorophyll-a). The data were tested as mean, median and 90. percentile of whole vegetation period (from April until September, 6 times). Today are used mean values of data sampled from April until October (sampled every month, 7 times per vegetation period).

Sampling procedure:

The free surface water is sampled and putted in the sampling bottles: Chlorophyll -a (1 Liter), quantitative analyse of phytoplankton 250 ml bottle. We also sample water for qualitative analyse of phytoplankton (100 ml), concentrated during plankton net with 10 micrometer mesh size. Results of qualitative analyses do not enter directly into ecological status assessment based on phytoplankton.

Analyse procedure:

Analyse procedure is divided into three steps.

Biomass: Chlorophyll – a is carried out according to ISO 10 260 (1992). The result of this analyze is **concentration of Chlorophyll in µg.l^{-1}**.

Quantitative analyses: of phytoplankton is undertaken according to Relevant National Guidance Standarts (STN 757715) using counting chamber Cyrus I. It is not sedimatation chamber as Utermohl chamber and others chambers mentioned in EN 15 204. Due to that fact we performed comparison test between two different counting analyses. These tests were performed both in artificial samples (cultures of selected algae) and natural samples from rivers in four level concentrations. The result was comparable according to statistical analyses (paired tests T-test, F-test). Small differences were found in case of small number of cells in samples (<500 cells per ml) together with very high number of cells (>20000 cells per ml) and with high portion of detritus. Chamber Cirus I. has 40 x 40 squares and the proportion of 125 x 125µm. The number of cells in one millilitre is recalculated according to the table and equation mentioned below.

Tab 1: Recalculation of number of organism per 1 ml in Cyrus 1

Part of chamber	Number of quadrates (n)	Number of cells or individuals	Number in 1 ml (X)
1 quadrate	1	a	a x 160 000
1 stripe	40	a	a x 4000
2 stripes	80	a	a x 2000
4 stripes	160	a	a x 1000
10 stripes	400	a	a x 400
20 stripes	800	a	a x 200
Whole chamber	1600	a	a x 100
1 mm^2	16		a x 10 000

$$X = \frac{axK}{nxzxV}$$

X – number of individuals /cells per 1 ml
a - number of individuals/cells in n quadrates
n – number of examined quadrates
z – concentration of sample (if not z =1)
K – number of all quadrates in chamber (for Cyrus 1, K = 1600)
V – volume of chamber in 1 ml (for Cyrus 1, V = 0,01ml)
For concentrating sample from 10 ml to a volume of 0,2 ml z = 50 (**It is or case**)

In our case, we do not calculate the number of cells per each algae species, but the individual phytoplankton groups are calculated. Nomenclature was made according to Marhold & Hindák (1998). The result of this analyse is **total abundance of phytoplankton in cells per 1 ml** and **the abundance of individual groups**: **Cyanophyta**, **Chromophyta** (sum of Cyanophyceae, Chrysophyceae, Xanthophyceae, Dinophyceae, Cryptophyceae and Bacilariophyceae) **Chlorophyta** (Chlorococcales, Volvocales, Ulotrichales and Conjugatophyceae) and **Euglenophyta.**

Qualitative analyse is not directly involved in ecological status assessment. It is made from free or concentrated water through the net with mesh size 10 µm (in samples with

low portion of phytoplankton). It is made with magnification from 400 to 1000. The results of this analyse is the **name of species, or genus and the degree of abundance** according to table set below. This list of species is only complementary to quantitative analyse.

Tab 2: The degree of abundance.

Verbal expression	Coverage of field view (%)	Degree of abundance
very rare	<1	1
rare	1- 3	2
prevailing	4 - 10	3
abundant	11- 20	5
very abundant	21- 40	7
mass	41 - 100	9

Boundary setting

Statistical values (mean of vegetation period of metrics abundance and biomass) were calculated for setting boundaries between classes. The boundaries for ratio of groups were set by expert judgement also according to mean value from vegetation period to which was added the percentage. In result of ecological status is involved only the group of algae with the worst value (lowest score).

Tab 3: Values of estimated boundaries

Class	**I**	**II**	**III**	**IV**	**V**
Score	**5**	**4**	**3**	**2**	**1**
Cyanophyta (%)	<2,5	<5,0	<10,0	<20,0	≥20,0
Chromophyta (%)	≥66,0	<66,0	<50,0	<35,0	<15,0
Chlorophyta (%)	<30,0	<40,0	<45,0	<50,0	≥50,0
Euglenophyta(%)	<2,0	<5,0	<10,0	<15,0	≥15,0
Abundance	<2000,0	<5000,0	<15000,0	<25000,0	≥25000,0
Biomass (Chlorophyll-a)	<15,0	<30,0	<50,0	<75,0	≥75,0

Consequently the EQR was calculated by equation:

$$EQR = \frac{score(abundance) + score(biomass) + score(worst\_group)}{\sum \max score}$$

The ∑ max score is 15.

Tab. 4: The boundaries between classes were estimated as equidistant EQR:

classes	I	II	III	IV	V
EQR	>0,8	>0,6	>0,4	>0,2	≤0,2
	high	**good**	**moderate**	**poor**	**bad**

Example of ecological status calculation in the Danube river - Bratislava

Tab. 5: Data for status estimation in the Danube river-Bratislava

Date	abundance	biomass	Cyanophyta	Chromophyta	Chlorophyta	Euglenophyta
02. 04.2012	4442	12.4	0	4330	112	0
14. 05.2012	5320	24.5	600	3640	1080	0
11. 06.2012	870	4.5	0	50	820	0
09.0 7.2012	1220	5.8	0	1000	220	0
06. 08.2012	760	4.8	0	500	260	0
03.09.2012	20	0.4	0	16	4	0
01. 10.2012	840	3.3	0	840	0	0
mean	**1925**	**8,0**	**86**	**1483**	**357**	**0**
%			**4.5%**	**77,0%**	**18,5%**	**0,0%**
class	**I**	**I**	**II**	**I**	**I**	**I**
score	**5**	**5**	**4**	**5**	**5**	**5**

1. average abundance= 1925 <2000 score=5
2. biomass (chlorophyll-a)= 8 <15 score=5
3. abundance of worst phytoplankton groups in %= Cyanophyta (4,45 %) has the lowest score = 4

$$EQR = \frac{5+5+4}{15} = 0{,}93$$ (High ecological status)

Literature:

Marhold , K. & Hindák, F. 1998. Checklist of non-vascular and vascular plants of Slovakia. Veda, Bratislava. 687 pp.

ISO 10 260(1992): Measurement of biochemical parameters. Spectrometric determination of the chlorophyll-a concentration.

Annex III: Overview of chlorophyll a boundaries of all countries

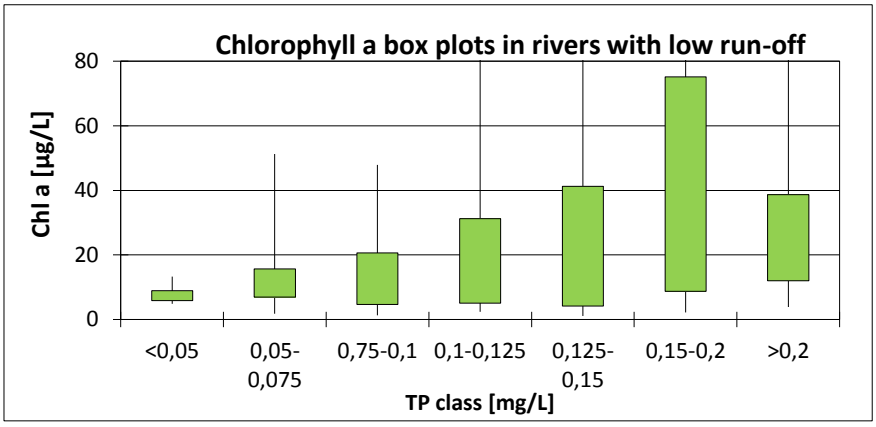
The national phytoplankton methods apply different chlorophyll a-boundaries within their assessment systems: The upper boundaries for the status class “high” (H/G) range between 5.9 to 32 µg/l chlorophyll a and for status class “good” (G/M) between 8.5 to 60 µg/l.

The strong differences might be caused partial by different sub-types within the very large river group. The XGIG expert group found out that the response of chlorophyll a is different in the sub-type “high run-off” and “low run-off” when analyzing with the pooled XGIG data (see figure Annex_III_1) depending on that the catchment area specific run-off is below or higher than 10 l s^{-1} km^{-2}.

In IC-exercise these potential type-differences were taken into account by benchmark standardizing the single metrics of the common metric for sub-type as a random effect in a mixed linear model.

Boundaries of the national methods are listed separately for sub-type groups (see tables III_1 and III_2).

Further river groups (see column 1 and 2 in tables) are for geographic regions (Baltic, Danube before and after Iron Gate etc.). In IC-exercise these potential country-differences were taken into account by benchmark standardizing the single metrics of the common metric for “sub-type & country” as a random effect in a mixed linear model.



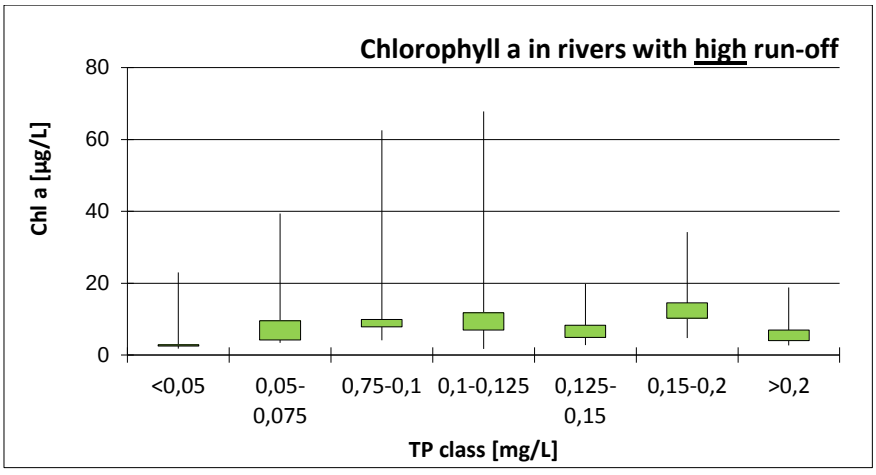


Figure Annex_III_1: Distribution of PP biomass (as chlorophyll a) to total phosphorus (TP) all in annual means as box plots for the same TP classes in very large rivers redawn from XGIG data base for rivers with low specfic run-off (N = 556;, upper graph) and high specific run-off (N = 480, lower graph).

Table III_1: Chlorophyll a boundary values for countries sharing the "low run-off type" and its median provided as upper boundary for each class (high = H/G; good = G/M; moderate = M/P; poor = P/B). In column "& applied" are listed those countries which apply methods from other countries (see column "country")

river test group	river group XGIG „low run-off type"	Coun-try	& app-lied	National type	H/G	G/M	M/P	P/B
I* + V	Very large rivers with low specific run-off	CZ		CZ 9	32	48	65	81
		DE*	BE(FL)	PP 10.2, 20.2	23.4	40.6	70.2	122
		PL		PL 21	25	60	95	130
		HU**		3 (HU river group)	5,9	10	18.3	27.6
II	Very large baltic lowland rivers	EE		EE_Narva	9	10	11	14
		LV		Daugave	5.9	9.6	25.6	31.5
IV	Very large lowland rivers assessed like smaller large lowland rivers	DE*	LT	PP 15.2+17.2	15.6	25.7	42.7	70.3

* DE boundaries recalculated chlorophyll a-uncorrected to Chla_ISO and similar used in updated method PhytoFluss 4.0
* * 10 HU stations of lower Tisza (downstream Balsa), Hármas Körös, Maros and Szamos have low run-off

Table III_2: Chlorophyll a boundary values for countries sharing the "high run-off type" and its median provided as upper boundary for each class (high = H/G; good = G/M; moderate = M/P; poor = P/B).

river test group	river group XGIG „high run-off type plus lower Danube"	Cou n-try	& app-lied	National type	H/G	G/M	M/P	P/B
V	Large to very large lowland rivers with high specific run-off	HU	HR	3 (HU river group)	5.9	10	18.3	27.6
III	Danube before Iron Gate & Rhine	DE*	AT /BG	PP 10.1, PP 20.1	7.9	13.5	23,2	39.8
		SK		9	15	30	50	75
		HU		4 (HU river group)	15	30	45	60
		HU	HR	5 (HU river group)	20	40	60	80
VI**	Danube and other very large rivers downstream Iron Gate with low specific run-off	RO		RO 12, 13, 14, 15	9	9	16	28
		RO		RO 11	8.3	8.5	14.9	25.7

* DE boundaries recalculated chlorophyll a-uncorrected to Chla_ISO and similar used in updated method Phytofluss 4.0
** Lower Danube sections below Iron Gate were decided to be included in this group because of low chlorophyll a response.

