Bulgaria

Report on fitting a classification method to the results of the completed intercalibration of the Med GIG (R-M1 and R-M2)

BQE: Macrophytes

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1 Introduction

The official intercalibration of macrophyte-based methods for ecological status assessment within the Mediterranean Geographical Intercalibration Group (MedGIG) was finalized. Bulgaria did not join the official IC round because the common river type was yet not recognized.

The MedGIG of river macrophytes involved 7 countries and 2 assessment methods with similar data acquisition and assessment concept: the Macrophyte Biological Index for Rivers (IBMR) in Cyprus, France, Greece, Italy, Portugal and Spain, and the River Macrophyte Index (RMI) in Slovenia.

The objective of this report is to declare that the present Bulgarian classification method of ecological status of Mediterranean river types, based on macrophytes is compliant with the WFD normative definitions and that its class boundaries are in line with the results of the completed intercalibration exercise.

In particular, the classification method (Reference Index Bulgaria; RI-BG; Gecheva et al., 2013) is an intercalibratable finalized method. The class boundaries were compared with agreed boundaries from the MedGIG intercalibration exercise following the instructions of the CIS Guidance Document n°30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise".

2 Typology

The biological analysis of macrophytes in MedGIG revealed a poor segregation between types RM1, RM2 and RM4 (Table 1), both for the reference sites (Global R of ANOSIM =0,263; significance level=0,1%) and for the all sites (Global R of ANOSIM =0,218; significance level=0,1%). **Therefore these types were treated together throughout the IC process.**

A validation of the national sites' allocation to the common IC river types was performed based on: mean altitude above sea level, catchment area and from qualitative information concerning hydrological features that were included in the dataset. The established national sites corresponded to 2 of the reported common intercalibration river types (or Mediterranean river types, Aguiar et al., 2014): R-M1 and R-M2.

National biological dataset was divided by the classification analyses into 2 subsets: first included medium rivers (average catchment 360 km^2) at low altitude (88-273 m a.s.l.) with mixed geology, i.e. R-M2. Recorded preferentials were aquatic vascular taxa *Lemna minor*, *Myriophyllum spicatum*, *Potamogeton nodosus*, *Ranunculus trichophyllus*, *Schoenoplectus lacustris*, *Berula erecta*, *Nasturtium officinale*, *Veronica beccabunga*; helophytes *Alisma lanceolatum*, *Eleocharis palustris*, *Sparganium erectum*, *Typha latifolia*; hygrophilous *Lysimachia nummularia*, bank species *Equisetum arvense*, *Carex pseudocyperus*, *Carex riparia*, *Cyperus longus*. These sites were characterized also by slowly to medium running water and sand and gravel as dominant substrate (Fig. 1).

The above described group is similar to the **Group e** recorded for the southeastern Mediterranean (Cyprus and Greece) (Feio et al., 2014).

The second subset included small rivers (catchment <100 km^2) at medium altitude (170-540 m a.s.l.), rapidly running water and coarser bottom, i.e. R-M1 (Fig. 1). These rivers were characterized by the taxa *Veronica anagallis-aquatica*, *Equisetum fluviatile*, helophyte *Agrostis stolonifera*, bank species *Petasites hybridus*, and aquatic mosses *Brachythecium rivulare, Cratoneuron filicinum, Platyhypnidium riparioides.* Bulgarian second group resembles **Group c** from the Med GIG (Feio et al., 2014) which was composed of highly seasonal rivers with mixed geology, clear running-waters and coarse substrates; with small catchments (51.1 ± 79.9 km^2). These sites were spread throughout the study area (from Spain to Cyprus) and *Platyhypnidium riparioides* was found to occupy large portions of the substrates (***Figure 1***).

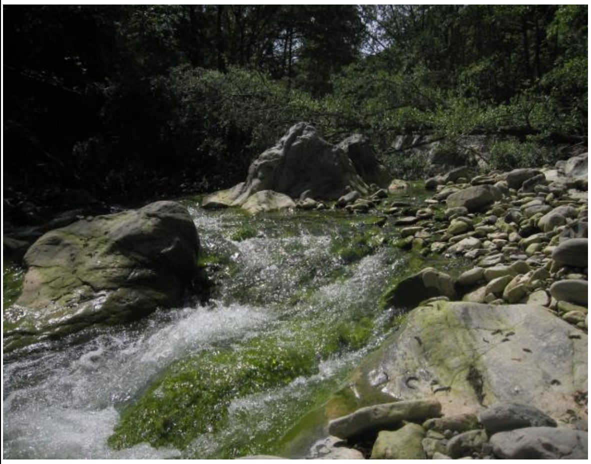
Examples from First subset, R-M2 / Group e (Feio et al., 2014)	**Second subset, R-M1 / Group c (Feio et al., 2014)**
	
Varbitsa River at Studen Kladenets	Varbitsa River after Zlatograd
	
Yerusalimovska River	Stara River

Figure 1: Photos of Bulgarian R14 rivers (national typology)

Based on the above, there are sufficient possibilities for intercalibration for R-M1 and R-M2, and intercalibration is feasible in terms of typology (*Table 1*).

Table 1: Overview of common intercalibration types in the Mediterranean rivers GIG and MS sharing the types.

Common IC type	Type characteristics	MS sharing IC common type
R-M1	catchment <100 km^2; mixed geology (except non-siliceous); highly seasonal	France, Italy, Portugal, Slovenia, Spain
R-M2	catchment 100-1000 km^2 ; mixed geology (except non-siliceous); highly seasonal	France, Greece, Italy, Portugal, Slovenia, Spain
R-M3	catchment 1000-10000 km^2 ; mixed geology (except siliceous); highly seasonal	Greece, Portugal, Spain *This type was not intercalibrated because assessment methods were not fully developed.*
R-M4	non-siliceous streams; highly seasonal	Cyprus, France, Greece, Italy, Spain
R-M5	temporary rivers	Cyprus, Italy, Portugal, Slovenia, Spain (*Subject to a separate data treatment due to large structural and functional differences: Aguiar et al., 2010.*)

3 Description of the national assessment method

3.1 Overview of the assessment method

The national macrophyte-based method - Reference Index Bulgaria (RI-BG; Gecheva et al., 2013) is established for assessment of ecological status of all river types in Bulgaria except for R6 Large Rivers (i.e. Danube). It is compliant with European standardisation legislation (EN 14184: 2014, EN 14996: 2006, CEN 230165). The evaluation and the calculation procedures meet the requirements of the Water Framework Directive (WFD) and are in line with the recommendations of the implementation groups CIS Working Group 2.3 and 2.A (REFCOND, ECOSTAT).

