REPORT

DEVELOPMENT OF A NATIONAL ASSESSMENT METHOD FOR THE ECOLOGICAL STATUS OF RIVERS IN GREECE, USING THE BIOLOGICAL QUALITY ELEMENT "BENTHIC MACROINVERTEBRATES", THE HELLENIC EVALUATION SYSTEM-2 (HESY-2), and HARMONISATION WITH THE RESULTS OF THE COMPLETED INTERCALIBRATION OF THE MED GIG (RM1, RM2, RM4, RM5)

BQE: Benthic macroinvertebrates

The present study has been prepared in the framework of the Greek National Water Monitoring Network, according to the Joint Ministerial Decision 140384/2011. The Network is supervised by the Directorate for the Protection and Management of Water Resources of the Special Secretariat for Waters of the Ministry of Environment and Energy.
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1. INTRODUCTION

The Mediterranean GIG successfully finalized the intercalibration (IC) for macroinvertebrates for rivers in 2012 and it was completed in two phases. The results of the first phase were included in the first Commission Decision (COM DEC 2008/915/EC) and the second phase in the Commission Decision (COM DEC 2013/480/EC) on intercalibration. Although Greece participated in the first intercalibration exercise for the types RM1, RM2 and RM4, it did not contribute with data to the second intercalibration exercise.

The scope of this report is to declare that the Greek assessment method of ecological status of rivers of the IC types (RM1, RM2, RM4 and RM5) according to benthic macroinvertebrates is compliant with the WFD normative definitions and its class boundaries are in accordance with the results of the completed intercalibration exercise.

In particular, the classification method was verified for WFD compliance and IC feasibility and the class boundaries were compared with agreed boundaries from the MED-GIG intercalibration exercise following the instructions of the CIS Guidance Document n^{o} 30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise".

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The ratio of an observed value of the Hellenic Evaluation System (HESY, Artemiadou & Lazaridou 2005) to the median reference values of the same river type (see below section 2.4) produced the new Hellenic assessment system for rivers, HESY2.

HESY has been intercalibrated for RM4 in 2008 (Artemiadou et al. 2008) and for RM1 and RM2 in 2013 (Ntislidou et al. 2013). This assessment was also used in the last national river basin management plan of Central Macedonia (WD 10) and Crete (WD 13). HESY is based on family identification level. It consists of families found in reference sites of Greek rivers (e.g. Nestos) which don't have a score in other European indices or metrics as well as in the component metric of the STAR ICMi index. Additionally, the Hellenic Evaluation System takes into consideration the tolerance, the abundance and the diversity/richness of benthic macroinvertebrates, which are required by the WFD. It is composed of the:

(a) Hellenic Evaluation Score (HES) which follows the BMWP-type score rationale (Armitage et al. 1983) (Annex I)

(b) Average HES (AHES) which is similar to ASPT (Annex I) and

(c) SemiHES, which is the final result of the Hellenic Evaluation System, is the semi-total of the HES and AHES values [Semi- HES = (standardized HES+standardized AHES)/2] standardized against the habitat diversity richness (WFD requirement of habitat) based on the Habitat Richness Matrix (GHRM) (Chatzinikolaou et al. 2006) (Annex II).

The SemiHES is interpreted at a five class scale (five water quality categories according to the WFD) (Artemiadou & Lazaridou 2005) (Annex I).

HESY was transformed to EQR (HESY2) according to the procedure described in section 2.4.

2.1. METHODS AND REQUIRED BQE PARAMETERS

The Hellenic Evaluation System is in accordance with the WFD compliance, as it takes into consideration all the indicative parameters which are mentioned in CIS Guidance document No 14 (Table 1).

Table 1. Overview of the metrics included in the Hellenic Evaluation System 2.

MS	Taxonomic composition	Abundance	Disturbance sensitive taxa	Diversity	Absence of major taxonomic groups	Taxa indicative of pollution
GR	x	x	x	x	x	x

2.2. SAMPLING AND DATA PROCESSING

The sampling is performed by wading in the river, when the depth is less than 1m, or along the riparian zone in deep rivers. Benthic macroinvertebrates are sampled using a 250 mm × 230 mm, D-shaped pond net (0.9 mm mesh size, ISO 7828:1985; EN 27828:1994) according to the semi-quantitative 3-min kick/sweep method (Armitage & Hogger 1994) plus 1 min when bank vegetation exists (Wright 2000, Kemitzoglou 2004). During the 3 min sampling event all available microhabitats are covered proportionally according to a matrix of possible river habitats (Chatzinikolaou et al. 2006) (Annex II). Sampling is conducted twice a year, during the high flow (spring) and low flow (summer, autumn).

Overview of sampling and data processing for HESY2 national assessment method

Parameter	**Description**
Sampling	Benthic macroinvertebrates are sampled using a 250 mm × 230 mm, D-shaped pond net (0.9 mm mesh size, ISO 7828:1985; EN 27828:1994)
Sampling methods	According to the semi-quantitative 3-min kick/sweep method (Armitage and Hogger, 1994) plus 1 min when bank vegetation existed (Wright 2000, Kemitzoglou 2004). During the 3 min all microhabitats are covered proportionally according to a matrix of possible river habitats (Chatzinikolaou et al., 2006) (Annex II). All benthic macroinvertebrate taxa and their abundance are taken into consideration.
Level of identification	Family level except of Ostracoda, Hydracarina, Aranae and Oligochaeta (apart from Tubificidae).
Frequency	Sampling is performed twice a year, during the high flow (spring) and low flow (summer, autumn).
Data processing	Taxa identification is carried out in the laboratory with the help of specialized taxonomic keys, and the relative abundance of each taxon is calculated.

2.3. NATIONAL REFERENCE CONDITIONS

Reference samples were selected from High (spring) and low flow (summer, autumn) according to MEDGIG 2012. Selection of reference sites was based on MEDGIG 2012 and on the following criteria as well:

I) Hydromorphological criteria

a) Degree of habitat AMENDING (Habitat Modification Score, HMS) ≤2 (Raven et al. 1998)

b) Land use coverage at catchment area (MEDGIG 2012, Feio et al. 2014) according to Corine Land Cover 2012:

- Forests and semi-natural areas ≥68%
- Intensive agriculture ≤11%
- Extensive agriculture% ≤ 32
- Artificial surfaces ≤ 1

c) Land use coverage at catchment area (Environment Agency 2005):

- Agriculture areas <20%
- Urban areas <20%

d) Land use coverage rate along the river (Environment Agency 2005):

- Agriculture areas <20%
- Urban areas <20%

II) Physico-chemical criteria

The selection of reference sites based on the physico-chemical criteria led to the creation of four databases which were tested in order to choose the appropriate typological system for Greece.

DATABASE A: Physico-chemical criteria recommended by Bonada et al. 2002:

- NH_4 < 0.5 mg/l, equivalent to N-NH4 <0.3889 mg/l
- N-NO_2 <0.01 mg/l
- P -PO_4 <0.05 mg/l

DATABASE B: Physico-chemical criteria recommended by Munné et al. 2006:

Maximum value NH_4 < 1 mg/l, equivalent to N-NH_4 < 0.7778 mg/l

Maximum value NO_3 < 20 mg/l, equivalent to N-NO_3 < 4.5162 mg/l

Maximum value PO_4 <1 mg/l, equivalent to P-PO_4 < 0.326 mg/l

DATABASE C: Physico-chemical criteria recommended by MEDGIG 2012:

DO 6.39-13.70 mg/l

O2 73.72-127.92 %

N-NH_4^+ ≤0.09 mg/l

N-NO_3^- ≤1.15 mg/l

P-PO_4^{3-} ≤0.06 mg/l

DATABASE D: Physico-chemical criteria recommended by Skoulikidis et al. 2006:

N-NH_4 <0.024 mg/l

N-$N0_2$ <0.003 mg/l

N-$N0_3$ <0.220 mg/l

P-PO_4<0.030 mg/l

In DATABASE D, the DO criteria was also taken into account (DO 6.39-13.70 mg/l) as specified in the MED GIG 2012.