Annex IV: R-script computing intercept offsets with linear mixed models

(redrawn from Birk et al. 2016, Annex 3)

```
#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

#----------------------------------Fit linear models (not necessary, for analytical purposes only)-------------------------

fit.lm1 <- lm(CommonMetric_xy ~ Pressure, data=data) # simple linear model

summary(fit.lm1)

fit.lm2 <-lm(CommonMetric _xy ~ Pressure + national_type, data=data) # linear model with national type as

fixed factor

summary(fit.lm2)

fit.lm3 <-lm(CommonMetric _xy ~ Pressure * national_type, data=data)# linear model with national type as
fixed

factor slope varies

summary(fit.lm3)

anova(fit.lm1,fit.lm2,fit.lm3)

AIC(fit.lm1,fit.lm2,fit.lm3)

#..... fit mixed model with intercept as random factors.....

fit.mm2 <- lmer(CommonMetric _xy ~ Pressure + (1| national_type),data=data)

summary(fit.mm2)

coef(fit.mm2)

ranef(fit.mm2) # random effects. values used as offset correction by national type
```

Variable specification for Phytoplankton exercise:

for single metric benchmark standardisation against combined_stressor (pressure) with “country&IC_sub-type” as random effect – results used for CM12 offset correction by country combined to IC sub-type (high or low specific run-off)

for national EQR benchmark standardisation against benchmark standardized common metric (CM12b) with “country” as random effect

Annex V: List of national delegates participating in the phytoplankton intercalibration exercise

Detelina Belkinova (BG)

Jan Błachuta (PL)

Gabor Borics (HU)

Wim Gabriels (BE-Flanders)

Ruxandra Garbea (RO)

Daša Hlúbiková (AT, BG)

Jolanta Jekabsone (LV)

Jurgita Stankeviciene (LT)

Ute Mischke (DE)

Libuse Opatrilova (CZ)

Maria Placha (SK)

Piotr Panek (PL)

Joanna Picińska-Fałtynowicz (PL)

Kai Piirsoo (EE)

Nicoleta Rotaru (RO)

Igor Stanković (HR)

Irja Truumaa (EE)

Jeroen VanWichelen (BE-Flanders)

Gabor Varbiro (HU)

Tomas Virbickas (LT)

Georg Wolfram (AT, BG)

Annex VI: Offsets in single common metrics per IC river type gained from benchmark standardising the common metrics against the pressure index

Global intercalibration exercise of phytoplankton methods

country & IC river sub-type	chla	TIP	Q_avg
AT_high run-off or extreme large	-8,559	-0,615	0,277
B_Fl_low run-off	-11,973	1,084	-0,156
BG_high run-off or extreme large	-5,087	-0,404	0,272
CZ_low run-off	-5,854	0,806	-0,135
DE_high run-off or extreme large	-11,727	-0,443	0,125
DE_low run-off	37,819	0,555	-0,036
EE_low run-off	-1,135	-0,813	-0,137
HR_high run-off or extreme large	-0,213	-0,397	0,198
HU_high run-off or extreme large	7,906	-0,347	0,091
HU_low run-off	-4,327	0,414	-0,047
LT_low run-off	11,704	0,656	-0,139
LV_high run-off or extreme large	0,592	-0,711	-0,118
LV_low run-off	-4,955	-0,609	-0,091
PL_low run-off	-2,088	0,785	-0,198
RO_high run-off or extreme large	-6,714	-0,480	0,104
RO_low run-off	4,612	0,519	-0,010
response direction of metric parameter to pressure	positive	positive	negative

Annex VII: Offsets in national EQR units gained from benchmark standardising the national EQR scores against the CM12b

For final version (calculation version 3b) the EQRs of the official methods listed in report Part A, table 1 are used.

country_ID	offset
AT_2*	-0,1001
B_FL	-0,0718
BG_2*	0,0098
CZ	-0,1345
DE	-0,0819
EE	0,0123
HR	0,0030
HU	-0,0760
LT	-0,0086
LV	-0,1035
PL	0,3545
RO	0,1967

List of alternative offsets in case Germany use the updated method (DE_2: see Annex II-D-2; calculation version 2e).

country_ID	offset
AT_2	-0.1044
B_FL	-0.0676
BG_2	0.0067
CZ	-0.1313
DE_2	-0.0755
EE	0.0075
HR	0.0006
HU	-0.0760
LT	-0.0047
LV	-0.1076
PL	0.3581
RO	0.1943

Alternative Table B4: In case Germany use the method version PhytoFluss 4.0: National class boundaries, boundary biases and adjusted boundary values if bias falls below -0.25.

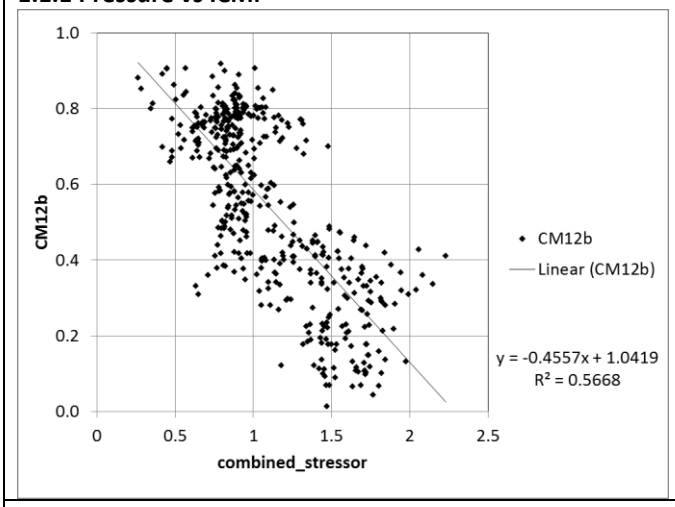
		original		adjusted	
country		H/G	G/M	H/G	G/M
AT_2	boundary	0,80	0,60		
AT_2	bias	0,522	0,522		
B_FL	boundary	0,80	0,60		
B_FL	bias	0,338	0,338		
BG_2	boundary	0,80	0,60		
BG_2	bias	-0,027	-0,034		
CZ	boundary	0,80	0,60		
CZ	bias	0,657	0,657		
DE_2	boundary	0,80	0,60		
DE_2	bias	0,378	0,378		
EE	boundary	0,80	0,75		
EE	bias	-0,030	-0,037		
HR	boundary	0,80	0,60		
HR	bias	-0,002	-0,003		
HU	boundary	0,80	0,60		
HU	bias	0,380	0,380		
LT	boundary	0,80	0,60		
LT	bias	0,023	0,023		
LV	boundary	0,80	0,60		
LV	bias	0,538	0,538		
PL	boundary	0,80	0,60	1,08	0,92
PL	bias	-0,593	-1,790	-0,250	-0,244
RO	boundary	0,80	0,60	0,91	0,76
RO	bias	-0,441	-0,971	-0,250	-0,244

Annex VIII: Partial regressions within type- and pressure-parameter

1.1 Correlations of all data together

All EQR data are transformed in that way, that the class boundaries are represented by the values 0.8 (high-good), 0.6 (good-moderate), 0.4 (moderate-poor) and 0.2 (poor-bad).

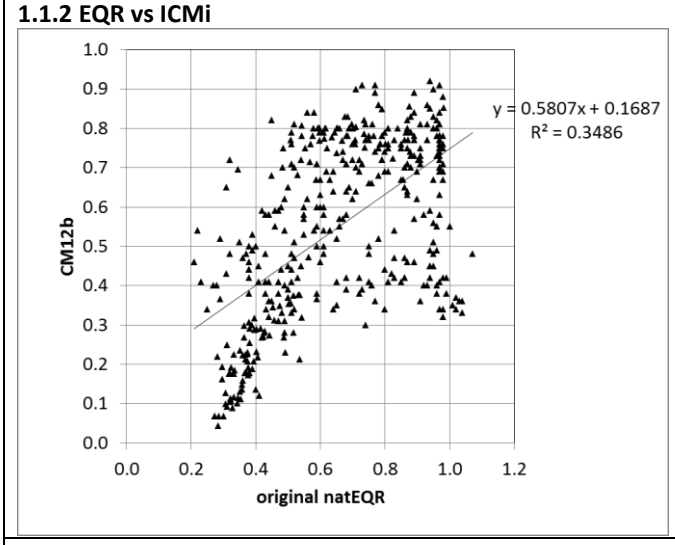
1.1.1 Pressure vs ICMi



Indices legend:

- "combined stressor" is sum of index values of normalized single stressor total phosphorus (TP), total nitrogen (TN) and log-transformed chloride (Cl^-) concentrations
- CM12b is multi-metric-index of single metrics "chl_a", trophic indicator index "TIP" and function group index "Q" after benchmark standardizing for country-type and normalization to whole XGIG data set.

1.1.2 EQR vs ICMi

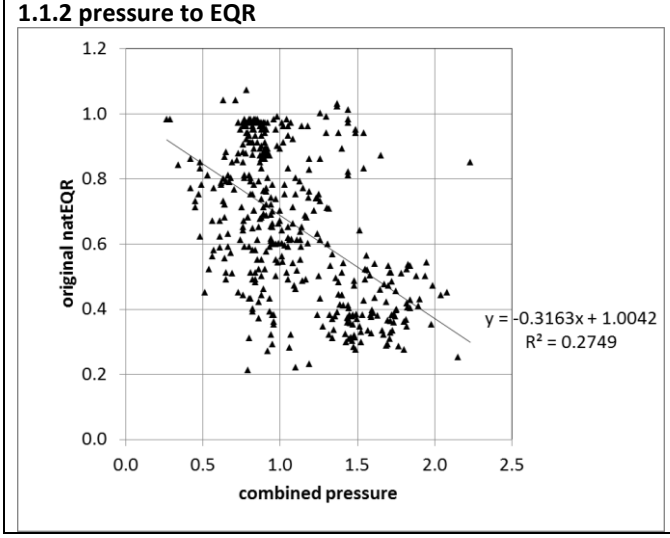


Indices legend:

- "Original natEQR"* = original national EQR is used before benchmark standardizing

*updated method (PhytoFluss 4.0) for countries DE_2, AT_2, BG_2

1.1.2 pressure to EQR



Indices legend:

See above

Partial regressions within type- and pressure-parameters

1.2 Scatter plots grouped by phytoplankton IC types

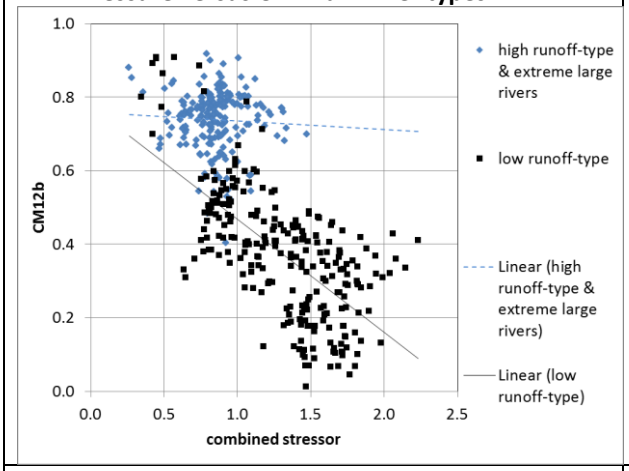
All sampling sites were assigned to one of the two very large river types, established in the ongoing IC exercise based on the typisation in German very large rivers (Mischke et al. 2011) modified by adding extreme large rivers (lower Danube >100.000km^2 catchment size) to river sub-type "high".

River type "high" = high area specific run-off when larger or equal to 10L /s/ km^2 or extreme large

River type "low" = low area specific run-off when smaller than 10L /s/ km^2

All in all: need to pool data of both IC-types to cover whole pressure range

1.2.1 Pressure versus CM12b in river types



Comments:

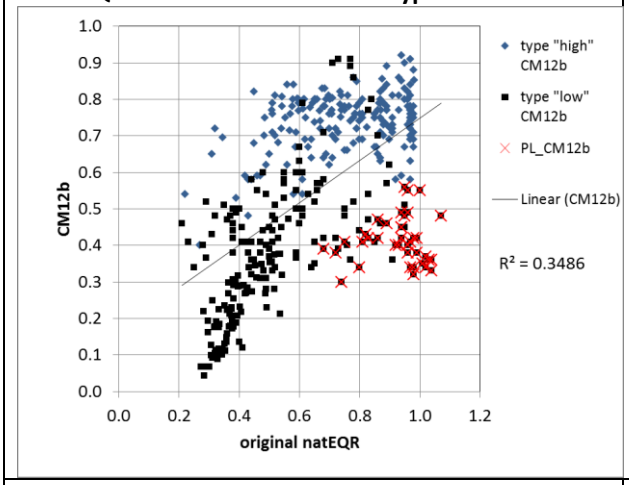
- types still differ strongly after benchmark standardizing for country-type

Rational 1: averaged pressure level differ in types

Rational 2: chlorophyll a response is limited by physical constrains in high run-off type resulting in high values of EQR_CM

Rational 3: Indices of the single metrics "TIP" and "Q_HU" are calculated slightly different for specific river types accordingly the metric description

1.2.2 EQR* versus CM12b in river types



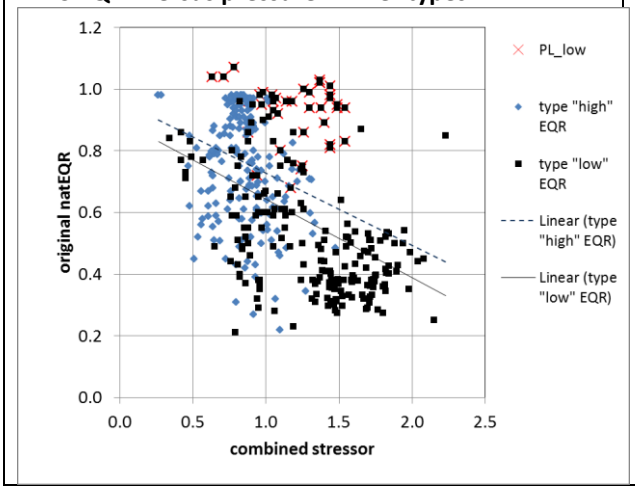
Comments:

- systematic differences between IC types CM12b assess samples in low run-off type more stringent

Rational 1: width of pressure range differ in types

Rational 2: Polish EQRs indicate "high" or "good" while CM indicate high pressure (red cross symbol)

1.2.3 EQR* versus pressure in river types



Comments:

- Assessment strictness are expected to differs between IC types, but differ only slightly

Rational 1: widths of pressure range differ in types and is compensated by different class boundaries in methods

Rational 2: Polish EQRs indicate "high" or "good" status while combined pressure index indicate strong pressure. This sample group influence "low" type regression to be more near to "high"-type regression

* Original EQR (before benchmark standardizing) is used and updated method (PhytoFluss 4.0) for countries DE_2, AT_2, BG_2

1.3 Scatter plots grouped by pressure parameters

The dominant pressure parameters acting on phytoplankton in rivers are all in the pressure group "physico-chemistry" (PC), comprising parameters for eutrophication and for saprobity.