The method was intercalibrated within the EC GIG for 3 river types in 2011: medium-sized lowland (R-E2) and large to very large lowland rivers (R-E3), and upland rivers (R-E4).

The macrophyte survey is carried out once during the main vegetation period (May-June). In each sampling site usually a 100 m long section is surveyed. The abundances of all single species are registered using a five-level scale (see below).

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

Field records also include estimation of 4 abiotic parameters: flow velocity, shading, substrate type and mean depth. Shading was noted based on a five-degree scale (1 = completely sunny, 2 = sunny, 3 = partly overcast, 4 half shaded, 5 = completely shaded) of Wörlein (1992). The other three parameters were determined after Schaumburg et al. (2004, 2006) in a semi-quantitative way using class scales, to enable a fast and easy field application. Velocity of flow was recorded via a six-point scale: I = not visible, II = barely visible, III = slowly running, IV = rapidly running (current with moderate turbulences), V = rapidly running (turbulently running), VI = torrential. The substratum conditions at the sampling site are classified in 5% steps according to an eight-point scale: % mud, % clay/loam (< 0.063 mm), % sand (0.063 - 2.0 mm), % fine/medium gravel (2.0 - 6.3/6.3 - 20 mm), % coarse gravel (20 - 63 mm), % stones (63 - 200 mm), % boulders (> 200 mm) and % organic/peat. Mean depth was noted on a three-degree scale (I = 0 - 30 cm, II = 30 - 100 cm, III > 100 cm).

3.2 Indicators used

Macrophyte taxonomic composition:

The taxonomic composition of aquatic macrophytes (Characeae, mosses and vascular plants) is assessed on a species level, except for *Chara*, *Chiloscyphus* and *Sphagnum*, which are determined on genus level. The indicators list is presented at Annex A.

Macrophyte abundance:

The abundance of each species is recorded according to Kohler’s five class scale (1978). The species composition uses 5 classes of abundance (***Table 2***).

Table 2: Abundance scale after Kohler (1978)

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

3.3 Assessment

Methods of calculation

The registered relative abundance of each indicator species is converted into metric quantities using the following function: macrophyte abundance³ = macrophyte quantity.

Taxa are assigned to indicator groups A (reference taxa) B (indifferent taxa) and C (disturbance indicators) – Annex A. The list of indicators, additional type-specific criteria and class boundaries were specified for national conditions as follow:

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

Bioindicative capabilities of individual plant species was assessed based on literature data, expert knowledge, analysis of river database;

(i) the list of indicators was verified in relation to its application in Bulgaria;
(ii) the RI-BG was officialy intercalibrated within EC GIG for 3 river types (R-E2, R-E3 and R-E4).

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

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

RI = *Reference Index*

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

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

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

nA = *Total number of taxa in group A*

nC = *Total number of taxa in group C*

ng = *Total number of taxa in all groups*

Equation 1: Calculation of the Reference Index.

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

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

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

M_{MP} = Module Macrophyte Assessment

RI = type specifically calculated Reference Index

Equation 2: Equation 2. Transformation of the module RI (Reference Index Macrophytes) on a scale from 0 to 1.

3.4 Description of boundary setting procedure

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

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

ES	Range of EQR	Definition given by the WFD	Interpretation
High	**>0.74 (Type R14)**	“The taxonomic composition corresponds totally or nearly totally to undisturbed conditions. There are no detectable changes in the average macrophytic […] abundance. […]”	The HG boundary was assumed as one quarter (0.25) below the maximum value at which only species of Group A taxa are represented.
Good	**>0.49**	“There are slight changes in the composition and abundance of macrophytic […] taxa compared to the type-specific communities. […]”	The GM boundary was the point at which Group B (indifferent taxa) is dominant and Group A is represented with about 30% share. Still no or very few representatives of disturbance indicators.
Moderate	**>0.20**	“The composition of macrophytic […] taxa differ moderately from the type specific communities and-are significantly more	The MP boundary was set as the average where the community is dominated by disturbance indicators (Group C). It is characterised by

ES	Range of EQR	Definition given by the WFD	Interpretation
		distorted than those observed at good quality. Moderate changes in the average macrophytic […] abundance are evident. […]”	disappearance of aquatic bryophytes.
Poor	**0≤EQR≤0.20**	Macrophyte “communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions”.	EQR values are very low. The PB boundary is a point at which macrophyte species are extinct due to anthropogenic pressures.
Bad	**-**	“Large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent”	Macrophyte depopulation due to anthropogenic pressure.

At ***Table 4*** an example for the macrophyte assessment is provided.

Table 4: An example for calculation of RI for Byala River (BG3MA00035MS1620, 2015), BG type R14

Taxa	abundance (0-5)/ quantity(0-125)	species group (see Annex A)	Calculation	EQR
Ranunculus trichophyllus	2/8	A	**RI = [(200-0)/209] * 100 = 95.69** **S = 14**	**0.98 (high)**
Carex pseudocyperus	3/27			
Eleocharis palustris	2/8			
Carex riparia	4/64			
Potamogeton nodosus	2/8	B		

Taxa	abundance (0-5)/ quantity(0-125)	species group (see Annex A)	Calculation	EQR
Lysimachia nummularia	1/1			
Polygonum sp.	2			
Typha latifolia	1/1			
Mentha spicata	2/8			
Alisma lanceolata	1/1	B		
Mentha longifolia	2/8			
Chara sp.	4/64	A		
Platyhypnidium riparioides	4/64	A		
Fontinalis hypnoides	4/64	A		

4 National data set

The national dataset contains data (both non-biological and biological) for 28 sites from the common intercalibration MedGIG river types: R-M1 and R-M2. Sites were selected to sufficiently cover the geographical area in which the common type occurs within BG (***Table 5***). Among sites, during the surveys in 2014-2015, 7 sites were assessed in high status, 15 in good, 5 in moderate, and 1 in poor status. It can be concluded that the dataset encompasses sampling sites covering almost the entire gradient of the pressure to be intercalibrated, and hence the complete ecological quality gradient (Wilby et al., 2014).

The dataset contains both non-biological (environmental, ***Table 6***) and biological data to conduct pressure-impact analyses, as well as non-biological data are contemporaneous with the accompanying biological data in time and space in order to be used for pressure-impact analyses.