In **DATABASE E** only the criteria proposed by Feio et al. 2014 were taken into consideration.

III) Biological quality

The biological quality under the Hellenic Evaluation System (Artemiadou & Lazaridou 2005) was also taken into consideration in the selection of reference sites; the value of SemiHes had to be greater than 4.0, so as the sites to have at least good ecological quality.

From all above physico-chemical databases, the last one (database D) was found to be more appropriate for Greece. However, the number of reference sites was less (Table 2) since the criteria were stricter.

Table 2. Number of reference samples in the tested typological systems (see Section 4.1).

Typological system	Database C	Database D	Database E
RM	73	54	147
RBMP	63	51	95
System A	63	50	145
System B′	73	54	147

2.4. NATIONAL BOUNDARY SETTING

The national boundary has been set for each IC river type using the EQR_Semi_HES (HESY 2) reference samples value from Database D. The value of 25th percentile of the reference values (i.e. the peak value of the lower quartile) is the boundary between the high and good (H/G) water quality. The range between zero value and high-good quality limit (i.e. higher value of the lower quartile - 0) is divided into four equal parts (quarters). The lower value of the first quarter (from the top) is the boundary between good and moderate quality (G/M) (Table 3).

Table 3. Summary of the class boundaries for the EQR_HESY values in the IC river types based on the DATABASE D.

	R-M1	R-M2	R-M4	R-M5
Reference values	1.100	1.000	1.000	1.000
High/Good Boundary	1.000	0.944	0.850	0.889
Good/Moderate Boundary	0.750	0.708	0.638	0.667
Moderate/Poor Boundary	0.500	0.472	0.425	0.444
Poor/Bad Boundary	0.250	0.236	0.213	0.222

2.5. PRESSURES ADDRESSED

In the finalized IC exercise a number of pressures were used by the countries. The GR assessment method responded to land use, organic pollution and hydro-morphological degradation (Table 4). Spearman correlation coefficient showed statistically significant relationships ($p<0.001$) between HESY2 and various pressures except of two case in RM5 (HMS, DO). So, the GR assessment method is comparable to the methods which have already been successfully intercalibrated.

Table 4. Spearman correlations of the EQR_HESY to the individual pressures. In bold are marked significant correlations with rho>0.5. Red color represents non significant correlations.

Pressures	**RM5**			**RM1+RM2+RM4**		
	Spearman rho	p	n	**Spearman rho**	p	n
near_natural_catchment	0.422**	0.000	163	**0.527****	**0.000**	**1056**
intens_agric_catchment	-0.441**	0.000	163	**-0.577****	**0.000**	**1056**
extens_agric_catchment	-0.194**	0.007	163	-0.245**	0.000	1056
artificial_catchment	-0.457**	0.000	163	**-0.539****	**0.000**	**1056**
urban_catchment	-0.256**	0.000	163	-0.366**	0.000	1056
Agriculture_catchment	-0.419**	0.000	163	**-0.532****	**0.000**	**1056**
HMS	*0.005*	*0.478*	*118*	-0.333**	0.000	754
N-NO2	**-0.528****	**0.000**	**145**	-0.511**	0.000	989
N-NO3	-0.261**	0.001	137	-0.365**	0.000	1008
N-NH4	-0.268**	0.001	141	-0.240**	0.000	980
P-PO4	-0.495**	0.000	159	-0.176**	0.000	1027
DO	*0.057*	*0.239*	*159*	0.316**	0.000	1034
BOD	-0.375**	0.000	82	-0.245**	0.000	790

3. WFD COMPLIANCE CHECKING

According to Guidance document No 14 (2011), only assessment methods meeting the requirements of the WFD can be intercalibrated. An important step in the intercalibration procedure is the evaluation 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 Guidance document No 14

(2011)]. The compliance check showed that the Greek method fulfils the requirements of the WFD (Table 5).

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

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes; High-Good classes boundary: 25th percentile of reference sites; the range below was divided in 4 equal classes; Good-Moderate = H/G x 0.75; Moderate-Poor = H/G x 0.50; Poor-Bad = H/G x 0.25
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	Yes; the assessment method takes into consideration the tolerance, the abundance/habitat and the diversity/richness of the biocommunity
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	Yes
The water body is assessed against **type-specific near-natural reference conditions**	Yes
Assessment results are expressed as **EQRs**	Yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	Yes- 2 occasions per year: Spring (high flow) and Summer/Autumn (low flow)
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes; in MED GIG all methods, except SI (Slovenia) use family level. SI method uses mostly genus/species level.

4. IC FEASIBILITY CHECKING

In the final MED-GIG Intercalibration exercise similar assessment concepts were used. The assessment method, which is presented here, also follows the IC exercise using the fit-in procedure which is considered feasible in terms of assessment concepts.

4.1. TYPOLOGY

Discriminant analysis (SPSS 22.0) was carried out (benthic macroinvertebrates were used as independent values and types as grouping values) in order to investigate which of the four typological approaches, used in Greece, was the most appropriate regarding the benthic fauna composition of reference conditions:

a) The intercalibration types (RM types) used in the last national basin management plans of Central Macedonia (WD 10) and Crete (WD 13).

b) The typological system, which has been used for the preparation of a number of the River Basin Management Plans (hereafter RBMP typology)
c) System A, as proposed by the Directive 2000/60/EC (hereafter System A)
d) System B′, used in Northern and Central Greece by Lazaridou et al. 2013 (hereafter System B)
The reference samples used were in accordance with the European Guidelines conditions (Wallin et al. 2003, European Commission 2007, van de Bund 2009) and referred either to high (Spring) or low (Summer, Autumn) flow period according to MedGIG 2012. For the selection of the reference conditions, the criteria described above (2.3 National reference conditions) were followed.
System A fails to take into account rivers with a small catchment size (<10Km^2), which are numerous in Greece. Moreover, system A incorporates the ecoregion descriptor and as Greece hosts ecoregions 6 and 7 and Axios river flows through both of them this system cannot be applied in Greece.
As to the RBMP typology system, some of the reference sites cannot be classified (e.g. ELATO from the national monitoring network) since the shapefiles provided do not cover the whole country due to the lack of parameters used in this typological system (e.g. annual runoff, annual rainfall and evapotranspiration and runoff index).
System RBMP (11 types), System B (25 types) as well as system A (13 types) consist of a large number of types, some of which are represented only by one or two reference sites (Table 6) which cannot lead to a correct estimation of the Ecological Quality Ratio (EQR).

For the selection of reference sites the criteria proposed are described in Section 2.3. National reference conditions. To compare the typological systems mentioned above, discriminant analysis (using the SPSS 22.0 program for Windows) was applied to benthic macroinvertebrates as independent values and types as grouping ones. According to the results, a higher accuracy was found in all typological systems as to the original grouping of the types using DATABASE D than DATABASE E (Table 6). However, according to the discriminant analysis results, the RM typological system, based on DATABASE D had a higher original but a lower cross-validation grouping (Table 6) than the DATABASE E (Table 6). Additionally, from Database D 54 samples (Table 2) were reference whereas 144 with Feio et al. (2014) criteria (Table 2).

Table 6. Results of the discriminant analysis of all the typology systems.

	RM	Typology of RBMP	System A	System b
DATABASE D				
Original grouped (%)	90.4	97.1	95.6	95.9
Cross-validated grouped (%)	35.6	29.0	13.2	13.7
DATABASE E				
Original grouped (%)	86.2	87.8	83.8	75.5
Cross-validated grouped (%)	46.8	26.8	19.0	22.9

The MED-GIG includes 5 types, all of which are applicable for Greece (Table 7). Among them, RM3 type cannot be intercalibrated due to the lack of comparability between MS methods because of insufficient number of reference sites.