Concentration of total phosphorus (TP) and total nitrogen (TN) were selected for nutrient parameters causing eutrophication.

Chloride concentration was used as a surrogate for house-hold emission which causes organic pollution when not treated.

The combination of TP, TN and Cl—indices were used as combined stressor scale for intercalibration.

Hydromorphological parameters were not included in the combined stressor scale (e.g. presence of upstream or downstream dams) since knowledge is lacking for identifying dam characteristic able to quantify the effect on phytoplankton (e.g. Distance to sampling site; length of backwaters; change in water residence time). No specific dam characters were collected. Furthermore, opposite effects of dams are observable: Iron-Gate dams in Danube are an example for loss of phytoplankton biomass assumed by sedimentation in the reservoirs, the dams in river Labe (CZ) are examples for dams causing an increase of phytoplankton.

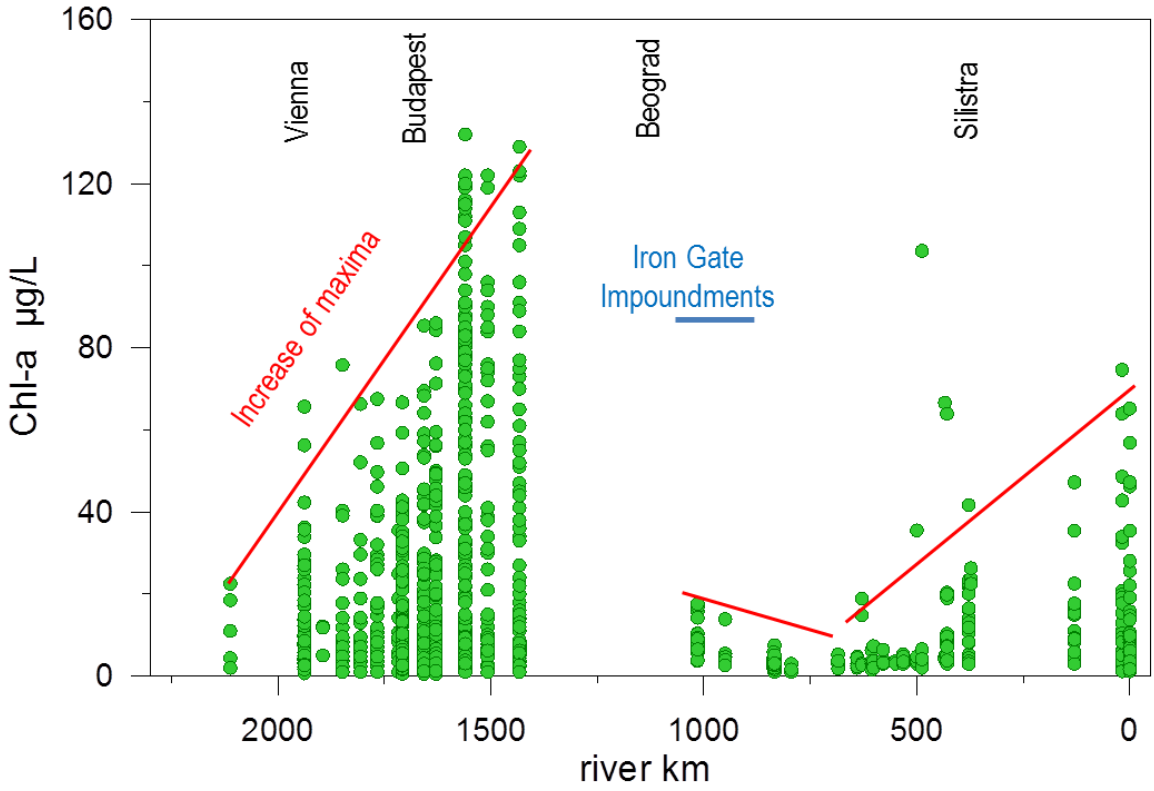
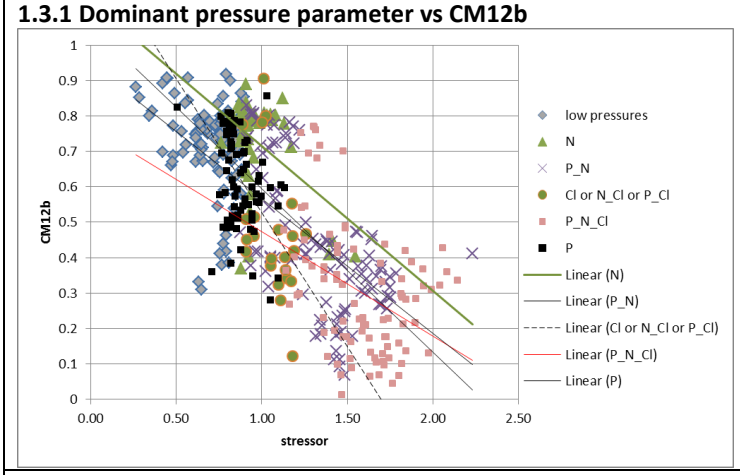
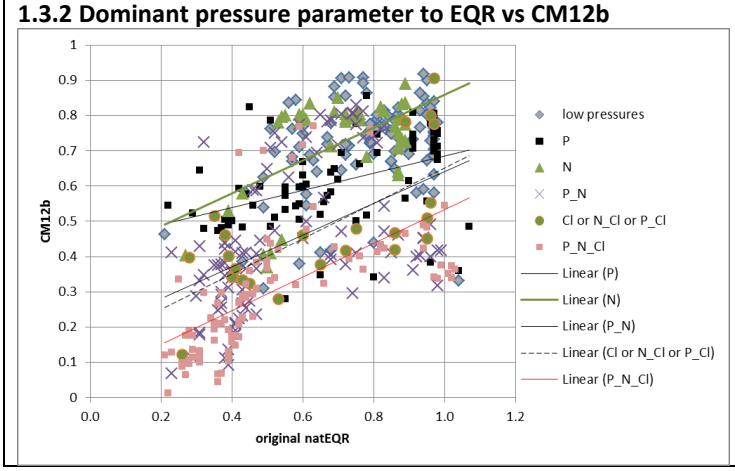


Figure 1.2.3.4: Distribution of single chlorophyll a values before and after chain of Iron Gate dams in river Danube based on XGIG data (analyzed by Georg Wolfram)

The dominant pressure acting at a sampling site for one year was identified using thresholds defined below. When concentrations at one site surpass threshold than pressure is relevant.

Pressure thresholds – Physico-chemistry (PC): total phosphorus >0.1 mg/l; total nitrogen >2 mg/l; chloride >50 mg/l

Applying this resulted in the following pressure groups each site was allocated to (including "low pressure" when no thresholds was surpassed): P=only TP pressure; N=only TN pressure; P_N=TP and TN pressure; Cl or N_Cl or P_Cl =only chloride or combined with nutrients; P_N_Cl = all pressures are acting.

1.3.1 Dominant pressure parameter vs CM12b 	**Comments:** Groups and single pressure parameter influence CM in comparable manner no separate regression for solely Cl-pressure (N = 8 annual mean only)
1.3.2 Dominant pressure parameter to EQR vs CM12b 	**Comments:** Sites under combined pressure of P, N and chloride are more stringent assessed with CM12b than with national EQRs of some countries

1.4 Scatter plots by countries

On the following pages separate graphs are given for all countries to give their covered pressure ranges and to enable to follow certain country samples when they are assessed by common metric (CM12b) of by nationalEQR.

The countries are in alphabetical order; in each graph one country is in focus; it is displayed in red, versus all other countries in black. For each country the regression equation and r-square is provided in the upper right grey box.

List of countries which have small own pressure gradient and/or few samples and which therefore take over methods of another country: AT, B-FL, BG, LT adopt German method and EE, HR, and LV adopt Hungarian methods.

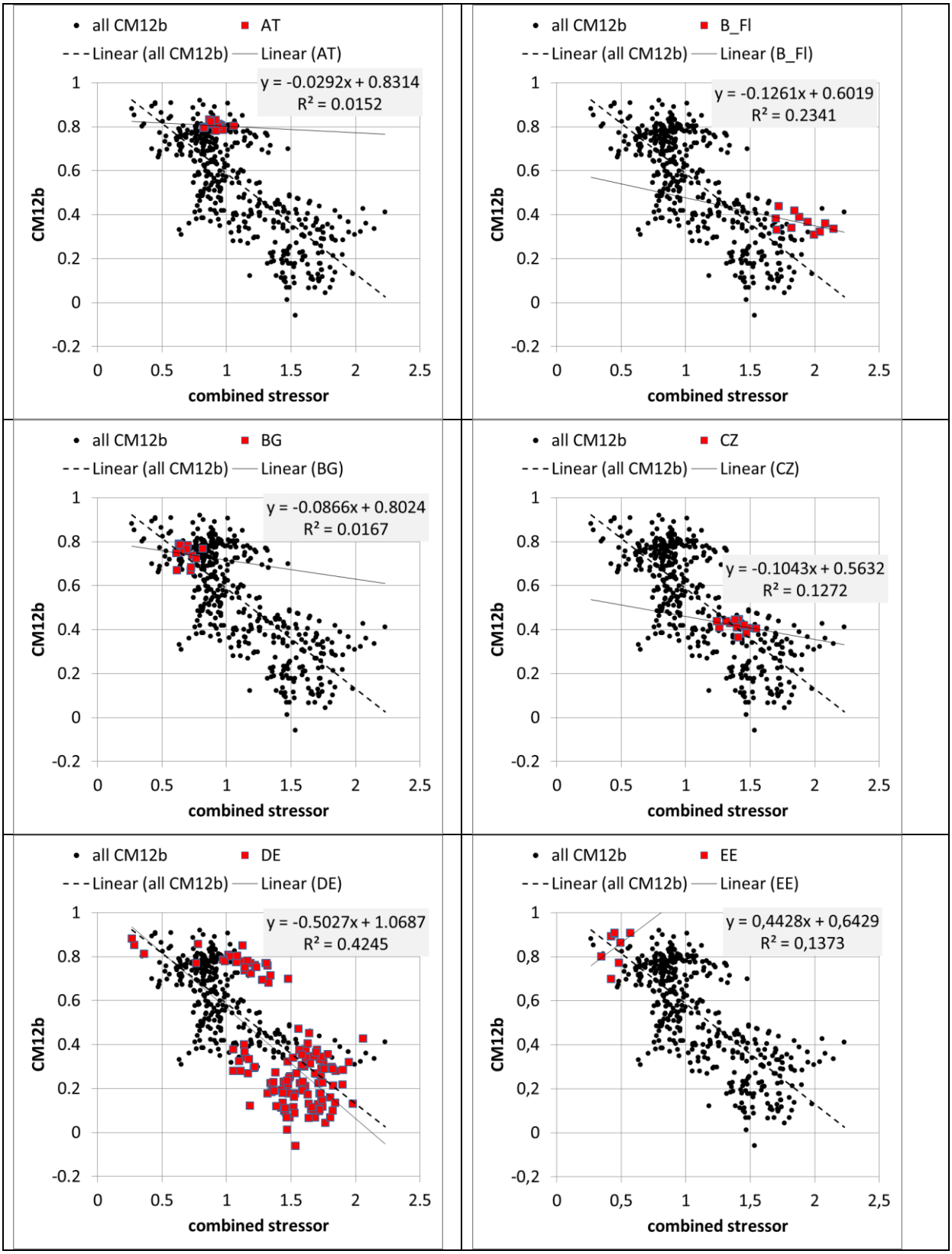
List of countries which have much more samples but not for applying the common metric: CZ and SK (SK = no samples).

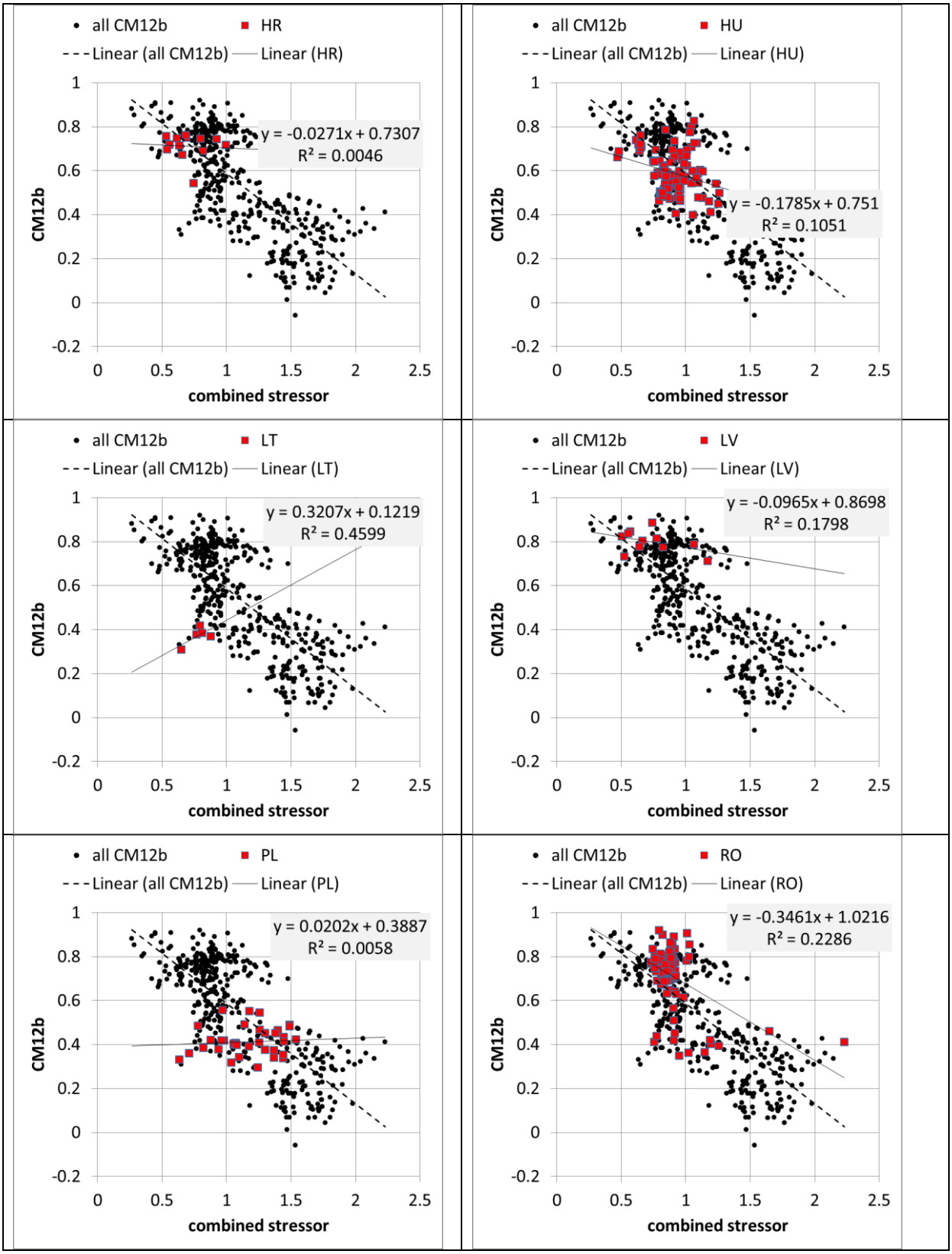
Number of original provided EQR might be reduced for one country because for some years there are no proper data to calculate combined stressor or CM12b.