Table 5: List of studied sites in Bulgaria

No	Site	MED GIG type	River Basin District
1	Brejanska14	R-M1	West Aegean
2	Stara14		West Aegean
3	Melnishka14		East Aegean

No	Site	MED GIG type	River Basin District
4	Manastirska14		East Aegean
5	Krumovitsa14		East Aegean
6	Melnishka15		East Aegean
7	Djebelska15		East Aegean
8	Nedelinska-Before15		East Aegean
9	Nedelinska-After15		East Aegean
10	Varbitsa-Zlato15		East Aegean
11	Sushichka15		West Aegean
12	Stara15		West Aegean
13	Kopriven15		West Aegean
14	Dereorman14	R-M2	East Aegean
15	Popovska14		East Aegean
16	Sokolitsa14		East Aegean
17	Yerusalimovska14		East Aegean
18	Byala14		East Aegean
19	Biserska14		East Aegean
20	Perperek14		East Aegean
21	Varbitsa-St.Klad14		East Aegean
22	Popovska15		East Aegean
23	Manastirska15		East Aegean
24	Sokolitsa15		East Aegean
25	Byala15		East Aegean
26	Krumovitsa15		East Aegean
27	Perperek-GK15		East Aegean
28	Varbitsa-St.Klad15		East Aegean

Table 6: List and range of selected environmental variables and physico-chemical parameters at river sites (n=28) listed at Table 5.

Variable/parameter	MIN	MAX	Variable/parameter	MIN	MAX
Altitude [m a.s.l.]	88	540	**COD-Cr** [mg L^{-1}]	<5	39.2

Catchment Size [km^2]	24.8	1,058.8	**TP** [mg L^{-1}]	<0.01	0.17
CLC_urbanization [%]	0	7.6	**SRP** [mg L^{-1}]	<0.03	0.14
CLC_intensive [%]	0	63.2	**TN** [mg L^{-1}]	0.1	9.4
CLC_non-intensive [%]	3.9	30.4	**NO_3-N** [mg L^{-1}]	0.07	9.1
CLC_natural [%]	20.9	88.7	**NO_2-N** [mg L^{-1}]	0	0.23
Conductivity [µS cm^{-1}]	206	1256	**NH_4-N** [mg L^{-1}]	0.04	0.25
DO [mg L^{-1}]	6.1	12			
BOD_5 [mg L^{-1}]	1.1	3.3	**RI-BG EQR**	0.07	0.97

5 Pressure-impact relationships

Table 7 below lists the pressures addressed by the national method.

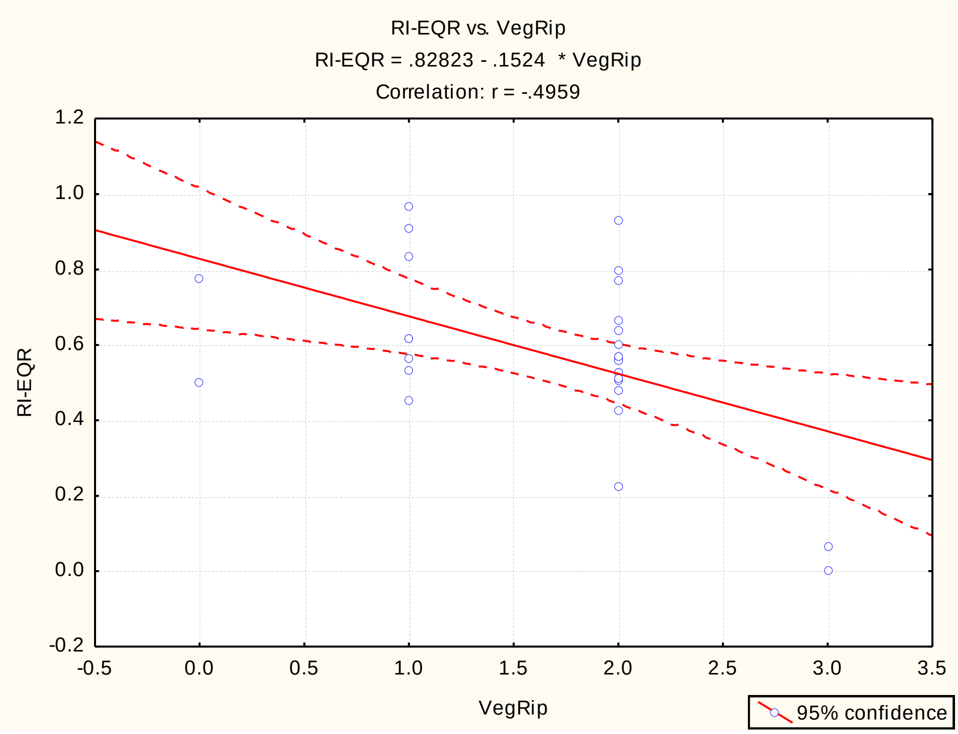
Table 7: Overview of the sensitivity to pressures of the national method

Method	Pressure
RI-BG	eutrophication, general degradation

Additionally, the national dataset was used to test the response to different types of pressures (***Table 8***). Responses to selected individual gradients are illustrated in ***Figure 2***. **It can be concluded that fitting procedure is feasible in terms of pressures.**

Table 8: Pressure-impact-relationships of national method and selected pressure variables

Pressure		Indicator tested	r^2	p
Eutrophication and general degradation				
1	Urban, Agricultural areas, Impoundment, Abstraction, Habitat alteration, Riparian Vegetation	Macrophyte-based EQR	0.563	<0.05
2	Physico-chemical parameters (BOD_5, TN)	Macrophyte-based EQR	0.431	<0.05