Table 7. IC types in the MED GIG.

Common IC Type	**River characterization**	**Catchment (km^2)**	**Geology**	**Flow regime**
RM1	Small Mediterranean streams	< 100	Mixed (except silicious)	Highly seasonal
RM2	Medium Mediterranean streams	100 - 1000	Mixed (except silicious)	Highly seasonal
RM3	Large Mediterranean streams	1000-10000 km^2	Mixed (except siliceous)	Highly seasonal
RM4	Mediterranean mountain streams		Non-silicious	Highly seasonal
RM5	Temporary streams			Temporary

4.2. PRESSURES ADDRESSED

The HESY2 addresses the general degradation of various pressures (see above Section "2.5 Pressures addressed"). To our knowledge the Bulgarian Report on fitting a new classification method to the results of the completed intercalibration of the EC GIG (R-E2, R-E3) and the Fitting of the new Lithuanian River Macroinvertebrate Index (LRMI) to the results of the Central-Baltic Geographical Intercalibration Group do also address these pressures.

4.3. ASSESSMENT CONCEPT

The HESY2 is based on multi-habitat sampling scheme (Annex II) and takes into consideration the tolerance, the abundance and the diversity/richness of the biocommunity (Annex II). It is standardized according to rich/poor habitats (Annex I). HESY2 responds effectively to various pressures (see above Section "2.5 Pressures addressed"). Additionally, when discriminant analysis was applied [benthic macroinvertebrates abundance represented the independent values of DA and quality class values from HESY2 (Table 8) and five other multimetric and biotic indices (Annex III), before the intercalibration exercise, the grouping values)] to find out how many samples have shifted from moderate to high and good quality (major importance for the WFD), it was found that no shift occurred in HESY2 in three out of five river types (RM1, 2, 5) in the high quality while only 5.6-5.7 % shifted from moderate to good in RM-1,-2 and RM-4 river types (Table 8).

Table 8. Number of samples shifted from moderate to good/high for HESY2 before the intercalibration exercise.

MODERATE	BAD	POOR	MODERATE	GOOD	HIGH	SUM
RM1-HESY_2		4	45	3	0	52
RM2-HESY_2		6	113	7	0	126
RM4-HESY_2		2	56	1	3	62
RM5-HESY_2		0	34	0	0	34

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The RM typology was chosen. The HESY2 is based on multi-habitat sampling (Annex II) and takes into consideration the tolerance, the abundance and the diversity/richness of the biocommunity (Annex I). It is standardized according to rich/poor habitats. The HESY2 addresses the general degradation of various pressures (see above Section "2.5 Pressures addressed"). It is concluded that the fitting of HESY2 to the results of the MED-GIG river intercalibration results was feasible.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

According to the flow diagram in the CIS Guidance No. 30 (Willby et al. 2014) to select the appropriate fitting procedure, option B (case A1) was applied for the assessment method using benthic macroinvertebrates as biological element in the MED GIG river types RM1, RM2, RM4 and RM5.

The requirements for case A1 are:

- Full details of the common metric
- A suitable *site x biology* dataset covering a range of environmental quality from which the national EQR and common metric can be calculated
- Accompanying pressure data in the same format as that used in the completed exercise.
- Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites
- Details of exactly how benchmarking was undertaken in the complete exercise. 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.
- 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.

The process of fitting the GR method to the completed IC exercise:

According to the Willby et al. 2014, the following steps should be followed:

- Calculate the common metric (CM) on the national dataset.
- 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.
- 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.

- 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.
- Predict the position of the national class boundaries (MP, GM, HG and reference) on the CM bm scale.
- Apply the comparability criteria as summarised in Chapter 6.

5.2. Description of IC dataset

Calculate the common metric (CM) on the national dataset

The Intercalibration Common Metric index (ICMi) was developed and used in the first intercalibration exercise and is described in detail by Buffagni et al. 2006.

The ICMi is a multimetric index covering the aspects of the normative definition for the ecological status classification (WFD Annex V, 1.2.1). The following 6 metrics are used: average score per taxon, log 10 (sel_EPTD+1), 1-GOLD, total number of taxa Families, number of EPT taxa (Families) and the Shannon-Wiener diversity index (Table 9).

Table 9. Description of the component Intercalibration Common Metrics (ICMs) and calculation of the Intercalibration Common Multimetric Index STAR_ICMi (Buffagni et al. 2006). EQR = Ecological Quality Ratios. The weight of each metric appears in the equation at the end of this table.

COMPONENT INTERCALIBRATION COMMON METRICS (ICMs)			
Information type	Metric	Description	EQR values (EQR ICMs)
Tolerance	ASPT	Whole community (family level)	EQR ASPT-2 = (ASPT-2)/median value of (ASPT-2) in reference samples
Abundance/Habitat	Log_{10}(Sel EPTD +1)	Log_{10}-transformed abundance of selected taxa of Ephemeroptera, Plecoptera, Trichoptera and Diptera (Heptageniidae, Ephemeridae, Leptophlebiidae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae, Stratyomidae, Dixidae, Empididae, Athericidae, Nemouridae)	EQR log(SelEPTD + 1) = log(SelEPTD + 1)/median value of log(SelEPTD + 1) in reference samples
	1-GOLD	1-(relative abundance of Gastropoda, Oligochaeta and Diptera)	EQR (1-GOLD) = (1-GOLD)/median value of (1-GOLD) in reference samples
	EPT	Number of Ephemeroptera, Plecoptera and Trichoptera families	EQR EPT = EPT/median value of EPT in reference sites
	N-families	Total number of taxa	EQR N-families = N-families/median value of N-families in reference samples
Richness & Diversity	Shannon-Wiener index	Diversity index	EQR Shannon index = Shannon index/median value of Shannon index in reference samples

INTERCALIBRATION COMMON MULTIMETRIC INDEX

STAR_ICMi = 0.333*EQR (ASPT-2) + 0.266*EQR Log_{10} (SelEPTD +1) + 0.067*EQR (1-GOLD) + 0.083*EQR EPT + 0.167*EQR N-families + 0.083*EQR Shannon

EQR STAR_ICMi = STAR_ICMi / median value of STAR_ICMi in reference samples

Identify benchmark sites in the national dataset

Sites selected as reference (benchmark) were based on criteria defined in section "2.3 National reference conditions"

Benchmark standardization

Following the procedure, which is described in Guidance document No 14, the mean and median values were calculated for the benchmark sites (DATABASE E) for each river type and the ICMi metrics and index (Table 10).

Table 10. Number of benchmark samples in each river type (DATABASE E) and statistical values for ICMi index and its metrics.

IC River type	Sample N	ASPT-2		Log_{10}(Sel EPTD +1)		1-GOLD		N-families		EPT		Shannon-Wiener index		ICMi	
		Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median
RM1	52	4.81	4.87	1.95	1.98	0.77	0.80	25.06	25.00	11.90	11.50	2.16	2.17	0.99	0.99
RM2	32	4.85	4.89	1.92	1.97	0.78	0.82	24.19	24.00	11.78	11.00	2.04	2.11	0.99	0.97
RM4	59	4.89	4.90	1.96	2.05	0.77	0.82	22.83	23.00	12.24	12.00	2.03	2.13	0.98	1.02
RM5	6	5.27	5.19	1.84	1.61	0.67	0.71	19.17	20.00	11.50	11.50	1.70	1.75	1.00	1.01

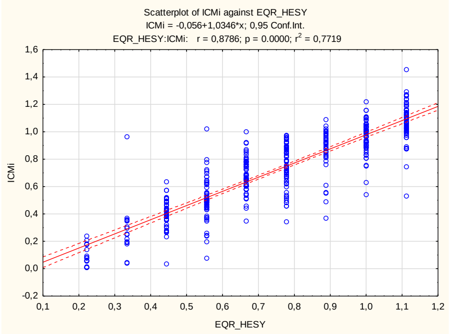
Ordinary least squares (OLS) regression

HESY 2 and ICMi were strongly correlated ($p<0.005$, $r> 0.5$) for each IC river type (Table 11 and Figure 1).