1.4.1 Pressure vs CM12b

With these graphs it can be judged if a CM assessment of samples of one country follows the same dose-response curve as the others. In that case the CM12b is comparable between the countries. When the benchmarking worked this should be the case and the data of each country should lie centred on the regression line of all countries together (dotted line).

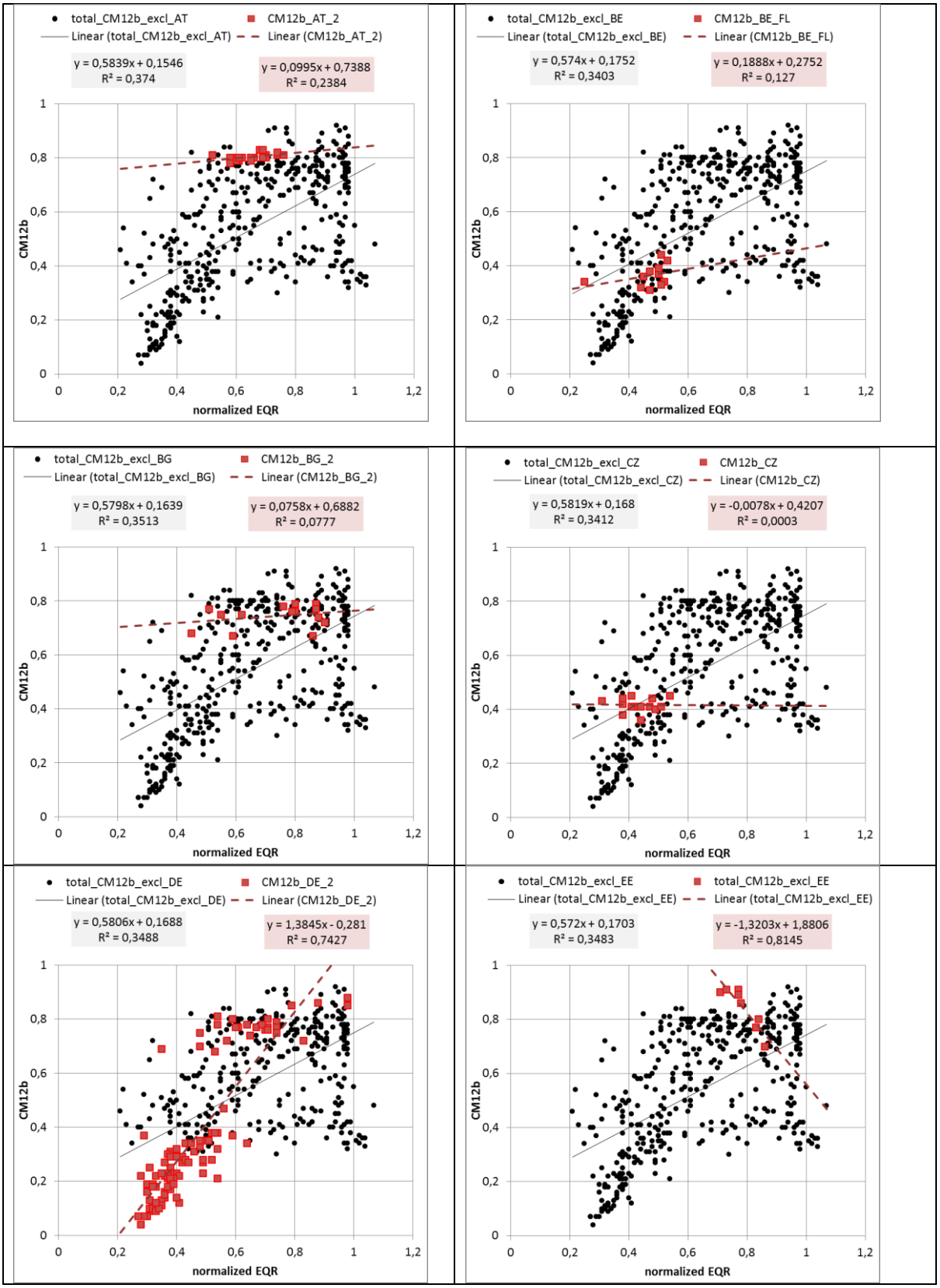
All in all: Benchmarking seems to be fine for all countries

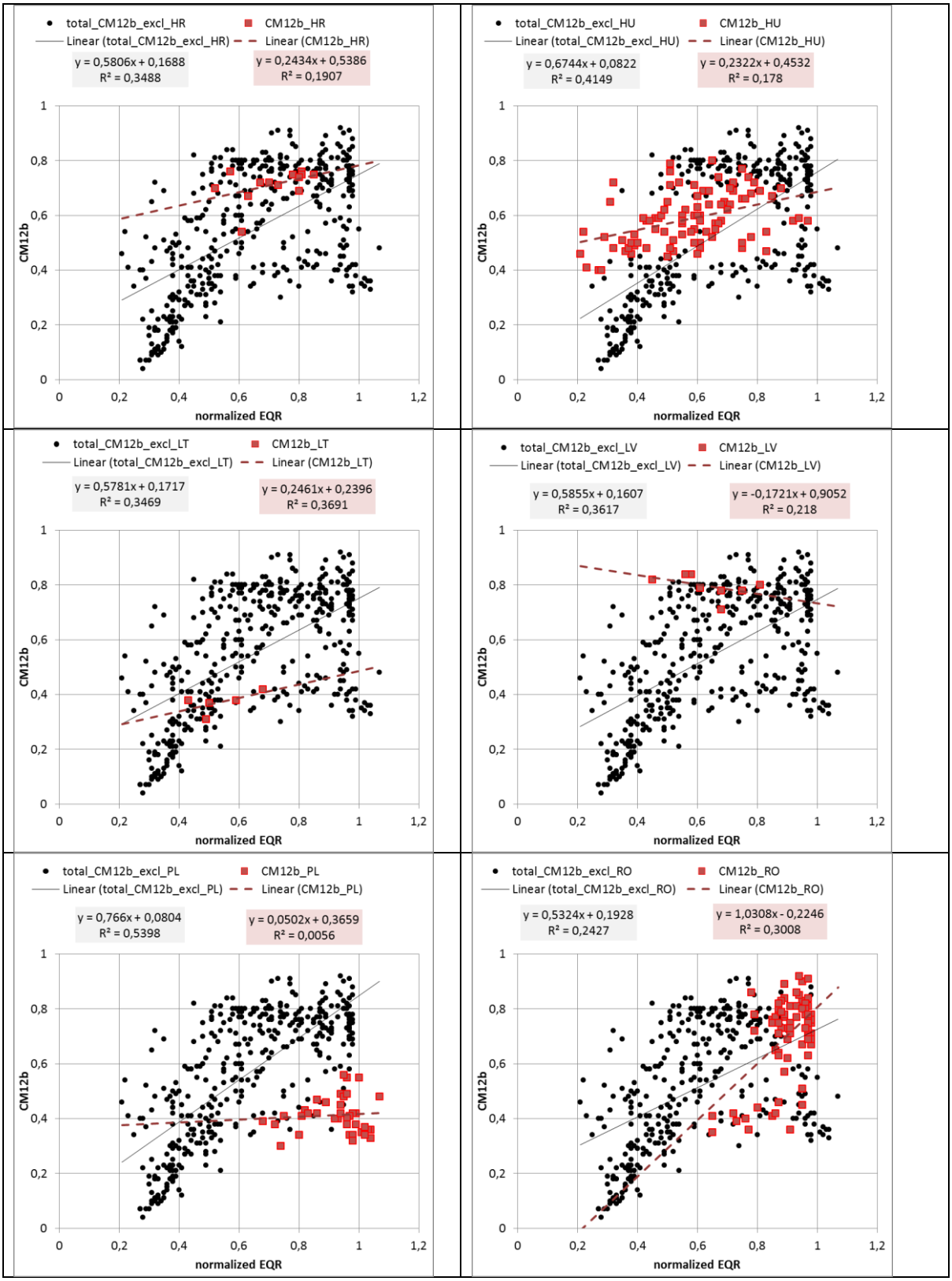

all CM12b
AT
Linear (all CM12b)
Linear (AT)
y = -0.0292x + 0.8314
R² = 0.0152
CM12b
combined stressor
all CM12b
B_FI
Linear (all CM12b)
Linear (B_FI)
y = -0.1261x + 0.6019
R² = 0.2341
CM12b
combined stressor
all CM12b
BG
Linear (all CM12b)
Linear (BG)
y = -0.0866x + 0.8024
R² = 0.0167
CM12b
combined stressor
all CM12b
CZ
Linear (all CM12b)
Linear (CZ)
y = -0.1043x + 0.5632
R² = 0.1272
CM12b
combined stressor
all CM12b
DE
Linear (all CM12b)
Linear (DE)
y = -0.5027x + 1.0687
R² = 0.4245
CM12b
combined stressor
all CM12b
EE
Linear (all CM12b)
Linear (EE)
y = 0,4428x + 0,6429
R² = 0,1373
CM12b
combined stressor


all CM12b
HR
Linear (all CM12b)
Linear (HR)
y = -0.0271x + 0.7307
R² = 0.0046
CM12b
combined stressor
all CM12b
HU
Linear (all CM12b)
Linear (HU)
y = -0.1785x + 0.751
R² = 0.1051
CM12b
combined stressor
all CM12b
LT
Linear (all CM12b)
Linear (LT)
y = 0.3207x + 0.1219
R² = 0.4599
CM12b
combined stressor
all CM12b
LV
Linear (all CM12b)
Linear (LV)
y = -0.0965x + 0.8698
R² = 0.1798
CM12b
combined stressor
all CM12b
PL
Linear (all CM12b)
Linear (PL)
y = 0.0202x + 0.3887
R² = 0.0058
CM12b
combined stressor
all CM12b
RO
Linear (all CM12b)
Linear (RO)
y = -0.3461x + 1.0216
R² = 0.2286
CM12b
combined stressor

1.4.2 EQR vs CM12b

Graphs gave a direct Option 2 comparison of countries EQRs to common metric. Please note that original EQR are used here without any benchmark standardizing. For final Option 2 with "continuous benchmarking" the national EQRs were corrected for random effect by country.





Remarks to regressions for national EQR to CM12 b:

Please note that the exclusion of PL and RO data had the most influence on regression of the remaining total XGIG data (solid black line).

Please note that the preliminary common metric (same single metrics but no benchmark standardizing for combined country and river_type random effect) is much better correlated to national EQRs, and was originally used for common metric selection (see table Annex VIII_1.