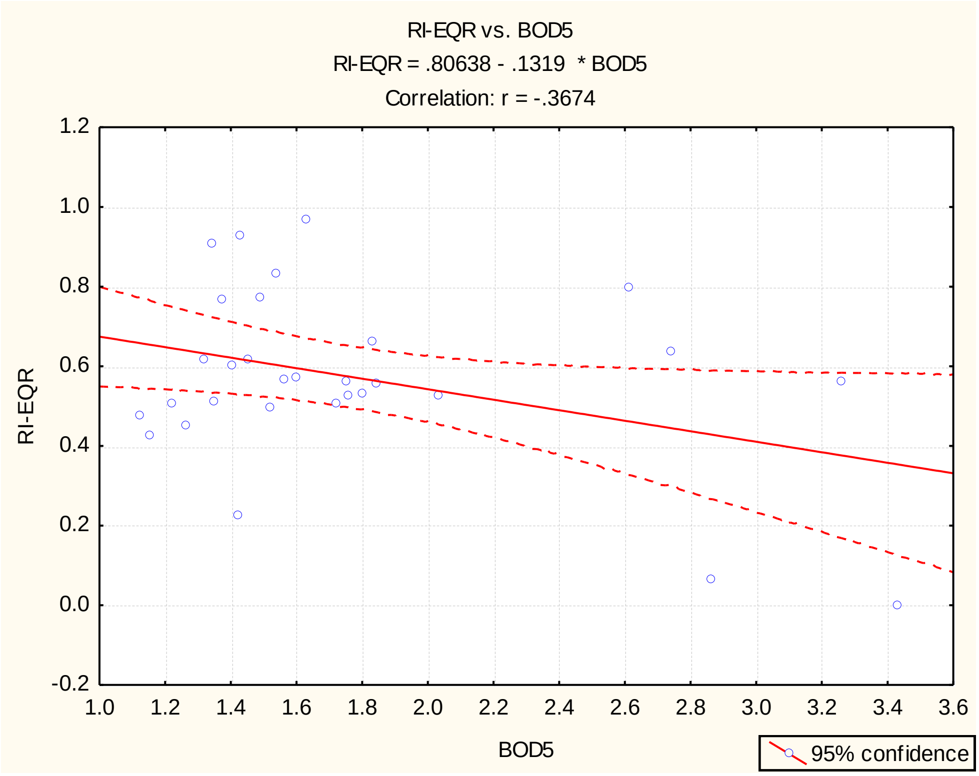


Figure 2: Response of assessment method (IC types R-M1 and R-M2) to individual pressures (Riparian Vegetation alteration and BOD_5).

6 Reference/benchmark setting process and boundary setting procedure

6.1 Description of reference/benchmark setting process

The setting of reference values was done based on alternative benchmark sites, since true reference sites are lacking. The procedure followed the approach of the Med GIG and developed common benchmark conditions. Thresholds established in the MedGIG for the final selection of benchmarks (***Table 9***) were applied to the national dataset.

Table 9: Thresholds applied for selection of benchmarks (after Feio et al., 2014)

Variables	Boundary
Channelization	≤2
Bank alteration (1–4)	≤2
Local habitat alteration (1–4)	≤2
Riparian vegetation (1–4)	≤2
Connectivity (1–4)	≤2
Stream flow (1–4)	≤2
Upstream dam influence (1–4)	≤2
Hydropeaking (1–4)	≤2
DO (mg/l)	6.39–13.70
N-NH4+ (mg/l)	≤0.09
N-NO3− (mg/l)	≤1.15
P-Total (mg/l)	≤0.07
P-PO4 3 − (mg/l)	≤0.06
% artificial areas (catchm)	≤1
% intensive agriculture (catchm)	≤11
% extensive agriculture (catchm)	≤32
% semi-natural areas (catchm)	≥68

Comparison with the established thresholds for benchmarks (Feio et al., 2014) showed that three five sites (Byala14, Byala15, Nedelinska-before Nedelino and Stara 14 and Stara 15) fulfilled them. These sites were with highest RI-EQR (0.969; 0.931; 0.870).

Macrophyte communities at these sites were bryophyte dominated (*Brachythecium rivulare*, *Platyhypnidium riparioides*, etc.). This observation corresponded with recorded loss and/or decrease in cover of bryophytes in conditions of declining ecological status (Aguiar et al., 2014).

Additional important metric of the macrophyte communities in benchmark sites is high species richness (S_{median} = 12).

Boundary setting

The H/G boundary was assumed as one quarter (0.25) below the maximum value at which only species of Group A taxa are represented. The G/M boundary was the point at which Group B (indifferent taxa) is dominant and Group A is represented with about 30% share. There are still no or very few representatives of disturbance indicators.

The M/P boundary was set as the average where the community is dominated by disturbance indicators (Group C). It is characterised by disappearance of aquatic bryophytes. The P/B boundary is a point at which macrophyte species are extinct due to anthropogenic pressures. EQR values are very low. Bad status is assigned in cases of macrophyte depopulation due to anthropogenic pressure.

Reference values and class boundaries

Based on the alternative benchmark, biological communities in undisturbed conditions at MED rivers in Bulgaria (IC types R-M1 and R-M2) are characterised by the dominance of aquatic bryophytes such as *Rhynchostegium riparoides*, *Brachythecium rivulare* and *Fontinalis* sp., indicating coarse substrates, higher current velocities and shaded river banks. Along with the constant presence of mosses, amphibious taxa (e.g. *Veronica* sp.) and helophytes (*Agrostis stolonifera*, *Mentha* sp., *Sparganium erectum*, *Typha* sp.) increase in abundance towards good status. At moderate and worse status myriophyllids (e.g. *Myriophyllum spicatum*), ceratophyllids (*Ceratophyllum demersum*) and parvopotamids (e.g. *Potamogeton crispus*, *P. nodosus*, *P. pusillus*) represent significant shares of the macrophyte assemblages.

The national dataset generally confirms the model described in Med GIG, i.e. in the reference sites of RM1, 2 and 4 a high diversity of bryophytes was observed (Aguiar et al., 2014). The moss cover (e.g. *Rhynchostegium riparioides*, *Fontinalis antipyretica*, etc.) was higher in the reference sites when compared with the borderline communities of G/M classes. Some species were not observed in these sites, or had a very low frequency of occurrence and/or abundance (e.g. *Potamogeton pectinatus*, *Scirpus lacustris*).

The reference values as defined in the previous chapter and the boundaries are summarized in ***Table 10***.

Table 10: Reference values and class boundaries for the Reference Index and the EQR value in the IC river types R-M1 and R-M2 (=R14)

Original (Before adjustment)	RI	EQR
Reference values	**100**	**1.00**
High / Good Boundary	**48**	**0.74**
Good / Moderate Boundary	**-2**	**0.49**
Moderate / Poor Boundary	**-60**	**0.20**
Poor / Bad Boundary	**-100**	**0**
Bad status	**Macrophyte depopulation**	

7 Checking of WFD compliance and evaluation of the assessment concept

WFD Compliance

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. All criteria for the compliance check are fulfilled (***Table 11***).