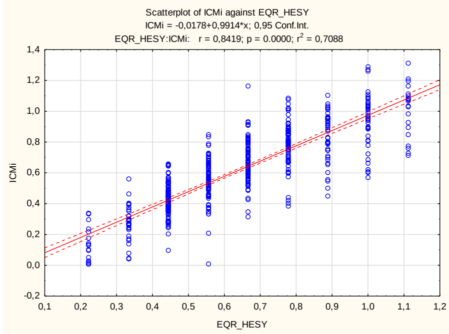
Table 11. OLS regression to ICMi and EQR_HESY_2 for each IC river type based on samples from DATABASE E.

IC river type	**Equation**	**p**	**r**	**n**
RM1	ICMi=-0.056+1.0346*[EQR_HESY2]	0.0000	0.8786	411
RM2	ICMi-0.0178+0.9914*[EQR_HESY2]	0.0000	0.8419	537
RM4	ICMi=-0.0218+1.0772*[EQR_HESY2]	0.0000	0.8719	394
RM5	ICMi=0.0742+0.587*[EQR_HESY2]	0.0000		

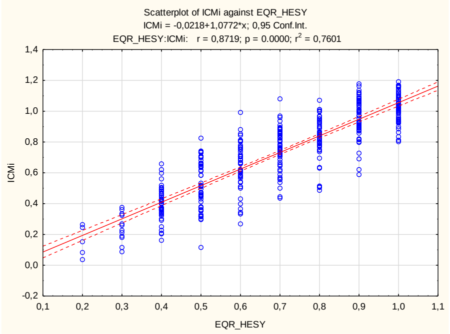
RM1



RM2



RM4



RM5

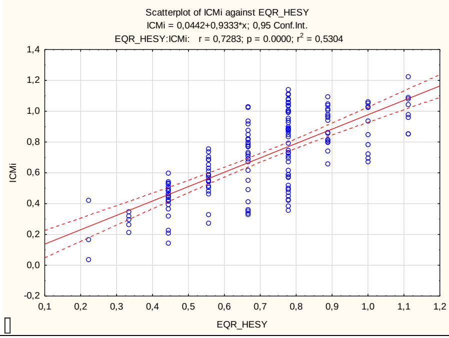


Figure 1. OLS regressions to establish the relationship between ICMi and the HESY 2 for each IC river type based on benchmark reference samples from DATABASE E.

5. Position of the national class boundaries on the ICMi scale

The predicted position of the national class boundaries (MP, GM, HG and reference) (see section 2.4) on the ICMi scale for each IC river type (Table 12 – 14).

Table 12. Reference values and High/Good class boundary of the ICMi values derived from the OLS regression (Figure 1) for each IC river type.

	RM1	RM2	RM4
HIGH Max (maximum of national EQR)	**1.082**	**0.974**	**1.055**
H/G Boundary + 0,25H	1.004	0.936	0.934
H/G Boundary (for MS)	**0.979**	**0.923**	**0.894**
H/G Boundary - 0.25H	0.914	0.865	0.836
H/G MedGIG Global mean	0.884	0.884	0.884
H/G quarter (+)	0.026	0.013	0.040
H/G quarter (-)	0.065	0.058	0.057

Table 13. Good/Moderate class boundary of the ICMi values derived from the OLS regression (Figure 1) for each IC river type

	RM1	RM2	RM4
Good/Moderate Max	0.979	0.923	0.894
G/M+0.25H	0.785	0.749	0.722
G/M Boundary (for MS)	**0.720**	**0.690**	**0.664**
G/M Boundary - 0.25H	0.655	0.632	0.607
M/P Min	0.461	0.453	0.436
G/M MedGIG Global mean	**0.704**	**0.704**	**0.704**
G/M quarter (+)	0.065	0.058	0.057
G/M quarter (-)	0.065	0.058	0.057

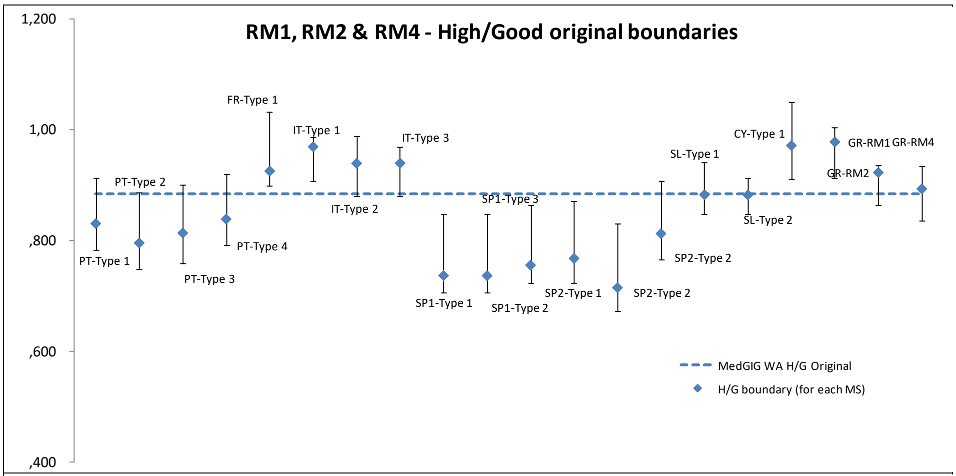
Table 14. Reference values, High/Good and Good/Moderate class boundary of the ICMi values derived from the OLS regression (Figure 1) for RM5 river type.

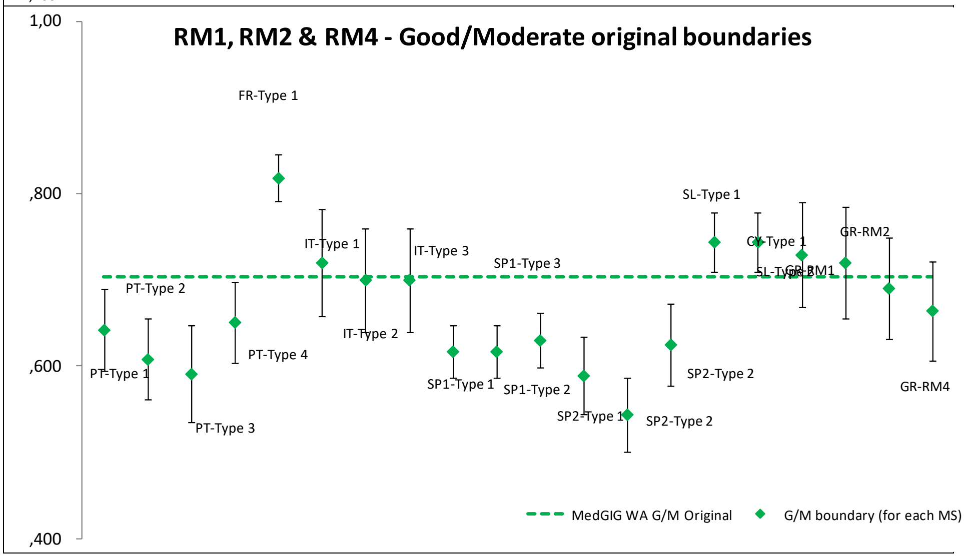
	RM5		RM5
HIGH Max (maximum of national EQR)	0.978	**Good/Moderate Max**	0.874
H/G Boundary + 0,25H	0.900	G/M+0.25H	0.719
H/G Boundary (for MS)	**0.874**	**G/M Boundary (for MS)**	**0.667**
H/G Boundary - 0.25H	0.822	G/M Boundary - 0.25H	0.615
H/G MedGIG WA	**0.990**	**G/M MedGIG WA**	**0.722**
H/G quarter (+)	0.026	G/M quarter (+)	0.052
H/G quarter (-)	0.052	G/M quarter (-)	0.052
		M/P Min	0.459

In Figure 2 is presented the comparison of H/G and G/M original boundaries values for the types RM1, RM2 & RM4 and RM5 with the other MS of the MED-GIG. The explanation of the typological codes used is given in Table 15.