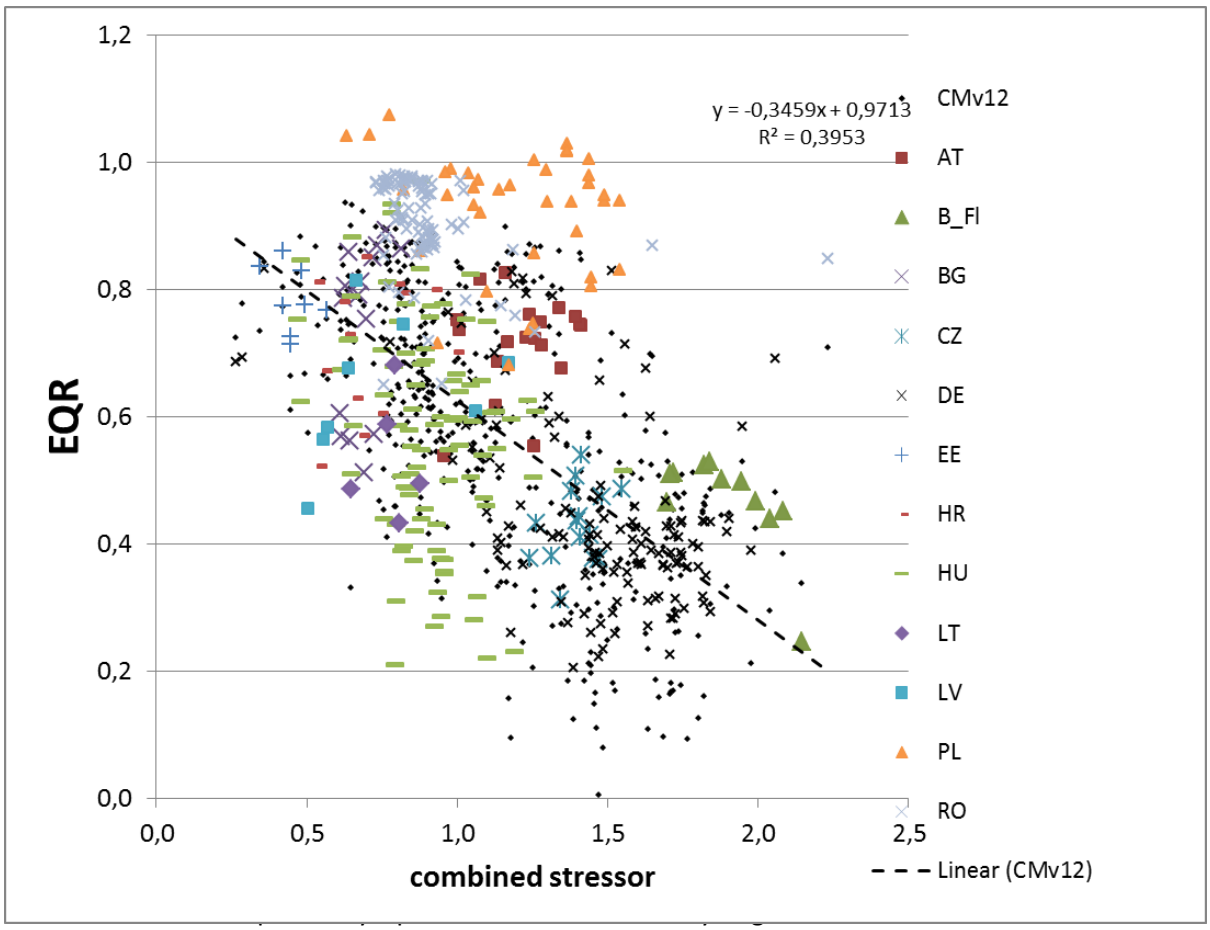
Table Annex VIII_1: Number of data (annual averages) and correlation coefficients btween national EQR and preliminary common metric (CM12 – before benchmark standardization)

country	N years	Pearson´s r	r2
AT	19	0,875	0,765
B_Fl	11	0,684	0,468
BG	15	0,644	0,415
CZ	15	0,682	0,465
DE	131	0,838	0,702
EE	8	0,777	0,604
HR	13	0,548	0,300
HU	90	0,702	0,493
LT	5	0,562	0,316
LV	8	0,934	0,873
PL	38	0,775	0,601
RO	93	0,792	0,627

1.4.3 Pressure vs EQR

While the previous graphs gave a direct Option 2 comparison of the countries, the causes of the country deviations can be seen in the following graph.

Note that this graph is independent of any benchmarking. They just show the national assessments (normalized original EQRs) in dependence of the pressure index. Informatively the common metric CM12 (normalized single metrics) without benchmark standardisation (no country and type offset correction) is shown (black dotted regression CM12 to combined stressor in global XGIG data set).



Annex IX: Complete list of sites and number of annual averages used in the IC exercise for phytoplankton

Table Annex IX_1: Sites used for option 2 (sites with taxa biovolume data, complete stressor and EQR data)

Country ID	river_name internat	river_name	sampling station	GIS_lat	GIS_long	national_wb_code
AT	Danube	Danube	Abwinden	48.249230	14.429310	410360007
AT	Danube	Danube	Jochenstein	48.521660	13.705070	303070000
AT	Danube	Danube	Langenzersdorf	48.298020	16.346220	409040009
AT	Danube	Danube	Linz	48.309600	14.250050	410360007
AT	Danube	Danube	Wildungsmauer	48.116390	16.807980	409040008
AT	Danube	Danube	Wolfsthal	48.141300	17.049410	411340000
AT	Danube	Danube	Ybbs	48.190610	15.068490	410360012
B_Fl	Bovenschelde	Bovenschelde	Bovenschelde	51.008467	3.733414	OMES_BS
BG	Danube	Danube	JDS67 (Novo selo)	44.164840	22.786840	BG1DU000R001
BG	Danube	Danube	JDS68 (before Vidin (Calafat))	44.010090	22.949390	BG1DU000R001
BG	Danube	Danube	JDS69 (after Kozloduy)	43.799190	23.678290	BG1DU000R001
BG	Danube	Danube	JDS70 (Baykal)	43.713770	24.406720	BG1DU000R001
BG	Danube	Danube	JDS72 (after the mouth of the river Iskar (Zagrazhden))	43.751580	24.570110	BG1DU000R001
BG	Danube	Danube	JDS73 (before the mouth of the river Olt)	43.690240	24.769600	BG1DU000R001
BG	Danube	Danube	JDS75 (after the mouth of the river Olt (Cherkovitsa, Nikopol))	43.705760	24.844590	BG1DU000R001
BG	Danube	Danube	JDS76 (before Belene (after Turnu Magurele/Nikopol))	43.670630	25.115860	BG1DU000R001
BG	Danube	Danube	JDS77 (after Svishtov (after Zimnitsa/Svishtov))	43.623700	25.450770	BG1DU000R001
BG	Danube	Danube	JDS79 (after the mouth of the river Yantra)	43.647350	25.576800	BG1DU000R001
BG	Danube	Danube	JDS80 (before Ruse)	43.814180	25.918440	BG1DU000R001
BG	Danube	Danube	JDS82 (after Ruse/Gyurgevo (0,6 км after Danube Bridge))	43.910390	26.064760	BG1DU000R001
BG	Danube	Danube	JDS86 (Silistra)	44.116070	27.242470	BG1DU000R001
CZ	Elbe_Labe	Labe	Děčín p.b.	50.726194	14.187641	OHL_0940
CZ	Elbe_Labe	Labe	Lysá nad Labem p.b.	50.181125	14.836639	HSL_1680
CZ	Elbe_Labe	Labe	Obříství p.b.	50.312191	14.497178	HSL_2090
CZ	Elbe_Labe	Labe	Schmilka p.b.	50.888481	14.234496	OHL_1150
DE	Aller	Aller	Aller, Verden	52.925822	9.225799	FG_W2
DE	Danube	Donau	Donau, Jochenstein Bay	48.521338	13.704044	FG_BAY_119
DE	Danube	Donau	Donau, Kelheim	48.917527	11.866127	FG_BAY_168
DE	Danube	Donau	Donau, Niederalteich	48.825916	12.961325	FG_BAY_515
DE	Danube	Donau	Donau, Schäfstall Pegel	48.718194	10.848057	FG_BAY_2962
DE	Elbe_Labe	Elbe	Elbe, Breitenhagen li	51.930806	11.949582	410001
DE	Elbe_Labe	Elbe	Elbe, Breitenhagen re	51.931947	11.952154	410002
DE	Elbe_Labe	Elbe	Elbe, Dömitz	53.142522	11.228861	205130014

Country ID	river_name internat	river_name	sampling station	GIS_lat	GIS_long	national_wb_code
DE	Elbe_Labe	Elbe	Elbe, Dommitzsch, links	51.649879	12.896772	FG_S_3
DE	Elbe_Labe	Elbe	Elbe, Geesthacht	53.425062	10.339036	FL_BfG16
DE	Elbe_Labe	Elbe	Elbe, Magdeburg li	52.128850	11.657305	410020
DE	Elbe_Labe	Elbe	Elbe, Magdeburg re	52.128850	11.657305	410021
DE	Elbe_Labe	Elbe	Elbe, Sandau, links	52.794319	12.033780	410060
DE	Elbe_Labe	Elbe	Elbe, Sandau, rechts	52.794319	12.033780	410061
DE	Elbe_Labe	Elbe	Elbe, Schmilka, rechts	50.892511	14.231985	FG_S_1
DE	Elbe_Labe	Elbe	Elbe, Schnackenburg	53.039712	11.571176	FL_ARGE_1
DE	Elbe_Labe	Elbe	Elbe, Tangermünde re	52.541028	11.985858	410051
DE	Elbe_Labe	Elbe	Elbe, Wahrenberg li	52.983266	11.685502	410090
DE	Elbe_Labe	Elbe	Elbe, Wittenberg	51.860649	12.649252	2110020
DE	Elbe_Labe	Elbe	Elbe, Zehren, links	51.210583	13.405765	FG_S_2
DE	Havel	Havel	Havel, Göttlin	52.632795	12.318794	BRB_20698
DE	Havel	Havel	Havel, Potsdam	52.401993	13.074757	12_1675
DE	Havel	Havel	Havel, uh Toppel	52.833971	12.064042	410720
DE	Main	Main	Main, Erlabrunn	49.856640	9.854816	FG_BAY_420
DE	Odra	Oder	Oder, Friedrichsthal	52.836858	14.124792	2_0001
DE	Odra	Oder	Oder, Lunower Dammhaus	52.836858	14.124792	2_0337
DE	Rhine	Rhein	Rhein, Bad Honnef	50.644709	7.212389	923102
DE	Rhine	Rhein	Rhein, Bimmen	51.848340	6.113088	923138
DE	Rhine	Rhein	Rhein, Duisburg	51.187210	6.776320	923126
DE	Rhine	Rhein	Rhein, Öhningen	47.650367	8.895431	FG_BW_101
DE	Rhine	Rhein	Rhein, Reckingen	47.571203	8.339471	FG_BW_7
DE	Saale	Saale	Saale, Bad Dürrenberg	51.295525	12.064406	310030
DE	Spree	Spree	Spree Sophienwerder	52.538563	13.213461	FLBL1
DE	Weser	Weser	Weser, Hemeln	51.501927	9.605759	FG_W8
DE	Weser	Weser	Weser, Hess. Old.	52.159377	9.249824	FG_W12
DE	Weser	Weser	Weser, Pegel Porta	52.256424	8.921353	702705
DE	Weser	Weser	Weser,uh KA Vlotho	52.224502	8.830494	702304
DE	Weser	Weser	Weser,Weserbrücke Minden	52.360844	8.980720	703000
EE	Narva River	Narva River	N-Jõesuu	59.450000	28.033333	1062200_2
HR	Danube	Danube	Batina	45.889417	18.827397	DDRI010002
HR	Danube	Danube	Ilok	45.232544	19.401700	DDRI010001
HR	Drava	Drava	Botovo	46.241600	16.938475	DDRI020004
HR	Drava	Drava	Donji Miholjac	45.783447	18.201053	DDRI020003
HR	Drava	Drava	Mouth	45.545308	18.912636	DDRN020001
HR	Drava	Drava	Terezino Polje	45.945597	17.461842	DDRI020004
HR	Mura	Mura	Goričan	46.412056	16.701056	DDRI030001
HU	Danube	Duna, lower	Duna, Hercegszántó közép	45.90903	18.81529	AEP445
HU	Danube	Duna, middle	Duna, Budapest alatt, sodor	46.19272	18.92792	AEP444
HU	Danube	Duna, middle	Duna, Budapest felett, bal part	46.19272	18.92792	AEP444

Country ID	river_name internat	river_name	sampling station	GIS_lat	GIS_long	national_wb_code
HU	Danube	Duna, middle	Duna, Budapest felett, sodor	46.19272	18.92792	AEP444
HU	Danube	Duna, middle	Duna, Dunaföldvár, közép	46.19272	18.92792	AEP444
HU	Danube	Duna, middle	Duna, Szob, bal part	46.19272	18.92792	AEP444
HU	Danube	Duna, middle	Duna, Szob, jobb part	46.19272	18.92792	AEP444
HU	Danube	Duna, middle	Duna, Szob, sodor	46.19272	18.92792	AEP444
HU	Danube	Duna, middle	Szentendrei-Dunaág, Szentendre alatt	46.19272	18.92792	AEP444
HU	Drava	Dráva, lower	Dráva, Barcs	45.950809	17.443616	AEP438
HU	Drava	Dráva, lower	Dráva, Drávaszabolcs			AEP438
HU	Drava	Dráva, upper	Dráva, Őrtilos-Botovo	48.432473	21.460123	AEP349
HU	Hármas Körös	Hármas Körös	Hármas-Körös, Békésszentandrás duzzasztó fölött	46.890836	20.99589	AEP567
HU	Hármas Körös	Hármas Körös	Hármas-Körös, Gyoma	46.890836	20.99589	AEP567
HU	Hármas Körös	Hármas Körös	Hármas-Körös, Szentes (Magyartés) bal part	46.890836	20.99589	AEP567
HU	Hernád, lower	Hernád, lower	Hernád alsó, Gesztely	48.108233	20.962027	AEP579
HU	Kettős-Körös	Kettős-Körös	Kettős-Körös, Békés, duzzasztó fölött	48.504822	21.264968	AEP668
HU	Kettős-Körös	Kettős-Körös	Kettős-Körös, Mezőberény, híd			AEP668
HU	Maros	Maros	Maros, Nagylak, bal part	46.161328	20.703025	AEP784
HU	Maros	Maros	Maros, Szeged (2.0 fkm)	46.203447	20.454909	AEP783
HU	Mura	Mura	Mura, Letenye	46.420306	16.694086	AEP816
HU	Sajó	Sajó, lower	Sajó alsó, Sajólád	47.966685	21.05082	AEP932
HU	Szamos	Szamos	Szamos, Csenger	47.841292	22.693345	AEP971
HU	Tisza	Tisza	Tisza, Aranyosapáti	48.32368	22.087017	AEQ057
HU	Tisza	Tisza	Tisza, Balsa	48.022575	21.321109	AEQ058
HU	Tisza	Tisza	Tisza, Keleti-főcsatornától Tiszabábolnáig, Tiszaújváros (Polgár)	47,785456	21,000852	AEQ059
HU	Tisza	Tisza	Tisza, Kiskörétől Hármas-Körösig, Kisköre	47,480936	20,514826	AEQ060
HU	Tisza	Tisza	Tisza, Kiskörétől Hármas-Körösig, Szolnok	47,480936	20,514826	AEQ060
HU	Tisza	Tisza	Tisza, Kiskörétől Hármas-Körösig, Tiszaug	47,480936	20,514826	AEQ060
HU	Tisza	Tisza	Tisza, Szeged (Tápé)	46.532853	20.164667	AEQ056
HU	Tisza	Tisza	Tisza, Szipa-főcsatornától Belfő-csatornáig, Zemplénagárd	48.32368	22.087017	AEQ057
HU	Tisza	Tisza	Tisza, Tiszabábolnától Kisköréig, Tiszafüred	47,641063	20,728179	AIW389
HU	Tisza	Tisza	Tisza, Tiszabecs			AEQ055
HU	Tisza	Tisza	Tisza, Tiszasziget (bal part)	46.532853	20.164667	AEQ056
HU	Tisza	Tisza	Tisza, Tiszasziget (jobb part)	46.532853	20.164667	AEQ056