Table 11: Compliance criteria assessment methods for Reference Index, IC river types R-M1 and R-M2 (BG type R14)

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by one of five classes (high, good, moderate, poor and bad)	yes
2. High, good and moderate ecological status are set in line with the WFD's normative definitions	The H/G boundary is one quarter below the maximum RI value (at which sensitive species Group A dominated). The G/M boundary is the point at which Group B (indifferent taxa) is dominant and Group A is represented with 30% share. Nearly no representatives of disturbance indicators. The M/P boundary was set as the average where the community is dominated by

Compliance criteria	Compliance checking conclusions
	disturbance indicators (Group C). It is characterised by disappearance of aquatic bryophytes. The P/B boundary is a point at which macrophyte species are extinct due to anthropogenic pressures.
3. All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A combination rule to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole.	Taxonomic composition and relative abundance. Characeae, bryophytes and vascular plants - assessed on a species level, except for Chara, Chiloscyphus and Sphagnum (determined on genus level). Possible forms of combination between phytobenthos and macrophytes were not addressed in the MedGIG exercise
4. Assessment is adapted to intercalibration common types that are defined in line with the typological requirements of the WFD Annex II and approved by WG ECOSTAT	Yes, the assessment method is compatible with intercalibration common types. The national typology has been verified according to the common types.
5. The water body is assessed against type-specific near-natural reference conditions	yes
6. Assessment results are expressed as EQRs	yes
7. Sampling procedure allows for representative information about water body quality/ ecological status in space and time	Sampling procedure of macrophytes allows representative information of annual cycle. Sites were surveyed once during the vegetation period (from May to July).
8. All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure	Both relevant parameters of BQE are covered (i.e. species composition and abundance). Sampling is performed before dry period in the summer, thus insuring a good representative image of the site.
9. Selected taxonomic level achieves adequate confidence and precision in classification	Species level is required which is considered adequate to achieve confidence and precision in classification. Genus level is accepted only for *Chara* and *Sphagnum* due to listing all species within the genus to Group A.

Evaluation of the assessment concept

National macrophyte-based method (RI-BG) is based on multihabitat sampling covering the whole river channel and banks. Reference Index defines type-specific reference and non-specific disturbance indicating taxa (stoneworts, bryophytes and vascular plants).

Intercalibrated methods in MedGIG RMI (Slovenia) and IBMR (Cyprus, Greece, France, Italy, Portugal, Spain) are also indicator species based methods.

It could be concluded that BG method (RI-BG) has similar acquisition data and assessment concept with RMI and IBMR, thus the intercalibration is feasible in terms of assessment concepts.

8 IC Procedure

The macrophyte MedGIG has finalized Intercalibration, but BG did not join the group. Thus BG has to perform a fit-in-procedure. First it had to be shown that the macrophyte-based national method is compliant with the WFD normative definitions (see chapter 7) and intercalibration is feasible in terms of typology (see chapter 2).. In a second step it has to be proven, that the RI class boundaries are in line with the results of the intercalibration exercise.

8.1 Selection of the appropriate fit in procedure

The MED-GIG Macrophyte-group successfully completed intercalibration for rivers in the second round (2013). Seven member states (Portugal, Greece, France, Spain, Cyprus, Slovenia and Italy) were included. With one exception, they all used the French IBMR (Indice Biologique Macrophytique en Rivière; Haury et al., 2006) as assessment system. Only Slovenia was using another method: the Slovenian RMI (River Macrophyte Index). Accordingly a combination of Option 1 (for all countries using the IBMR-method) and Option 3 (median boundary values of all countries against the Slovenian RMI-method) was used for intercalibration. However, concerning the intercalibration of the Slovenian method no “real” option 3 was performed. As the harmonised median values of the H/G boundary and the G/M boundary of the countries intercalibrated previously via Option 1 was used as fixed benchmarks (Aguiar et al. 2013), the way of intercalibration in reality corresponded to a “fit-in procedure” (Aguiar, pers. comm.).

In case Option 3 was applied in the previous finalised intercalibration exercise, the current guidance for the fit-in-procedure “Instruction manual to fit new or revised national classifications to the completed IC exercise” stipulates Case B1 “IC Option 3, using reference/benchmark sites” as to be used for the fit-in procedure. However, as described above, this in reality was not the case in the MED-GIG. For this reason we decided not to follow Case B1. Furthermore, during testing to apply the Slovenian method on Bulgarian data, another problem arose: It turned out that due to the low number of indicator species, an application of the Slovenian Index for many of the Bulgarian sites was not possible. A similar situation was in the MED-GIG itself: Besides the Slovenian sites only some sites from France, Spain and Cyprus could be assessed with the Slovenian method.

For this reasons we decided to follow Case A1 for the fit-in procedure: **“IC Option 1 or 2 using reference/benchmark sites”.**

8.2 Requirements for using the procedure described as “Case A1”: IC Option 1 or 2 using reference/benchmark sites

Requirements for using the A1 approach

- *Full details of the common metric (e.g. species scores and metric weights).*

  The method used for the Option 1 intercalibration within the MED-GIG was the French IBMR (Haury et al., 2006). The details on how this method was applied as common metric for the MED-GIG intercalibration are given in the MED-GIG IC report (Aguiar et al., 2013).) and in Aguiar et al. (2014).

- *A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated.*

  A total of 28 sites, covering four quality classes (high to poor status), was available (see chapter 4 “National Dataset”).

- *Accompanying pressure data in the same format as that used in the completed exercise.*

  All relevant pressure parameters are available (see chapter 4 “National dataset”).

- *Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites (e.g. human population density, extent of agricultural land in catchment, nutrient concentrations etc.).*

  The benchmark criteria established in the MED-GIG macrophyte-group are given in chapter 6.1 “Description of reference/benchmark setting process”.

- *Details of exactly how the benchmarking was undertaken in the completed exercise (e.g. creation of a common metric EQR by dividing the observed value by the median common metric value of a set of national reference or benchmark sites). If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method.*

  The details exactly how benchmark standardisation was performed in the MED-GIG were provided by the MED-GIG lead Francisca Aguilar (Portugal).

- *Values of the global mean view of the HG and GM boundaries on the common metric scale for Member States who participated in the completed exercise.*

  Mean H/G-boundary (relevant for RM-1, RM-2, RM-4): 0.9127

  Mean G/M-boundary (relevant for RM-1, RM-2, RM-4): 0.7543

8.3 Process of fit the BG method to the completed IC exercise

Step 1: Calculate the common metric (CM) on the national dataset.

The common MED-GIG method IBMR (in the following: “common metric”=ICM) was calculated for the national dataset (see ***Table 12*** in comparison with the national RI-BG-EQR).