Table 15 - Typological codes used in the boundary bias analysis.

Code	*MS Type*	*Code*	*MS Type*
PT-Type 1	N1≤100	SP1-Type 2	IBMWP R-M2
PT-Type 2	N2	SP1-Type 3	IBMWP R-M4
PT-Type 3	N3	SP1-Type 4	SP1 R-M5
PT-Type 4	N1≥100	SP2-Type 1	IMM R-M1
PT-Type 5	S1<100	SP2-Type 2	IMM R-M2
PT-Type 6	S3	SP2-Type 3	IMM R-M4
FR-Type 1	FR R-M1	SP2-Type 4	SP2 R-M5
IT-Type 1	IT R-M1	SI-Type 1	SL R-M1
IT-Type 2	IT R-M2	SI-Type 2	SL R-M2
IT-Type 3	IT R-M4	SI-Type 3	SI R-M5
IT-Type 4	IT R-M5	CY-Type 1	CY R-M4
SP1-Type 1	IBMWP R-M1	CY-Type 2	CY R-M5


RM1, RM2 & RM4 - High/Good original boundaries
1,200
1,00
,800
,600
,400
PT-Type 1
PT-Type 2
PT-Type 3
PT-Type 4
FR-Type 1
IT-Type 1
IT-Type 2
IT-Type 3
SP1-Type 1
SP1-Type 2
SP1-Type 3
SP2-Type 1
SP2-Type 2
SP2-Type 2
SL-Type 1
SL-Type 2
CY-Type 1
GR-RM1
GR-RM2
GR-RM4
MedGIG WA H/G Original
H/G boundary (for each MS)


RM1, RM2 & RM4 - Good/Moderate original boundaries
1,00
,800
,600
,400
FR-Type 1
PT-Type 1
PT-Type 2
PT-Type 3
PT-Type 4
IT-Type 1
IT-Type 2
IT-Type 3
SP1-Type 1
SP1-Type 2
SP1-Type 3
SP2-Type 1
SP2-Type 2
SP2-Type 2
SL-Type 1
SL-Type 2
CY-Type 1
GR-RM1
GR-RM2
GR-RM4
MedGIG WA G/M Original
G/M boundary (for each MS)

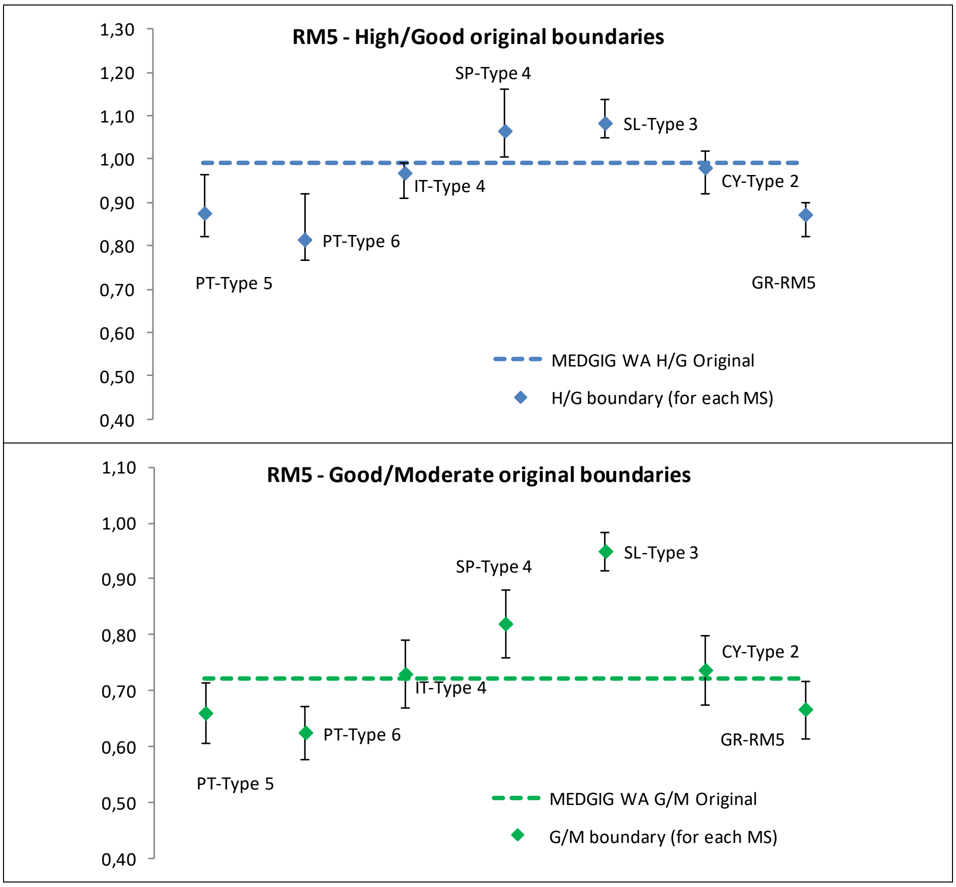


Figure 2. Comparison of H/G and G/M original boundaries values for the types RM1, RM2 & RM4 and RM5 with the other MS.

6. Application of the comparability criteria

The adjustment of the boundaries follows the fit according to the guidance of chapter 6 (Willby et al. 2014). The main principle is that H/G or G/M statistic must not be >|0.25|. These values do not meet this criterion in all cases (see red color in Table 16) and there is an obligation to make adjustments in the class boundaries.

Table 16. H/G and G/M statistic for each IC river type. Red color represents the statistic >|0.25|.

Boundary	RM1	RM2	RM4	RM5
H/G statistic	-0,91	0,78	0,06	1,12
G/M statistic	0,06	0,06	0,17	-0,27

After the harmonization of the boundaries, the new harmonized boundaries are presented in Tables 17 – 19 for H/G boundary and G/M boundary, respectively.

Table 17. Reference values and harmonized High/Good class boundary of the ICMi values derived from the OLS regression (Figure 1) for each IC river type.

	RM1	RM2	RM4
HIGH Max (maximum of national EQR)	**1.082**	**0.974**	**1.055**
H/G Boundary + 0,25H	0.961	0.932	0.934
H/G Boundary (for MS)	**0.920**	**0.918**	**0.894**
H/G Boundary - 0.25H	0.855	0.860	0.837
H/G MedGIG WA	**0.879**	**0.879**	**0.879**
H/G quarter (+)	0.041	0.014	0.040
H/G quarter (-)	0.065	0.058	0.057
H/G statistic	0.20	-0.17	-0.06

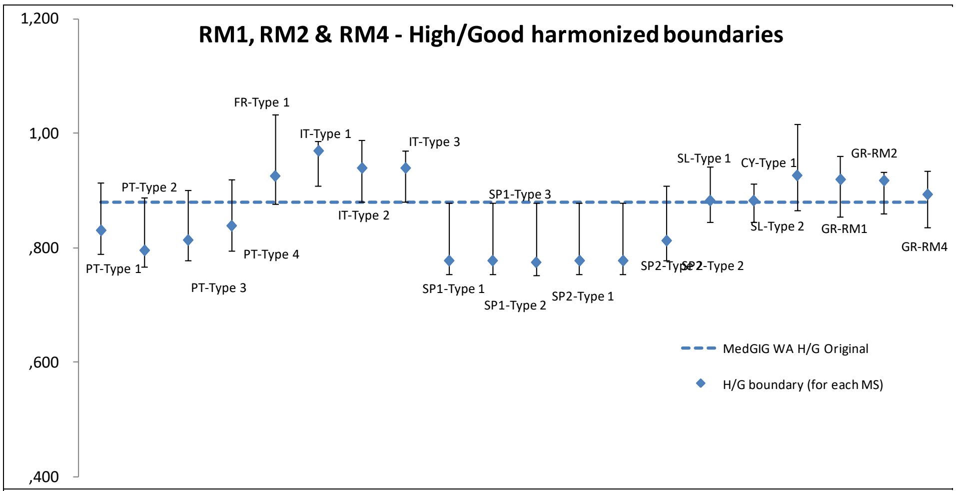
Table 18. Harmonized Good/Moderate class boundary of the ICMi values derived from the OLS regression (Figure 1) for each IC river type.