Country ID	river_name internat	river_name	sampling station	GIS_lat	GIS_long	national_wb_code
HU	Tisza	Tisza	Tisza, Tiszasziget (sodor vonal)	46.532853	20.164667	AEQ056
HU	Tisza	Tisza	Tisza, Záhony	48.32368	22.087017	AEQ057
LT	Nemunas	Nemunas	Nemunas_R1	54.030680	23.969140	R1
LT	Nemunas	Nemunas	Nemunas_R13	55.274720	21.408890	R13
LT	Neris	Neris	Neris_R43	54.838730	25.742040	R43
LV	Daugava	Daugava	Daugava, at border Latvija - Belarus	55.794800	27.440170	D500
LV	Daugava	Daugava	Daugava, at Dole	56.860230	24.257810	D413SP-2
LV	Daugava	Daugava	Daugava, at Rumbula	56.868210	24.249050	D413SP-1
LV	Lielupe	Lielupe	Lielupe, Gates caurteka	56.895410	23.631760	L100SP-1
PL	Biebrza	BIEBRZA	PL01S0801_1340	50.898160	24.050130	PLRW200024262999
PL	Bug	BUG	PL01S1101_1528	51.545830	21.841670	PLRW2000212663999
PL	Bug	BUG Gnojno	PL01S1101_3225	51.988530	15.065390	PLRW2000212665533
PL	Bug	BUG Krzyczew	PL01S1101_1529	50.122100		PLRW2000212665533
PL	Bug	BUG Sławatycze	PL01S1101_1527	51.972647	18.791256	PLRW2000212663939
PL	Bug	BUG Terespol	PL01S1101_1528	49.993652	18.287756	PLRW2000212663999
PL	Bug	BUG Włodawa	PL01S1101_1526	51.712790	18.648038	PLRW200021266359
PL	Narew	NAREW	PL01S0801_1344	53.226940	21.864440	PLRW20002426199
PL	Narew	NAREW	PL01S0801_1350	51.520674	20.222867	PLRW20002126539
PL	Notec	NOTEC	PL02S0401_0677	53.430380	18.594790	PLRW600021188899
PL	Notec	NOTEC	PL02S0401_1632	52.735494	15.405568	PLRW600021188971
PL	Odra	ODRA	PL02S0401_0658	52.577890	14.631740	PLRW60002117999
PL	Odra	ODRA	PL02S0401_0658	52.657818	19.133366	PLRW60002117999
PL	Odra	ODRA	PL02S0401_0661	52.133180	14.681920	PLRW60002117999
PL	Odra	ODRA	PL02S0401_0661	52.657818	19.133366	PLRW60002117999
PL	Odra	ODRA	PL02S1301_1124	50.423998	21.326302	PLRW600019117159
PL	Odra	ODRA	PL02S1301_1139	52.281390	23.169440	PLRW600019117159
PL	Odra	ODRA East Szczecin-Most Cłowy	PL02S0101_0478	53.468760	14.602081	PLRW6000211971
PL	Odra	ODRA Krajnik D.	PL02S0101_0456	52.843171	14.123660	PLRW60002119199
PL	Odra	ODRA Osinów	PL02S0101_0457	53.339645	14.498340	PLRW60002119199
PL	Odra	ODRA West Autobahn	PL02S0101_0463	53.397585	14.614428	PLRW6000211971
PL	Odra	ODRA West Mescherin	PL02S0101_0464	53.255103	14.442012	PLRW6000211971
PL	Odra	ODRA Widuchowa	PL02S0101_0455	53.034680	14.312370	PLRW60002119199
PL	Pilica	PILICA	PL01S0901_2077	51.510250	23.617440	PLRW200019254799
PL	San	SAN	PL01S1601_1955	50.709048	21.870348	PLRW20002122999
PL	San	SAN	PL01S1601_2238	53.137203	14.384698	PLRW2000192259
PL	Vistula	WISLA	PL01S0601_0979	52.735920	15.405540	PLRW20002127911
PL	Vistula	WISLA	PL01S0601_0979	53.273880	22.459330	PLRW20002127911
PL	Vistula	WISLA	PL01S0601_0980	53.215810	22.554410	PLRW20002127935

Country ID	river_name internat	river_name	sampling station	GIS_lat	GIS_long	national_wb_code
PL	Vistula	WISLA	PL01S0601_1054	52.731740	15.420510	PLRW20002129999
PL	Vistula	WISLA	PL01S0601_1054	53.215810	22.554410	PLRW20002129999
PL	Vistula	WISLA	PL01S0601_1055	53.144290	18.173010	PLRW2000212939
PL	Vistula	WISLA	PL01S1601_1874	49.954944	22.847889	PLRW20002121799
PL	Warta	WARTA	PL02S0401_0669	52.745145	18.963344	PLRW60002118799
PL	Warta	WARTA	PL02S0401_0676	52.577890	14.631740	PLRW6000211899
PL	Warta	WARTA	PL02S0401_0682	53.430380	18.594790	PLRW6000211899
PL	Warta	WARTA	PL02S0401_0693	52.603060	15.479510	PLRW60002118799
PL	Warta	WARTA	PL02S0901_0947	52.099720		PLRW600019183159
PL	Warta	WARTA	PL02S0901_0948	51.761670	23.558060	PLRW600019183199
PL	Wieprz	WIEPRZ	PL01S1101_1606	52.099720		PLRW20001924999
RO	Arges	Arges	Clatesti	44.145290	26.598810	RORW10.1_B7
RO	Danube	Dunare	Bazias_left	44.815900	21.373700	RORW14.1_B1
RO	Danube	Dunare	Bazias_middle	44.815900	21.373700	RORW14.1_B1
RO	Danube	Dunare	Bazias_right	44.815900	21.373700	RORW14.1_B1
RO	Danube	Dunare	Chiciu_left	44.129440	27.273330	RORW14.1_B4
RO	Danube	Dunare	Chiciu_middle	44.129440	27.273330	RORW14.1_B4
RO	Danube	Dunare	Chiciu_right	44.129440	27.273330	RORW14.1_B4
RO	Danube	Dunare	Gruia_left	44.263100	22.688900	RORW14.1_B3
RO	Danube	Dunare	Gruia_middle	44.263100	22.688900	RORW14.1_B3
RO	Danube	Dunare	Gruia_right	44.263100	22.688900	RORW14.1_B3
RO	Danube	Dunare	Modelu	44.182788	27.385040	RORW14.1_B4
RO	Danube	Dunare	Oltenita_left	44.059000	26.616700	RORW14.1_B3
RO	Danube	Dunare	Oltenita_middle	44.059000	26.616700	RORW14.1_B3
RO	Danube	Dunare	Oltenita_right	44.059000	26.616700	RORW14.1_B3
RO	Danube	Dunare	Pristol_left	44.214000	22.681500	RORW14.1_B3
RO	Danube	Dunare	Pristol_middle	44.214000	22.681500	RORW14.1_B3
RO	Danube	Dunare	Pristol_right	44.214000	22.681500	RORW14.1_B3
RO	Danube	Dunare	Reni_left	45.458060	28.247500	RORW14.1_B4
RO	Danube	Dunare	Reni_middle	45.458060	28.247500	RORW14.1_B4
RO	Danube	Dunare	Reni_right	45.458060	28.247500	RORW14.1_B4
RO	Danube	Dunare	Sf. Gheorghe_left	44.884720	29.609440	RORW14.1_B7
RO	Danube	Dunare	Sf. Gheorghe_middle	44.884720	29.609440	RORW14.1_B7
RO	Danube	Dunare	Sf. Gheorghe_right	44.884720	29.609440	RORW14.1_B7
RO	Danube	Dunare	Sulina_left	45.458060	29.670560	RORW14.1_B5
RO	Danube	Dunare	Sulina_middle	45.458060	29.670560	RORW14.1_B5
RO	Danube	Dunare	Sulina_right	45.458060	29.670560	RORW14.1_B5
RO	Danube	Dunare	Svinita	44.490800	22.092500	RORW14.1_B1
RO	Danube	Dunare	Valcov_left	45.404440	29.551390	RORW14.1_B6
RO	Danube	Dunare	Valcov_middle	45.404440	29.551390	RORW14.1_B6
RO	Danube	Dunare	Valcov_right	45.404440	29.551390	RORW14.1_B6
RO	Jiu	Jiu	Zaval	43.842500	23.845400	RORW7.1_B148
RO	Mures	Mures	Nadlac	46.145480	20.727540	RORW4.1_B11

Country ID	river_name internat	river_name	sampling station	GIS_lat	GIS_long	national_wb_code
RO	Olt	Olt	Islaz	43.816700	24.666700	RORW8.1_B12
RO	Prut	Prut	Sivita	45.552440	28.158300	RORW13.1_B5
RO	Siret	Siret	Sendreni	45.402770	27.935830	RORW12.1_B9
RO	Somes	Somes	Dara	47.815010	22.720140	RORW2.1_B7

Table Annex IX_2: Additional sites with abundance data for phytoplankton taxa

Country ID	river_name internat	river_name	sampling station	GIS_lat	GIS_long	national_wb_code
CZ	Berounka	Berounka	Lahovice	49.994978	14.398594	BER_0940
CZ	Divoká Orlice	Divoká Orlice	Čestice	50.122798	16.147863	HSL_0530
CZ	Elbe_Labe	Labe	Děčín	50.726194	14.187641	OHL_0940
CZ	Elbe_Labe	Labe	Hradec Králové	50.213930	15.828769	HSL_0440
CZ	Elbe_Labe	Labe	Kolín pod	50.057754	15.176712	HSL_1340
CZ	Elbe_Labe	Labe	Litoměřice	50.524758	14.207129	OHL_0030
CZ	Elbe_Labe	Labe	Lysá nad Labem	50.181125	14.836639	HSL_1680
CZ	Elbe_Labe	Labe	Němčice	50.095509	15.807922	HSL_0930
CZ	Elbe_Labe	Labe	Nymburk	50.184760	15.054096	HSL_1480
CZ	Elbe_Labe	Labe	Obříství	50.312191	14.497178	HSL_2090
CZ	Elbe_Labe	Labe	Schmilka l.b.	50.888481	14.234496	OHL_1150
CZ	Elbe_Labe	Labe	Valy	50.034039	15.619460	HSL_1180
CZ	Elbe_Labe	Labe	Veletov	50.023891	15.304353	HSL_1320
CZ	Jizera	Jizera	Příšovice	50.572344	15.061916	HSL_1960
CZ	Jizera	Jizera	Vinec	50.397236	14.877296	HSL_2040
CZ	Lužnice	Lužnice	Bechyně	49.289152	14.471816	HVL_1010
CZ	Lužnice	Lužnice	Veselí n.Luž.	49.177895	14.698802	HVL_0680
CZ	Morava	Morava	Lanžhot	48.688028	16.990461	MOV_1430
CZ	Mže	Mže	Plzeň	49.751456	13.376506	BER_0170
CZ	Mže	Mže	Stříbro	49.753007	13.009347	BER_0110
CZ	Nežárka	Nežárka	Veselí nad Lužnicí	49.182269	14.710186	HVL_0850
CZ	Odra	Odra	Bohumín	49.920917	18.328706	HOD_0720
CZ	Orlice	Orlice	Nepasice	50.207280	15.957142	HSL_0780
CZ	Otava	Otava	Topělec	49.351167	14.144880	HVL_2410
CZ	Sázava	Sázava	Pikovice	49.879113	14.428500	DVL_0720
CZ	Sázava	Sázava	Zruč nad Sázavou	49.743011	15.102352	DVL_0320
CZ	Svratka	Svratka	Vranovice	48.950848	16.619543	DYJ_0800
CZ	Thaya	Dyje	Pohansko	48.724038	16.886645	DYJ_1260
CZ	Vltava	Vltava	Hluboká nad Vltavou	49.049172	14.447570	HVL_0460
CZ	Vltava	Vltava	Vrané	49.942740	14.390062	DVL_0730
CZ	Vltava	Vltava	Zelčín	50.319026	14.442324	DVL_0820
SK	Danube	Danube	Dunaj-Bratislava stred	48.138333	17.107186	SKD0019
SK	Danube	Danube	Dunaj-Medveďov	47.791372	17.655931	SKD0017
SK	Danube	Danube	Dunaj-Szob stred	47.813400	18.853202	SKD0018

Annex X: Justification for excluding specific BQE or sub-BQE (Gap 4) for the countries Sweden, Finland and Norway

Prepared by:

Stina Drakare (SE, SLU), Jonas Svensson (SE, Swedish Agency for Marine and Water Management), Marko Järvinen, Ansa Pilke, Jukka Aroviita (FI, SYKE), and Birger Skjelbred and Anne Lyche Solheim (NO, NIVA)

Member States: FINLAND, SWEDEN and NORWAY

- BQE or sub-BQE: PHYTOPLANKTON IN RIVERS
- Water body category (type): RIVERS (N-GIG)

See full document (1-9 pages)

Annex XI: Justification for excluding specific BQE or sub-BQE (Gap 4) for the country Italy

Prepared by:

Member States: Italy

- BQE or sub-BQE: PHYTOPLANKTON IN RIVERS

- Water body category (type): VERY LARGE RIVERS

See full document (1-5 pages)

Template for reporting

Justification for excluding specific BQE or sub-BQE (Gap 4)

JUSTIFICATION FOR EXCLUDING SPECIFIC BQE OR SUB-BQE

1. INTRODUCTION

- Member States: FINLAND, SWEDEN and NORWAY
- BQE or sub-BQE: PHYTOPLANKTON IN RIVERS
- Water body category (type): RIVERS (N-GIG)

Stina Drakare (SE, SLU), Jonas Svensson (SE, Swedish Agency for Marine and Water Management), Marko Järvinen, Ansa Pilke, Jukka Aroviita (FI, SYKE), and Birger Skjelbred and Anne Lyche Solheim (NO, NIVA)

Not all of the quality elements listed in Annex V of the WFD (EC 2000, Annex V, 1.1.1) can be applied for the assessment of rivers in Nordic countries. Assessment method 'Phytoplankton in rivers' has not been developed in the N-GIG member states Finland (FI), Sweden (SE) and Norway (NO), because the use of this element is not relevant in Fennoscandian boreal rivers due to the natural conditions of the rivers.