Table 12: List of national IC sites with national EQR-values (RI-BG) and corresponding ICM-EQR-vaules

Site No	Site	RI-BG-EQR	ICM-EQR
1	Popovska15	0,6373	0,7936
2	Melnishka15	0,5714	0,9940
3	Manastirska15	0,4548	0,6689
4	Sokolitsa15	0,5067	0,7483
5	Byala15	0,9690	0,9392
6	Krumovitsa15	0,5102	0,9274
7	Perperek-GK15	0,4271	0,6931
8	Varbitsa-St.Klad15	0,2252	0,7803
9	Djebelska15	0,6036	0,9892
10	Nedelinska-Before15	0,9310	1,0673
11	Nedelinska-After15	0,8000	0,7385
12	Varbitsa-Zlato15	0,9091	1,2519
13	Sushichka15	0,0676	0,4836
14	Stara15	0,6176	0,8921
15	Kopriven15	0,5132	0,8587
16	Dereorman14	0,5635	0,9875
17	Popovska14	0,4797	0,6950
18	Melnishka14	0,7706	1,0323
19	Manastirska14	0,7746	1,0221
20	Sokolitsa14	0,5706	0,8364
21	Yerusalimovska14	0,6633	0,9243

22	Byala14	0,6159	0,8706
23	Brejanska14	0,5000	0,9274
24	Stara14	0,8704	1,0608
25	Krumovitsa14	0,5286	0,9699
26	Biserska14	0,5344	0,9532
27	Perperek14	0,5562	0,9528
28	Varbitsa-St.Klad14	0,5286	0,8587

<u>Step 2: Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.</u>

The procedure followed the approach of the Med-GIG and the developed common benchmark conditions. Thresholds established in the Med-GIG for the final selection of benchmarks (***Table 13***) were applied to the national dataset. Comparison with the established thresholds for benchmarks (Feio et al., 2014) showed that five sites (Byala14, Byala15, Nedelinska-before Nedelino15, Stara14 and Stara 15) fulfilled them.

Table 13: Thresholds of the MED-GIG (after Feio et al., 2014) applied for selection of benchmarks-sites out of the Bulgarian dataset

Variables	Boundary (after Feio et al., 2014)	BG Benchmark sites		
		Byala	Stara	Nedelinska-Before Nedelino
Channelization	≤2	0	1	2
Bank alteration (1–4)	≤2	1	0	0
Local habitat alteration (1–4)	≤2	1	0	1
Riparian vegetation (1–4)	≤2	1	1	2
Connectivity (1–4)	≤2	0	0	0
Stream flow (1–4)	≤2	0	0	0
Upstream dam influence (1–4)	≤2	0	0	0
Hydropeaking (1–4)	≤2	0	0	0
DO (mg/l)	6.39–13.70	8.1	11.8	8.1
N-NH4+ (mg/l)	≤0.09	0.04	0.04	0.04
N-NO3− (mg/l)	≤1.15	0.1	0.11	0.24
P-Total (mg/l)	≤0.07	<0.01	0.01	0.01
P-PO4 3 − (mg/l)	≤0.06	<0.03	<0.03	<0.03
% artificial areas (catchm)	≤1	0.94	0	0
% intensive agriculture (catchm)	≤11	9.29	0.29	2.17
% extensive agriculture (catchm)	≤32	10.77	21.09	13.05
% semi-natural areas (catchm)	≥68	79	78.62	84.79

Step 3: Standardise the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise. If benchmark standardisation was concluded not to be required in the completed exercise the mean CM value of the joining method's benchmark sites must lie inside the range of mean values of the benchmark sites of the methods already intercalibrated for this conclusion to remain applicable. If the joining method's benchmark sites lie outside of this range the joining method must benchmark standardise its sites relative to the global mean CM value of the benchmark sites included in the completed exercise. These scenarios are illustrated in Table 1 and 2.

Table 14 provides an overview on how benchmark standardisation was carried out in the MED-GIG.

Table 14: Benchmarking approach in the MED GIG and benchmarking of the Joining BG method

	MS	No of BM sites	Mean ICM		Conclusion
Com-pleted Exercise	Spain	21	1,04	0.05	MSs benchmark-standardised the ICM by subtracting the offset of their benchmark sites
	Portugal	13	0,98	-0.01	
	Italy	15	1,02	0.03	
	France	16	0,97	-0.02	
	Greece	10	0,91	-0.08	
	Cyprus	3	1,03	0.04	
	Slovenia	0			
	Global Mean		**0,99**		
	Range		**0,91 – 1,04**		
Joining MS	Bulgaria	5	0,89	-0.10	The joining BG method benchmark-standardises the ICM by subtracting the offset from the global meen in accordance with the completed exercise

Step 4: Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x). A specialist case is that when a joining method relies exclusively on the common metric developed in the completed exercise for its classification rather than devising an original method (then being more like Option 1). In such cases a regression would

be meaningless as y is directly dependent on x. The goal for an MS choosing to use the CM as the basis for their method is simple – after any benchmarking their boundaries must simply lie within one quarter of class of the global mean view.

The regression fulfils the minimum criteria defined by the EC, 2011 (r2 >0.25; p<0.001; r >0.5; ***Figure 3***). The following graph shows the regression between CM and RI-EQR.

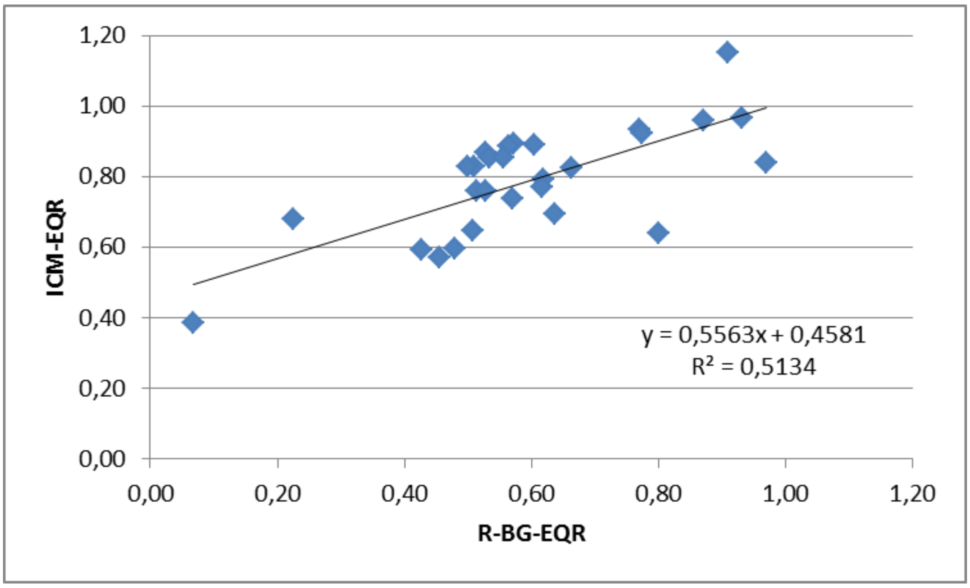


Figure 3: Regression between ICM (IBMR) and national RI-BG-EQR.