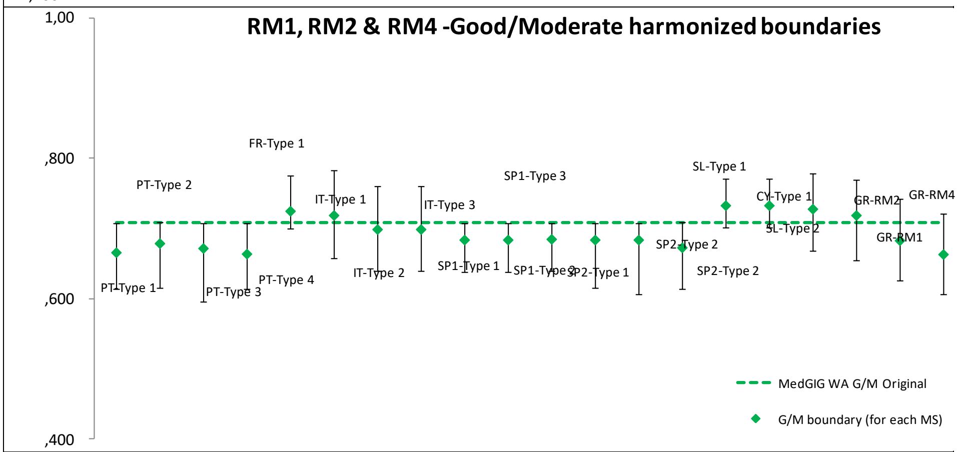
	RM1	RM2	RM4
Good/Moderate Max	0.920	0.918	0.894
G/M+0.25H	0.770	0.743	0.722
G/M Boundary (for MS)	**0.720**	**0.684**	**0.664**
G/M Boundary - 0.25H	0.655	0.626	0.607
M/P Min	**0.461**	**0.450**	**0.436**
G/M MedGIG WA	0.708	0.708	0.708
G/M quarter (+)	0.050	0.058	0.057
G/M quarter (-)			
G/M statistic	0.05	0.10	0.19

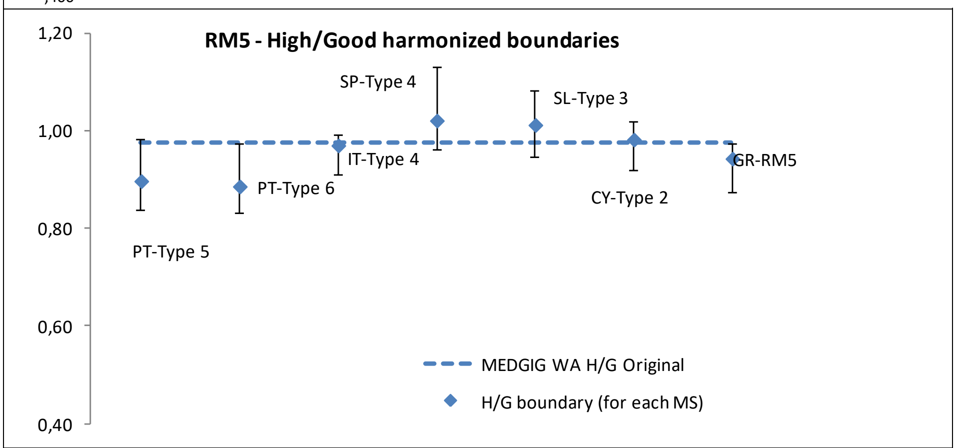
Table 19. References values and harmonized High/Good and Good/Moderate class boundaries of the ICMi values derived from the OLS regression (Figure 1) for RM5 IC river type.

	RM5		**RM5**
HIGH Max (maximum of national EQR)	**1.071**	**Good/Moderate Max**	**0.943**
H/G Boundary + 0.25H	0.975	G/M+0.25H	0.740
H/G Boundary (for MS)	**0.943**	**G/M Boundary (for MS)**	0.672
H/G Boundary - 0.25H	0.875	G/M Boundary - 0.25H	0.604
H/G MedGIG WA	**0.975**	**G/M MedGIG WA**	**0.722**
H/G quarter (+)	0.032	G/M quarter (+)	0.068
H/G quarter (-)	0.068	G/M quarter (-)	0.068
		M/P Min	0.459
H/G statistic	0.25	G/M statistic	-0.18

In Figure 3 is presented the comparison of H/G and G/M harmonized boundaries values for the types RM1, RM2 & RM4 and RM5 with the other MS of the MED-GIG. The explanation of the typological codes used is given in Table 15.


RM1, RM2 & RM4 - High/Good harmonized boundaries
1,200
1,00
,800
,600
,400
FR-Type 1
IT-Type 1
IT-Type 3
PT-Type 2
IT-Type 2
SP1-Type 3
SL-Type 1
CY-Type 1
GR-RM2
SL-Type 2
GR-RM1
GR-RM4
PT-Type 1
PT-Type 3
PT-Type 4
SP1-Type 1
SP1-Type 2
SP2-Type 1
SP2-Type 2
SP2-Type 2
MedGIG WA H/G Original
H/G boundary (for each MS)


RM1, RM2 & RM4 -Good/Moderate harmonized boundaries
1,00
,800
,600
,400
FR-Type 1
PT-Type 2
SP1-Type 3
SL-Type 1
IT-Type 1
IT-Type 3
CY-Type 1
GR-RM2
GR-RM4
SL-Type 2
GR-RM1
SP2-Type 2
SP2-Type 2
SP1-Type 1
SP1-Type 2
SP2-Type 1
IT-Type 2
PT-Type 4
PT-Type 1
PT-Type 3
MedGIG WA G/M Original
G/M boundary (for each MS)


RM5 - High/Good harmonized boundaries
1,20
1,00
0,80
0,60
0,40
SP-Type 4
SL-Type 3
IT-Type 4
GR-RM5
PT-Type 6
CY-Type 2
PT-Type 5
MEDGIG WA H/G Original
H/G boundary (for each MS)

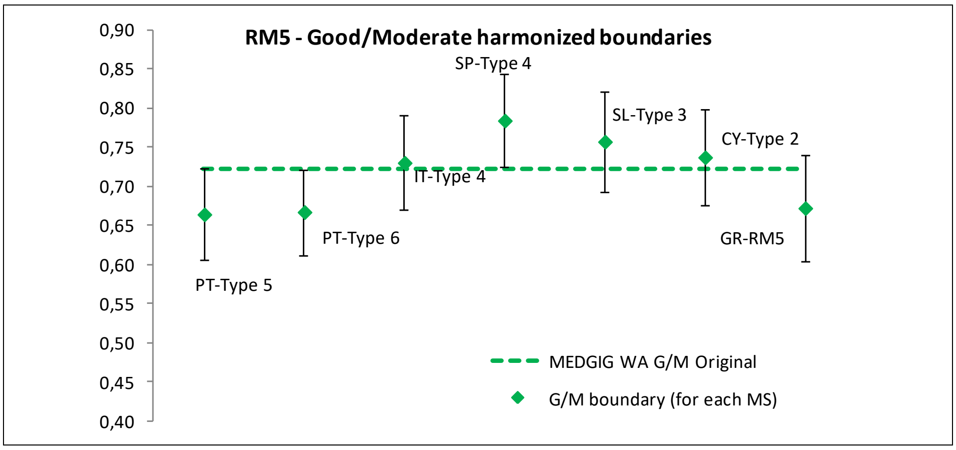


Figure 3. Comparison of HG and G/M harmonized boundaries values for the types RM1, RM2 & RM4 and RM5 with the other MS. The explanation of the typological codes is denoted in Table 15.