This document includes explanations and arguments why 'Phytoplankton in rivers' is not considered relevant and is therefore excluded from the FI, SE and NO assessment systems.

2. ARGUMENTS USED FOR EXCLUDING SPECIFIC BQE

2.1 Background

WFD lists as one Biological element for rivers 'Composition and abundance of aquatic flora' (EC 2000). This element, representing primary producers in rivers, includes components phytobenthos, macrophytes, and phytoplankton.

Phytoplankton growth and biomass in rivers strongly depend on flow conditions; for instance in fast-flowing rivers, local phytoplankton populations cannot develop (Whitton 1985, Wehr &Descy 1998, Mischke et al. 2011). There is strong evidence that in running waters phytoplankton biomass is restricted by short residence time (references above; see also Mustonen et al. 2016). Moreover, there is published evidence that phytoplankton responses to environmental factors in rivers are often difficult to assess (Wu et al. 2011).

The use of 'River phytoplankton' as an assessment method varies among the EU member states (MS). It is used as part of the classification system in some Central European MS (e.g. Borics et al. 2007, Piirsoo et al. 2010, Mischke et al. 2011), where large, slow-flowing rivers are characterized with long residence times. In the N-GIG member states Finland, Sweden and Norway 'River Phytoplankton' is not used as biological quality element for assessing ecological status in river water bodies. The BQE's/parameters used at present for FI, SE and NO rivers are listed in Table 1.

Table 1 *Biological Quality Elements (BQE) used in the assessment systems for rivers in Finland, Sweden and Norway (Vuori et al. 2009, Aroviita et al. 2012, SwAM 2013, Norwegian classification guidance 2013).*

BQE	FI	SE	NO
Phytoplankton	no	no	no
Phytobenthos	yes	yes	yes
Macrophytes	x	x	x
Benthic invertebrates	yes	yes	yes
Fish	yes	yes	yes

x = method under development

2.2 Justification for not developing assessment methods for phytoplankton in rivers

The main reasons why phytoplankton is not used to assess ecological status of Nordic rivers are the short residence time and presence of lake phytoplankton from upstream lakes, which prevent the reliable use of river phytoplankton for assessing impacts of nutrient pollution pressure on ecological status.

These reasons are further elaborated below:

- In the Nordic countries, a specific feature is the river-lake-chains, where neighboring lakes are connected to each other by streams and rivers (Eloranta 2004). This means that outflowing lake water strongly contributes to phytoplankton abundance and composition in the downstream rivers (e.g. Heinonen 1980). Therefore, the phytoplankton in these rivers indicates the environmental conditions of the upstream lakes, rather than those of the rivers. This interferes with the detection of "true" river phytoplankton that could reflect the ecological status of the rivers. This naturally high geographic density of lakes in most river basins in the Nordic countries also makes it virtually impossible to establish reliable type-specific natural reference conditions for phytoplankton in Nordic rivers.
- Finland, Sweden and Norway have a few very large rivers (CA > 10000 km^2). The large and very large rivers in FI, SE and NO are fast-flowing (for more details, see Chapter 2.3) due to steep slopes and relatively high precipitation (in relation to evaporation). High discharge

reduces the possibilities of true river phytoplankton to develop, and limits the possibility to use phytoplankton for the assessment purposes.

- The available information of river phytoplankton (mainly Chl-*a*) shows only a weak correlation with nutrient pressure. This is discussed in more detail in the following Chapter 2.3 using available common Nordic datasets.
- Aquatic flora is assessed in rivers in FI, SE and NO by intercalibrated BQE phytobenthos methods (Kelly et al. 2012), and by BQE macrophytes that is currently being intercalibrated (Table 1). Therefore, for the abovementioned reasons that are in more detail described below, inclusion of river phytoplankton in Nordic countries would only decrease the accuracy and sensitivity of the assessment systems.

2.3 Evidence from available national data sources to support justification

In this chapter we provide data-based evidence why the method 'River phytoplankton' has not been developed in FI, SE and NO, and the reasons for its low ecological significance in boreal Nordic rivers.

There are phytoplankton data available as Chlorophyll-*a* concentration from a limited number of Finnish rivers from the years 1974-2008 (Table 2). This and additional more recent (year 2015, March, April, August-October) data from seven Swedish large rivers (samples, n=36; see also Table 3) were used for the analysis to support and demonstrate justifications presented in Chapter 2.2. The Finnish data includes Chl-*a* results from rivers with differing size and national types. However, in the analysis more emphasis was given to large and very large rivers, and the summer growing period of May-September.

Table 2 *Phytoplankton (Chlorophyll-a) data available from the rivers in Finland (source: Hertta database of SYKE; Open data: http://www.syke.fi/en-us/Open_information).*

Decade	Years	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
1970's	1974-1979	-	2	-	5	106	128	95	44	23	-
1980's	1980-1989	2	3	2	321	1598	2289	2468	1186	150	6
1990's	1990, 1992, 1995	-	2	-	9	10	6	7	6	-	-
2000's	2000, 2006-2008					95	58	81	10	18	-
2010's	-	-	-	-	-	-	-	-	-	-	-

No phytoplankton data are available from Norway, but data will be collected in 36 rivers in August and September 2016. The generally steeper slopes and wetter climate, which are characteristic of Norwegian rivers compared to rivers in Sweden and Finland (Table 3), makes it even less probable to find river-generated phytoplankton in Norway than in the other Nordic countries.

2.3.1. High discharge of Nordic rivers

The large (CA >1000 km^2) and very large (CA>10000 km^2) rivers in FI, SE and NO have typically rather high slopes and high flow rates (Table 3), which do not favor phytoplankton occurrence (e.g. Whitton 1985, Reynolds 2006). Accordingly, the proportion of significant low-flowing river parts and pools is small. This is one reason why phytoplankton has been little studied and monitored in Nordic rivers. A mean run-off of >10 l sec^{-1} km^{-2} has been used to indicate high flow rivers in

Germany in Central Europe (Mischke et al. 2011). In these high flow German rivers, chlorophyll vs. total phosphorus (tot-P) was clearly lower than in low flow rivers. The rather clear relationship between Chl-*a* and tot-P, found for German rivers, is missing in Nordic boreal rivers (see Chapter 2.3.2).

Table 3 *Mean, median, minimum and maximum discharge of a) large (catchment area (CA) >1000 km²) and b) very large (CA >10000 km²) rivers in Finland (data from Räike et al. 2012), Sweden and Norway (Skarbøvik et al. (2010), NVE). A mean flow of >10 l sec⁻¹ km⁻² has been used to indicate high flow rivers in Germany (Mischke et al. 2011).*

a) Rivers CA > 1000 km^{-2}

Country	Discharge (l s^{-1} km^2)			Catchment area	no of rivers
	mean	median	min - max	km^2	
Finland	10	10	7 - 14	1088 - 61466	23
Sweden	11	10	6 - 18	3340 - 48193	9
Norway	31	22	13 - 72	1497 - 41967	9

b) Rivers CA > 10000 km^{-2}

Country	Discharge (l s^{-1} km^2)			Catchment area	no of rivers
	mean	median	min - max	km^2	
Finland	11	11	8 - 12	14191 - 61466	7
Sweden	12	12	6 - 18	15387 - 48193	5
Norway	20	18	17 - 25	10812 - 41967	3

2.3.2. Weak correlation against the pressure

Phytoplankton abundance shows a weak correlation with eutrophication, using available river Chl-*a* and tot-P data from FI and SE (Fig. 1, Table 4). Log-transformed tot-P explained only 7-29% of the variance in the log-transformed Chl-*a* data (r^2=0.07-0.29; Table 4). Similarly, the respective relationship between log-transferred soluble reactive P (SRP) and Chl-*a* was low (r^2=0.03-0.26; Table 4). For comparison (Table 5), for river phytobenthos, the relationship with TP explains a much larger proportion of the variance (e.g. Kelly et al. 2012: r^2=0.25-0.73 for intercalibrated phytobenthos metrics in N-GIG; Eloranta & Soininen 2002: r^2=0.74 for diatoms inferred TP). The relationship between river phytobenthos and SRP is also stronger (for R-C1, R-C3 and R-L river types r^2 is 0.42, 0.43 and 0.36, respectively; Phillips et al. 2016) than observed between the river phytoplankton and SRP using the Finnish data is this data analysis (r^2=0.03-0.26, Tables 4 & 5). In lakes, there is a very strong linear relationship between Chl-*a* and tot-P, in which tot-P typically explains 50-80% of the Chl-a variance (Carvalho et al. 2013, Lyche Solheim et al. 2014, Phillips et al. 2008; Table 5). Also, the linear regression between the percentage of agricultural land (representing nutrient pressure) in very large Finnish rivers and phytoplankton (Chl-*a*) is weak (r^2=0.08; Fig. 2, Table 4). Thus, a weak correlation with the nutrient pressure is also a strong justification for excluding phytoplankton from the assessment systems for rivers in the Nordic N-

GIG countries. A further argument is that the phytoplankton found in Nordic rivers most likely comes from upstream lakes, so the regressions may not represent the situation in the rivers, as such.

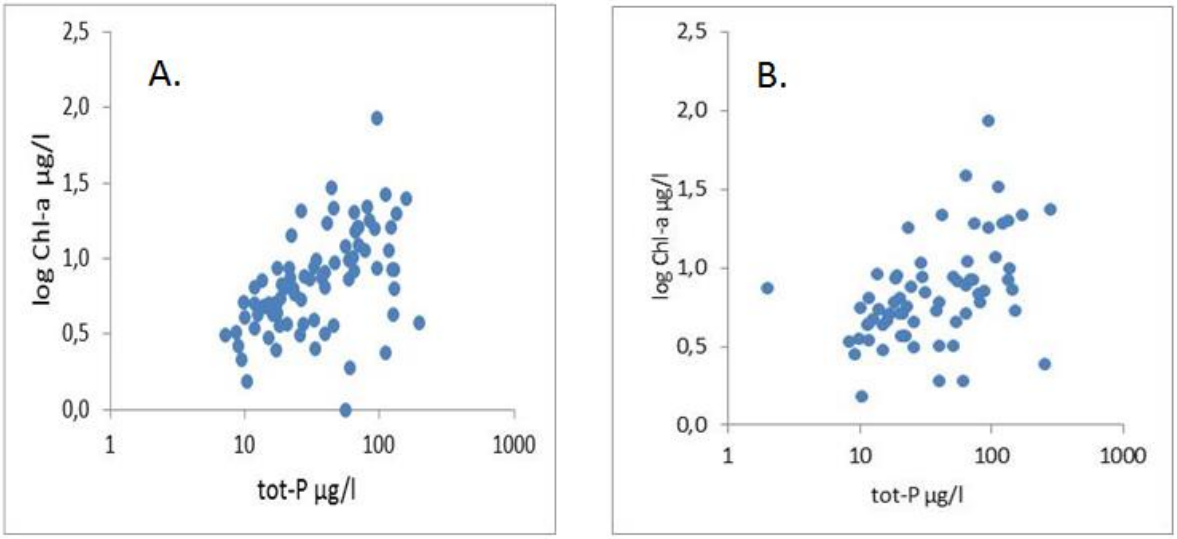
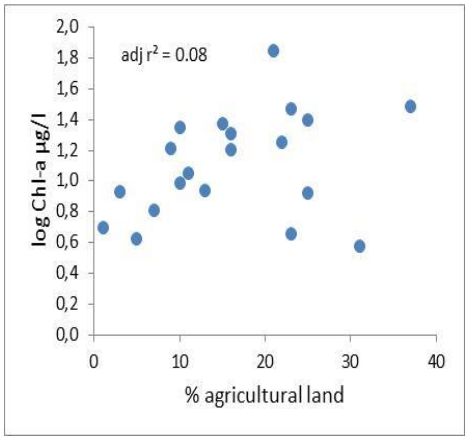


Figure 1 *Response of phytoplankton biomass (Chl-a) to eutrophication (expressed as log tot-P) in Finnish (n=74) and Swedish (n=7) large and very large rivers. (A)The plot represents log-transferred average Chl-a values against respective tot-P values (FI: May-September, SE: August-October). (B) Respective relationship between Chl-a and tot-P, but using August-October data of FI and SE large and very large rivers (n=63+7).*