5. Predict the position of the national class boundaries (MP, GM, HG and reference) on the CM_bm scale.

Using the formula given in ***Figure 3*** the national boundaries can be converted to ICM-boundaries as follows (***Table 15***).

Table 15: Conversion of the national boundaries into ICM-boundaries

Boundary / Reference	RI-BG-EQR	ICM-EQR predicted	ICM-EQR MED-GIG	Bias
Max	1.00	1.0144		
H/G	0.74	0.8809	0.9127	-0.0318
G/M	0.49	0.7307	0.7543	-0.0236
M/P	0.20	0.5694		

6. Apply the comparability criteria as summarised in Chapter 6.

6.1. Determine the direction of deviation of the national HG and GM boundaries of the joining method on the common metric scale relative to the global mean view defined in the completed exercise.

The national H/G and G/M-boundaries fall below the global view (see ***Table 15***).

*6.2. If the national GM boundary on the common metric scale falls below the global view, calculate the amount of this deviation and express it as a proportion of the width of the good status class on the common metric scale. If this value is ≤0.25 the boundary meets the comparability criteria. If >0.25, the GM boundary must be raised until the deviation between the national GM boundary on the common metric scale and the global view on the same scale is ≤0.25 class widths (**Table 16**).*

Table 16: Calculation of the amount of G/M bias expressed as proportion of class width

	Class width	Bias abs	Bias rel
G/M	0.1502	-0.0236	0.1572

The amount of the deviation expressed as proportion of class width is ≤0.25 and therefore meets the comparability criteria.

6.3. If the national GM boundary on the common metric scale falls above the global view,…

Not relevant.

*6.4. These steps should then be repeated for the HG boundary. Thus, if the national HG boundary on the common metric scale falls below the global view, calculate the amount of this deviation and express it as a proportion of the width of the high status class on the common metric scale. If this value is ≤0.25, the boundary meets the comparability criteria. If >0.25 the HG boundary must be raised until the deviation between the national HG boundary on the common metric scale and the global view on the same scale is ≤0.25 class widths. If the national HG boundary on the common metric scale falls above the global view, calculate the amount of this deviation and express it as a proportion of the width of the good status class on the common metric scale. If this value is ≤0.25, the boundary meets the comparability criteria. If >0.25, the HG boundary can be lowered until the deviation between the national HG boundary on the common metric scale and the global view on the same scale is ≤0.25 class widths. However, there is no obligation to make this adjustment. If the deviation is equivalent to >0.5 of the good class width, an adjustment is strongly recommended since this implies that the global view of the HG boundary of the countries that completed the exercise is closer to the GM boundary of the joining Member State (**Table 17**).*

Table 17: Calculation of the amount of H/G bias expressed as proportion of class width before and after harmonisation of the H/G-boundary

	Class width	Bias abs.	Bias rel.

H/G original	0.1446	-0.0429	0.2968
H/G adjusted	0.1335	-0.0318	0.2383

The H/G boundary had to be adjusted from 0.74 to 0.76.

Conclusions

The sampling campaigns of the joint BG-AT project have enabled establishing a comprehensive dataset for the national river type R14. Based on the relationships and following the CIS Guidance Documents, a reference value and class boundaries were defined for the Reference Index, which is used for assessing R14 rivers based on BQE macrophytes.

The national assessment method was compared with the finalized IC exercise of the Med GIG following the fit-in procedure of Willby et al. (2014). The analysis revealed a good agreement of the national method with the methods from other member states of the Med-GIG. Following the criteria defined in the fit-in-procedure of Wilby et al. (2014), the national assessment method of Bulgaria with a slight adjustion of the H/G boundary is considered as comparable with the existing macrophyte-based method (IBMR).

9 References

Aguiar F.C., Cambra J., Chauvin C., Ferreira T., Germ M., Kuhar U., Manolaki P., Minciardi M.R. & Papastergiadou E. 2010. Floristic and functional gradients of river plant communities: a bio geographical study across the Mediterranean Basin. XV Congress of the Iberian Association of Limnology, 5-7 July 2010, Azores: 55.

Aguiar F.C., Segurado P., Urbanič G., Cambra J., Chauvin C., Ciadamidaro S. et al., 2013. Meditteranean River GIG – Macrophytes. 2012 Intercalibration Technical Report, unpublished.

Aguiar F.C., Segurado P., Urbanič G., Cambra J., Chauvin C. & Ciadamidaro S., Dörflinger, G., Ferreira, J., Germ, M., Manolaki, P., Miniciardi, M.R., Munné, A., Papastergiadou, E. & Ferreira, M.T. 2014. Comparability of river quality assessment using macrophytes: A multi-step procedure to overcome biogeographical differences. Science of the Total Environment, 476–477: 757–767.

Feio M.J., Aguiar F.C., Almeida S.F.P., Ferreira J., Ferreira M.T., Elias C. et al., 2014. Least Disturbed Condition for European Mediterranean rivers. Science of the Total Environment, 476-477: 745-56.

Gecheva G., Dimitrova-Dyulgerova I. & Cheshmedjiev S. 2013. Macrophytes. In: Belkinova D. & Gecheva G. (eds.), Biological analysis and ecological status assessment of Bulgarian surface water ecosystems, Plovdiv University Publishing House, 127-146 (in Bulgarian).

Haury J, Peltre M-C, Trémolières M, Barbe J, Thiébaut G & Bernez I, et al. 2006. A new method for assess water trophy and organic pollution — theMacrophyte Biological

Index for Rivers (IBMR): its application to different types of rivers and pollution. Hydrobiologia 570: 153–8.

Kohler A. 1978. Methoden der Kartierung von Flora und Vegetation von Süßwasserbiotopen. Landschaft & Stadt., 10, 73–85.

Schaumburg J., Schranz C., Foerster J., Gutowski A., Hofmann G., Meilinger P., Schneider S. & Schmedtje U. 2004. Ecological classification of macrophytes and phytobenthos for rivers in Germany according to the Water Framework Directive. Limnology, 34, 283–301.

Schaumburg J., Schranz C., Stelzer D., Hofmann G., Gutowski A. & Foerster J. 2006. Instruction Protocol for the Ecological Assessment of Running Waters for Implementation of the EC Water Framework Directive: Macrophytes and Phytobenthos. Munich: Bavarian Environment Agency.

Willby N., Birk S., Poikane S. & van de Bund, W. 2014. Water Framework Directive Intercalibration Manual. Procedure to fit new or updated classification methods to the results of a completed intercalibration. JRC. doi: 10.2788/37057.

ANNEX A. Specified Reference Index indicators list for R-M1 and R-M2 (R14).