Table 21 indicates the final class boundaries, EQR values and ICMi after the harmonization process. In RM1 river types, the national H/G boundary was higher than the harmonization value (see Table 12) and in accordance with the IC Guidance Document (Step 7: Boundary adjustment), in this case the MS are not obliged to change their boundaries. For this occasion, the H/G boundary of RM1 river maintain the original boundary. For all other cases, the boundaries adopted the harmonized values.

Table 21. Final class boundaries of ICMi and HESY2after harmonization.

	R-M1		R-M2		R-M4		R-M5	
	ICMi	**HESY2**	ICMi	**HESY2**	ICMi	**HESY2**	ICMi	**HESY2**
Reference values	1.082	**1.100**	0.974	**1.000**	1.055	**1.000**	1.071	**1.100**
High/Good Boundary	0.920	**0.943**	0.918	**0.944**	0.894	**0.850**	0.943	**0.963**
Good/Moderate Boundary	0.720	**0.750**	0.684	**0.708**	0.664	**0.637**	0.672	**0.673**

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

Benthic macroinvertebrates are presented in Annex I according to their sensitivity standardized by their abundance. High-Good status is presented from 80-120 score, moderate from 50-78 and less than moderate from 1-40 score.

To describe the biological communities in the different status Similarity Percentages Analysis (SIMPER, using Primer 6) was applied to all data in RM1+RM2+RM4+RM5 intercalibration types. The similarity and dissimilarity results of macroinvertebrates for High, Good and Moderate status are presented below.

Group High Status Average similarity: 43.05 Species	Average Abundance	Contribution %	Cummulative contribution %
Chironomidae	4.37	13.09	13.09
Baetidae	4.36	13.05	26.14
Heptageniidae	3.47	9.5	35.63

Group Good Status Average similarity: 33.91 Species	Average Abundance	Contribution %	Cummulative contribution %
Chironomidae	3.82	18.56	18.56
Baetidae	3.74	18.2	36.76
Heptageniidae	2.19	8.52	45.28

Group Moderate Status Average similarity: 29.19 Species	Average Abundance	Contribution %	Cummulative contribution %
Chironomidae	4.1	26.17	2617
Baetidae	3.2	17.32	43.49
Caenidae	2.06	7.8	51.29

Groups Good & High Status Average dissimilarity = 63.83 Species	Group Good Average Abundance	Group High Average Abundance	Contribution %	Cummulative contribution %
Heptageniida e	2.19	3.47	3.58	3.58
Simuliidae	2.09	2.36	3.43	7.01
Caenidae	1.82	2.11	3.41	10.42

Groups Good & Moderate Status Average dissimilarity = 71.39 Species	Group Good Average Abundance	Group Moderate Average Abundance	Contributio n %	Cummulative contribution %
Baetidae	3.74	3.2	4.7	4.7
Chironomidae	3.82	4.1	4.59	9.29
Caenidae	1.82	2.06	4.13	13.42
Simuliidae	2.09	1.66	4.12	17.54

Groups High & Moderate Status Average dissimilarity = 70.85 Species	Group High Average Abundance	Group Moderate Average Abundance	Contribution %	Cummulative contribution %
Heptageniidae	3.47	1.08	4.19	4.19
Baetidae	4.36	3.2	3.56	7.75
Hydropsychidae	2.95	1.54	3.56	11.31

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ANNEX I

Hellenic Evaluation System (Artemiadou & Lazaridou 2005)

Sensitivity	Taxa	Present (0-1%)	Common (1.01-10%)	Abundant (>10%)
Taxa sensitive to to organic pollution	**a)** Capniidae, Chloroperlidae, **b)** Siphlonuridae, **c)** Aphelocheiridae, **d)** Blephariceridae **e)** Phryganeidae, Molanidae, Odontoceridae, Bareidae, Lepidostomatidae, Thremmatidae, Brachycentridae, Helicopsychidae	100	110	120
	a) Leuctridae, Perlodidae, Perlidae, **b)** Sericostomatidae, Goeridae, **c)** Neoephemeridae	90	97	100
	a) Nemouridae, Taeniopterygidae, **b)** Ephemeridae, Heptageniidae, Leptophlebiidae, **c)** Leptoceridae, Polycentropodidae, Psychomyidae, Philopotamidae, Limnephilidae, Rhyacophilidae, Glossosomatidae, Ecnomidae, **d)** Aeshnidae, Lestidae, Corduliidae, Libelulliidae, **e)** Athericidae, Dixidae, **f)** Helodidae, Gyrinidae, Hydraenidae, **g)** Sialidae, **h)** Grapsidae, Potamonidae (Brachyura) **i)** Astacidae, (Macrura)	80	86	90
Taxa medium sensitive to organic pollution	**a)** Potamanthidae, **b)** Calopterygidae, Cordulegasteridae **c)** Stratiomyidae, **d)** Hydrobiidae	70	75	78
	a) Platycnemididae, Gomphidae, **b)** Tabanidae, Ceratopogonidae, Empididae, **c)** Elmidae, **d)** Viviparidae, Neritidae, **e)** Unionidae,	60	64	67
	a) Caenidae, Oligoneuriidae, Polymitarcidae, Isonychiidae, **b)** Hydropsychidae, **c)** Ancylidae, Acroloxidae, **d)** Gammaridae, Corophidae, **e)** Atyidae, **f)** Planariidae, Dendrocoelidae, Dugesiidae, **g)** Dryopidae, Helophoridae, Hydrochidae, Clambidae, **h)** Psychodidae, Simuliidae,	50	53	56
Taxa tolerant to organic pollution	**a)** Ephemerellidae, Baetidae, **b)** Hydroptilidae **c)** Tipulidae, Dolichopodidae, Anthomyidae, Limoniidae, **d)** Haliplidae, Curculionidae, Chrysomelidae, Hydroscaphidae **e)** Hydracarina **f)** Piscicolidae, Glossiphonidae	40	38	35
	a) Coenagrionidae, **b)** Chironomidae (not red), **c)** Dytiscidae, Hydrophilidae, Hygrobiidae, **d)** Corixidae, Hebridae, Veliidae, Mesoveliidae, Hydrometridae, Gerridae, Nepidae, Pleidae, Naucoridae, Notonectidae, Belostomatidae, **e)** Asellidae, Ostracoda, **f)** Physidae, Bythiniidae, Bythinellidae, Molaniidae, Ellobiidae, **g)** Hirudinidae, **h)** Sphaeriidae **i)** Oligochaeta (except for Tubificidae)	30	25	20
	a)Chironomidae (red), Rhagionidae, Culicidae, Muscidae, Thaumaleidae, Ephydridae, Chaoboridae **b)** Lymnaeidae, Planorbidae, **c)** Erpobdellidae	20	12	3
	a)Tubificidae, **b)** Valvatidae, **c)** Syrphidae	10	2	1