River name	Area	Water	Field	Forest	Urban	Peatland	Open
	km²	%	%	%	%	%	%
VUOKSI	61466	19	5	45	3	14	13
VIROJOKI	357	3	15	54	4	10	15
KYMIJOKI	37159	18	7	49	4	8	13
PORVOONJOKI	1273	2	31	38	10	3	17
VANTAANJOKI	1686	2	25	37	20	4	13
KISKONJOKI	1047	6	23	48	7	5	12
AURAJOKI	874	1	37	33	12	7	11
EURAJOKI	1336	13	23	39	7	8	10
KOKEMÄENJOKI	27046	11	16	46	6	9	12
LAPVÄÄRTINJOKI	1098	0	13	50	3	22	11
NÄRPIÖNJOKI	992	1	21	47	4	16	11
KYRÖNJOKI	4923	1	25	36	5	19	13
LAPUANJOKI	4122	3	22	39	5	18	14
PERHONJOKI	2524	3	10	38	3	30	16
LESTIJOKI	1373	6	11	39	2	25	17
KALAJOKI	4247	2	16	47	3	17	15
PYHÄJOKI	3712	5	10	50	3	18	13
SIIKAJOKI	4218	2	9	45	2	32	10
OULUJOKI	22845	12	3	44	2	23	17
KEMIJOKI	51127	4	1	52	1	24	18

Figure 2 *Response of phytoplankton biomass (Chl-a*, log-transformed*) to eutrophication pressure (expressed as percentage of agricultural land; Field %) in Finnish large and very large rivers (national river types SSa, Sk, St, ESk, and ESt). The plot represents average May-September Chl-a values against land-use (n = 20 rivers, see Table in right panel, and also Table 4).*

Table 4 *Linear pressure response relationships (adjusted r^2) between phytoplankton (Chl-a) and eutrophication (tot-P, soluble reactive P (SRP) and % agricultural land (% of fields)) for different river types in Finland, and for combined FI-SE data. Results are based on log-transformed data.*

	TP		SRP	
Chl-a vs. TP / SRP	r^2	n	r^2	n
(i) Finnish Rivers (May-Sept, 1974-2008)				
Individual samples	0.19	4176	0.05	939
All rivers	0.29	278	0.17	193
Large and very large rivers:				
- all types	0.25	74	0.26	58
- mineral types	0.21	33	0.15	22
- humic types	0.18	37	0.14	33
(ii) Finnish Rivers (May-Sept, 1995-2008)				
All rivers	0.07	26	0.03	26
(ii) FI+SE Rivers (August-October)				
Large and very large rivers:	0.16	68	0.19	48
Chl-a vs. %-of agricultural land (fields)	r^2	n		
(iv) Finnish Rivers (May-Sept data)				
Large and very large rivers, all types	0.08	20		

Table 5 Overview of *pressure response relationships for river phytoplankton, river phytobenthos and lake phytoplankton (for more detail, see Chapter2.3.2).*

Data / Results	Parameter	vs.	tot-P r^2	SRP r^2	Reference
River phytoplankton					
- Nordic data (FI-SE, this document)	Chl-*a*		0.07-0.25	0.03-0.26	*Table 4*
River phytobenthos					
- Intercalibration results	Mean IC metric TISI		0.25-0.73	0.69	*Kelly et al. 2012*
	PIT perifyton trophic index (NO)		0.75-0.76		*Kelly et al. 2012 (Annex)*
- Diatom inferred tot-P	Phoshorus diatom equation (PDE)		0.74		*Eloranta & Soininen 2002*
- Pressure relationhip (R-C1. R-C3, R-L)	Phytobenthos EQR's			0.36-0.43	*Phillips et al. 2016*
Lake phytoplankton					
- Large European dataset (>1000 lakes)	Chl-*a*		0.52-0.81		*Phillips et al. 2008*
- N-GIG IC report (FI, SE, NO results)	Standardised EQR's		0.19-0.71		*Lyche-Solheim et al. 2014*
- WISER European dataset	Chl-a, PTI trophic index		0.63-0.67		*Carvalho et al. 2013*

In addition, algal blooms are not common phenomena in Nordic boreal rivers. This is for example evidenced by results of Algal Bloom Monitoring in Finland (see Rapala et al. 2010). Algal bloom situation (mainly cyanobacteria) has been visually monitored since 1998 during June-August (see Lakewiki web-system, www.jarviwiki.fi/wiki/Main_page?setlang=en). The emphasis in bloom monitoring has been in lakes and the Baltic Sea, but also records of bloom situations from rivers are collected. With some exceptions, algal bloom observations from rivers have not been detected or reported during 1998-2015. This is further supported by the sparse data of quantitative microscopic analysis results of phytoplankton from very large Finnish rivers (years 1963-1985, n of samples =163). In the dataset, only in 6 out of 163 samples (mainly from one river and the summer of 1982) cyanobacteria biomass constituted >25% of total phytoplankton biomass (median cyanobacteria biomass in these samples 0.7 mg l^{-1}, min-max 0.5-3.2 mg l^{-1}, n=6). The maximum cyanobacteria biomass was >1 mg l^{-1} only in two individual samples (1.3 and 3.2 mg l^{-1}) which exceeds the WHO low risk level of 1mg l^{-1}.

3. Conclusions

Nordic MS Finland, Sweden and Norway have not developed assessment method 'Phytoplankton' for rivers, because of the natural conditions of the rivers:

1) Large and very large rivers in FI, SE and NO have generally high discharge, due to steep slopes and wet and cool climate compared to Central Europe
2) The phytoplankton found in Nordic rivers are mainly imported from upstream lakes, due to naturally high geographic density of lakes in most Nordic river basins
3) The pressure response of phytoplankton is weak in Nordic rivers
4) FI, SE and NO use other, more applicable, aquatic flora elements in the assessment of rivers

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Template for reporting

Justification for excluding specific BQE or sub-BQE (Gap 4)

JUSTIFICATION FOR EXCLUDING SPECIFIC BQE OR SUB-BQE

1. INTRODUCTION

- Italy
- Phytoplancton
- Very large rivers

"*Composition and abundance of aquatic flora*" is a Biological Quality Element (BQE) for rivers (Annex V of the Water Framework Directive, WFD: EC, 2000) and includes phytobenthos, macrophytes and phytoplankton.

Italy has already defined the criteria for the classification of the ecological status of rivers based on macrophytes and phytobenthos BQEs. These criteria have been intercalibrated and the results included in the Commission Decision 2013/480/EU (EU, 2013) with the exception of macrophytes for the Alpine Geographical Intercalibration Group for which the BQE was found not applicable to all Member States.

With specific regard to very large rivers, Italy is participating to the ongoing intercalibration exercise with phytobenthos that is sensitive to very large rivers's trophic status. The results will be recommended to be included in the EC Decision on Intercalibration during the ECOSTAT meeting in the Netherlands (20-21 October 2016).

2. ARGUMENTS USED FOR EXCLUDING PHYTOPLANKTON

Although Italy has three rivers with a catchment size > 10,000 km^2 (i.e. the rivers Adige, Tiber and Po with catchments respectively of 12,200, 17,374 and 71,000 km^2), only river sections thereof are characterized as *very large rivers*.

This mainly because of Italian the geographical configuration: narrow shape, surrounded by the sea, with about 60% of the territory which consists of mountains and characterized by Alpine conditions in the Northern part and by Mediterranean peninsular conditions in the central and southern parts.

In this document we detail the reasons why the use of the BQE phytoplankton is not relevant to assess the ecological status of our very large rivers considered their main characteristics.

In fact, as reported also in <<Large River Intercalibration Exercise "*Overview of national assessment methods, including pressure-impact relationships and WFD compliance checking*" – BQE:

Phytoplankton>>, planktonic communities in large rivers are harshly constrained by water discharge and other variable directly linked to water fluxes, primarily turbidity (Reynolds & Descy, 1996; Wehr & Descy 1998; Mischke *et al.* 2011), rather than by chemical and biological conditions (Harris 1986; Reynolds, 2006).

ADIGE

In relation to the Adige, the short residence time that characterizes the river's flow prevents the optimal development of phytoplankton a fact which results in a weak correlation between the biological element and the nutrient concentrations.

More specifically, the Adige is for the most part a fast-flowing river characterized by a faster flow in the warmer months just when the conditions for algal growth would be better. For these natural features 55% of the Adige river is characterized, by the competent Authorities, in compliance with the Annex II of the WFD 2000/60/EC (transposed in the Annex 3, Part III of Italian Legislative Decree 152/2006, as amended), as national typology corresponding to the "IC types RW-R-A1 and RW-R-A2 (*Pre-Alpine and Alpine, small to medium, high altitude calcareous and siliceous*)".

The Adige river is characterized by a low production of algal biomass (see data evidence below) and about this topic the above-mentioned document of XGIG states : "*In order to take into account multi-factor limitations, different strategies to improve the demonstrating of the relationship were used by the MS in LR-XGIG: (……) They exclude all samples from pressure-impact analysis, in which the phytoplankton biomass remain below a certain threshold (e.g. LV; chl_a < 18µg/L), assuming that other factors than nutrient limit phytoplankton (e.g. light limitation by occasionally high amount of suspended solids) ".*

DATA EVIDENCE

1. ***Phytoplankton biomass***

   Maximum values of Chl a and biovolume were 5.7 lg l-1 and 2,356 mm³ m-3 (station 1 in the middle reaches of Adige river: station CA), and 6.9 lg l-1 and 3,210 mm³ m-3 (station 2 in the lower reaches of Adige river: station BP). Average concentrations of Chl a and total biovolume over the whole period (mean ± SD) were 2.1 ± 1.7 lg l-1, and 583 ± 557 mm³ m-3 (CA), and 2.3 ± 1.6 lg l-1, and 785 ± 740 mm³ m-3 (BP).

2. ***Response to pressures***

   Ordination of phytoplankton samples by Nonmetric Multidimensional Scaling (NMDS): *The gradient of species composition was strongly associated to water discharge and suspended materials, and, in the opposite direction, to phytoplankton biomass and light availability. Moreover, the samples of the two stations (CA e BP) were separated along a gradient strongly associated with phosphorus and dissolved inorganic nitrogen.*

REFERENCE (Annex 1, pag. 27; Fig. 7c):

At the extreme of physical gradients: phytoplankton in highly flushed, large rivers. Nico

Salmaso & Andrea Zignin, **Hydrobiologia (2010) 639:21–36**.

PO

About 85% of the water bodies of the river Po were characterized, by the competent Authority in compliance with the Annex II of WFD 2000/60/EC, as national typology corresponding to the "IC type RW-R-C5 (*Central / Baltic, large, lowland, mixed*)".

In these lowland river, where nitrogen and phosphorus loads are often critical, phytoplankton is rarely limited by phosphorus and/or nitrogen (Wehr & Descy, 1998; Piirsoo *et al*., 2008 and reference therein), which reveals the prominent role of the hydrological regime and other nutrients, e.g. dissolved reactive silica (DRSi). This is also the case for the Po river, where phosphorus and mainly nitrogen concentrations are always above the thresholds for phytoplankton growth (Wehr & Descy, 1998; Reynolds, 2006).

In this case a research on the river Po (Taverinini *et al*. 2011: Annex 2), conducted in a study area representative of the IC type RW-R-C5, shows that the "*discharge rates, water temperature and dissolved reactive silica can modify phytoplankton composition and biomass, with obvious implications on primary production and biogeochemical cycles in the river itself and all the ecosystem connected. On the other hand the influence of phosphorus and nitrogen was less evident".*

DATA EVIDENCE

1. ***Environmental gradients and their relation to phytoplankton in Po river***

Results of a statistical analysis (Canonical Correspondence Analysis) performed on phytoplankton samples and physical and chemical variables (i.e. average daily discharge, water temperature, dissolved reactive silica – DRS).

The ordination stressed the importance of seasonal changes of temperature (P = 0.002), DRSi (P = 0.002) and water discharge (P = 0.006) for algal assemblages. The first two ordination axes accounted for 35.2% of the total species variability. The species–environment correlations were 0.927 for axis 1 and 0.844 for axis 2, respectively. The Monte Carlo permutation test showed that the model was significant (P\0.05).

REFERENCE (Annex2, pag. 220-221):

Physical factors and dissolved reactive silica affect phytoplankton community structure and dynamics in a lowland eutrophic river (Po river, Italy). S. Tavernini, E. Pierobon & P. Viaroli, **Hydrobiologia (2011) 669: 213- 225.**

TEVERE

The Tiber river is 405-kms long and its catchment area is 17,374 km^2 and does not greatly exceed the 10,000 km^2. 67% of the river was characterized as national typology corresponding to the "IC type RW-R-L2 (*Very large medium to high alkalinity rivers*)". For the remaining part the Tiber's water bodies belong to the IC types RW-R-M1 and M2 - *Mediterranean, small, mid-altitude and Mediterranean, medium, lowland.*

In addition all Tiber's water bodies were designated as Heavily Modified (HMWB). The HMWBs are currently excluded from the intercalibration exercise ("*The aim of the large river exercise is to intercalibrate the national methods that classify the ecological status (not: potential) of large rivers ….*"[1])..

CONCLUSIONS:

The reason why phytoplankton is not used to assess ecological status in Italian very large rivers is because of their natural conditions:

- Adige is characterized by Alpine and pre-Alpine river conditions, with relatively fast flow rates specially during the summer months; high discharge rates reduce the possibility of river phytoplankton to develop, and limit the possibility to use phytoplankton for the assessment purpose.
- In the Po river we detect, in relation to phytoplankton growth, a prominent role of the hydrological regime, water temperature and other nutrients, e.g. dissolved reactive silica (DRSi) and a less evident influence of phosphorus and nitrogen;
- The Tiber has a small catchment area, of 17,374 km^2, and just a part of the river is characterized as a IC type *Very large river* and its water bodies are HMWBs. These water bodies are not included in the ongoing intercalibration exercise;
- The pressure response of phytoplankton is weak in our large rivers;
- Italy uses other, more applicable, aquatic flora elements in the assessment of rivers.

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