Taxa	R14 Group
***Agrostis gigantea* Roth**	A
***Agrostis stolonifera* L.**	B
***Alisma lanceolatum* With.**	B
***Amblystegium serpens* (Hedw.) Schimp.**	B
***Angelica sylvestris* L.**	B
***Apium nodiflorum* (L.) Lag.**	B
***Apium repens* (Jacq.) Lag.**	B
***Azolla filiculoides* Lam.**	B
***Berula erecta* (Huds.) Coville**	A
***Brachythecium rivulare* Schimp.**	A
***Bryum turbinatum* (Hedw.) Turner**	B
***Butomus umbellatus* L.**	B
***Calliergon cordifolium* (Hedw.) Kindb.**	B
***Calliergon giganteum* (Schimp.) Lindb.**	A
***Callitriche cophocarpa* Sendtn.**	B
***Callitriche platycarpa* Kütz.**	A
***Callitriche stagnalis* Scop.**	A
***Ceratophyllum demersum* L.**	C
***Ceratophyllum submersum* L.**	C
***Chara* spp.**	A
***Chiloscyphus* Corda spp. (*C. pallescens*; *C. polyanthos*)**	A
***Cinclidotus aquaticus* (Hedw.) Bruch & Schimp.**	B
***Cinclidotus fontinaloides* (Hedw.) P. Beauv.**	A
***Cyperus fuscus* L.**	B
***Cyperus longus* L.**	B
***Conocephalum conicum* (L.) Dumort.**	B
***Cratoneuron filicinum* (Hedw.) Spruce**	A

Taxa	R14 Group
***Dichodontium pellucidum* (Hedw.) Schimp.**	A
***Drepanocladus aduncus* (Hedw.) Warnst.**	A
***Drepanocladus sendtneri* (Schimp.) Warnst.**	A
***Eleocharis acicularis* (L.) Roem. & Schult.**	A
***Elodea canadensis* Michx.**	B
***Elodea nuttallii* (Planch.) H.St.John**	B
***Equisetum fluviatile* L.**	A
***Equisetum palustre* L.**	B
***Fissidens adianthoides* Hedw.**	B
***Fissidens crassipes* Wilson ex Bruch & Schimp.**	A
***Fissidens fontanus* (Bach.Pyl.) Steud.**	B
***Fissidens pusillus* (Wilson) Milde**	B
***Fissidens rivularis* (Spruce) Schimp.**	A
***Fontinalis antipyretica* Hedw.**	B
***Fontinalis hypnoides* C.Hartm.**	A
***Galium palustre* L.**	B
***Glyceria fluitans* (L.) R.Br.**	B
***Glyceria maxima* (Hartm.) Holmb.**	B
***Groenlandia densa* (L.) Fourr.**	A
***Hippuris vulgaris* L.**	A
***Hydrocharis morsus-ranae* L.**	B
***Hygroamblystegium fluviatile* (Hedw.) Loeske**	A
***Hygroamblystegium humile* (P.Beauv.) Vanderp. et al.**	B
***Hygroamblystegium tenax* (Hedw.) Jenn.**	B
***Hygroamblystegium varium* (Hedw.) Mönk.**	B
***Hygrohypnum luridum* (Hedw.) Jenn.**	A
***Juncus articulatus* L.**	B
***Juncus effusus* L.**	B
***Lemna gibba* L.**	C

Taxa	R14 Group
***Lemna minor* L.**	C
***Lemna trisulca* L.**	B
***Leptodictyum riparium* (Hedw.) Warnst.**	B
***Leskea polycarpa* Hedw.**	B
***Lythrum salicaria* L.**	B
***Lunularia cruciata* (L.) Lindb.**	B
***Marchantia polymorpha* L.**	B
***Marsupella emarginata* (Ehrh.) Dumort.**	A
***Mentha aquatica* L.**	B
***Myosotis scorpioides* L.**	B
***Myriophyllum spicatum* L.**	B
***Myriophyllum verticillatum* L.**	B
***Nardia compressa* (Hook.) Gray**	A
***Nasturtium officinale* R.Br.**	A
***Nuphar lutea* Sm.**	B
***Nymphaea alba* L.**	B
***Palustriella* spp. (*P. commutata, P. decipiens*)**	A
***Paspalum paspalodes* (Michx.) Scribn.**	C
***Phalaris arundinacea* L.**	B
***Platyhypnidium riparioides* (Hedw.) Dixon**	A
***Polygonum amphibium* L.**	A
***Polygonum hydropiper* L.**	C
***Polygonum mite* Schrank**	B
***Potamogeton acutifolius* Link**	A
***Potamogeton berchtoldii* Fieber**	C
***Potamogeton crispus* L.**	C
***Potamogeton friesii* Rupr.**	C
***Potamogeton gramineus* L.**	A
***Potamogeton lucens* L.**	A

Taxa	R14 Group
***Potamogeton natans* L.**	A
Potamogeton natans x nodosus	B
***Potamogeton nodosus* Poir.**	B
***Potamogeton pectinatus* L.**	C
***Potamogeton perfoliatus* L.**	A
***Potamogeton polygonifolius* Pourr.**	A
***Potamogeton praelongus* Wulfen**	A
***Potamogeton pusillus* L.**	B
***Potamogeton trichoides* Cham. & Schltdl.**	C
***Racomitrium aciculare* (Hedw.) Brid.**	A
***Ranunculus flammula* L.**	A
***Ranunculus penicillatus* (Dumort.) Bab.**	B
***Ranunculus repens* L.**	B
***Ranunculus trichophyllus* Chaix**	A
***Riccia fluitans* L.**	A
***Ricciocarpos natans* (L.) Corda**	C
***Sagittaria sagittifolia* L.**	B
***Scapania undulata* (L.) Dumort.**	A
***Schistidium rivulare* (Brid.) Podp.**	A
***Scirpus lacustris* L.**	B
***Sciuro-hypnum plumosum* (Hedw.) Ignatov & Huttunen**	A
***Sparganium emersum* Rehmann**	B
***Sparganium erectum* L.**	B
***Sparganium minimum* Wallr.**	A
***Sphagnum* L. spp.**	A
***Spirodela polyrhiza* (L.) Schleid.**	C
***Stratiotes aloides* L.**	A
***Trapa natans* L.**	C
***Utricularia vulgaris* L.**	A

Taxa	R14 Group
***Veronica anagallis-aquatica* L.**	B
***Veronica beccabunga* L.**	B
***Warnstorfia fluitans* (Hedw.) Loeske**	A
***Zannichellia palustris* L.**	C