Chironomidae (not red) and Oligochaeta (except for Tubificidae) scored as above but the relative abundance categories 0–10% for "present" (P), 10.01–20% for "common" and over 20% "abundant" (A)

Rich Habitat Diversity Sites

HES	X	AHES	Y
>1532	5	>64.72	5
1326-1532	4	54.57-64.72	4
830-1325	3	45.82-54.56	3
341-829	2	31.73-45.81	2
0-340	1	0-31.72	1

Poor Habitat Diversity Sites

HES	X	AHES	Y
>1053	5	>55.69	5
756-1052	4	45.18-55.69	4
389-755	3	35.33-45.17	3
167-388	2	27.50-35.32	2
0-166	1	0-27.49	1

Semi-HES=HES+AHES/2	Interpretation
5	**High**
4,5	**High**
4	**Good**
3,5	**Good**
3	**Moderate**
2,5	**Moderate**
2	**Poor**
1,5	**Poor**
1	**Bad**

ANNEX II

GREEK HABITAT RICHNESS MATRIX (GHRM)

(Chatzinikolaou et al. 2006)

Table of habitats ✓ *When existed the type of habitat*	Macrophytes>10% of total	Natural substrate					Slough	Artificial substrate		Woody snag
1. Riffle **(**swallow depth with fast flow)		CPOM	FPOM	Coarse**	Mixture*	Fine***		Other	Concrete	
Channel margin										
Island margin										
Main channel										
2. Run (all the others except of 1 and 3)										
Channel margin										
Island margin										
Main channel										
3. Pool] (deep depth, with no or slow flow)										
Channel margin										
Island margin										
Main channel										

* Mixture: Variant substrate composition that cannot be classified as coarse or fine
**Coarse: Substrate composition >70% of boulders and/or cobbles and/or pebbles
***Fine: Substrate composition >70% of gravel and/or sand and/or silt

At least one √	Rich station
	Poor station

ANNEX III

To form the best Hellenic assessment index (biotic or multimetric) the following metrics or indices were used:

1. All commonly used metrics -in Europe-, (most of them are referred in ASTERICS program (http://www.fliessgewaesser-bewertung.de/en/download/berechnung/) describing various features of the benthic fauna (Table Annex IIIa):

a) The number of taxa
b) Their relative abundance (%)
c) The presence of tolerant/sensitive taxonomic groups

Table Annex IIIa. Parameters used and tested for the creation of the index/indices.

Category	Parameter	Notes
Number of taxonomic groups	Total number of taxa	Number of taxa/ Total number of taxa
	Number of taxa Ephemeroptera	
	Number of taxa Plecoptera	
	Number of taxa Trichoptera	
	Number of taxa Coleoptera	
	Number of taxa Diptera	
	Number of taxa Odonata	
	Number of EPT taxa	
	Number of taxa EPTC	
	Log10(Sel EPTD +1)	Calculation of the decimal logarithm of the sum of the individuals from the families: Ephemeroptera, Plecoptera, Trichoptera and Diptera (Heptageniidae, Ephemeridae, Leptophlebiidae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae, Stratyomidae, Dixidae, Empididae, Athericidae, Nemouridae) (Buffagni et al., 2004; Buffagni & Erba, 2004)
	1-GOLD	1-(relative abundance of Gastropoda, Oligochaeta and Diptera) (Pinto et al., 2004)
	Pielou's index	Evenness index (Pielou 1966)
	Shannon-Wiener index	Diversity index (Hering et al., 2004; Böhmer et al., 2004)
Benthic fauna composition	Relative abundance of Ephemeroptera	Abundance (%) of a taxon/ sample
	Relative abundance of Plecoptera	
	Relative abundance of Trichoptera	
	Relative abundance of Coleoptera	
	Relative abundance of Diptera (A)	All families of Diptera
	Relative abundance of Diptera (B)	The family Chironomidae is not included
	Relative abundance of Odonata	Abundance (%) of a taxon/ sample
	Relative abundance of Chironomidae	
	Relative abundance of Oligochaeta	
	Relative abundance of EPT	
	Relative abundance of EPTC	
Resistance /sensitivity to pollution	Number of persistent taxa	Tolerant/sensitive taxa according to Annex I.
	Relative abundance of persistent taxa	
	Number of sensitive taxa	
	Relative abundance of sensitive taxa	

	Relative abundance of Hydropsychidae	In relation to the total number of Trichoptera
	Relative abundance of Baetidae	In relation to the total number of Ephemeroptera
	ASPT-2	Average score per taxon (whole community)
	HES	From Hellenic Evaluation System (Artemiadou & Lazaridou 2005)
	AHES	From Hellenic Evaluation System (Artemiadou & Lazaridou 2005)
	SemiHES	Hellenic Evaluation System (HESY) (Artemiadou & Lazaridou 2005)

2. The methodology to choose adequate metrics/indices is described in the following steps:

- Establishment of indices, polymeric indices and biotic indices based according to their response to reference conditions (excellent and good quality stations), moderate, and polluted (bad/ poor quality). The application of the above indices (biotic or multimetric) to the Greek rivers' data. Three categories were selected according to the intercallibration reports and the current literature: the reference conditions criteria are described in section 2.3.; for the moderate and poor/bad conditions the UK Environmental Agency (UK Environmental Agency, 2005b) assessment method was followed in order to reveal the existence or not of significant pressures from morphological alterations/modifications along a water body (Table IIIb).

Table IIIb. Risk characterization according to the percentage of a water body flowing along (i) agricultural areas, (ii) coniferous forests, (iii) improved pasture and (iv) urban areas.

Percentage (%)	**Risk Characterization**
< 20	*Not risk*
20 - 40	*Low*
40 - 60	*Moderate*
> 60	*High*

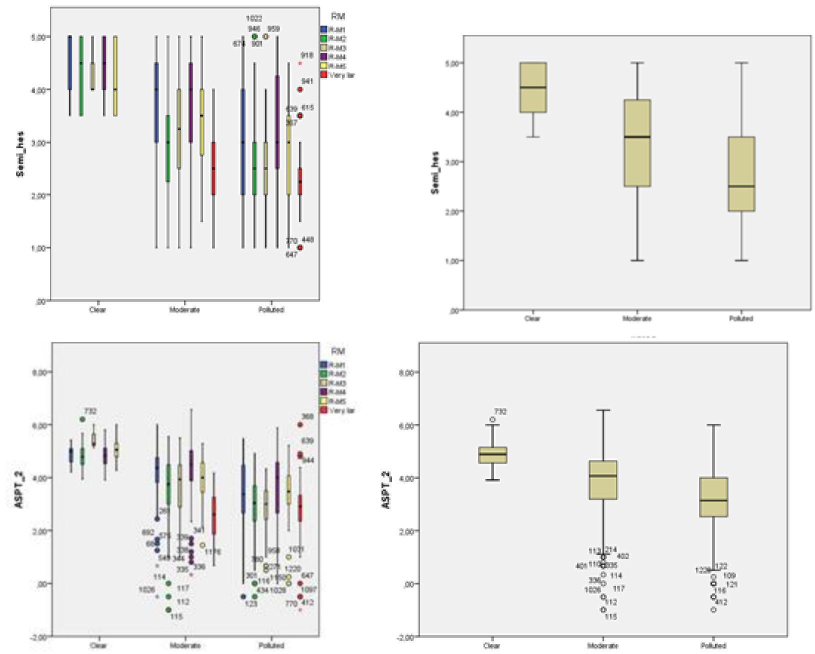

Figure annex IIIb, The response of SemiHES (from HESY) and ASPT-2 (from STARICMi) to reference conditions (excellent and good quality stations), moderate, and polluted (bad/ poor quality) through Box and Whisker plots (wherein is shown the median, the values spread and the extreme values of the metrics used per RM and separately).

- A metric's response to pollution conditions was checked through the overlap of values between polluted, medium polluted and not polluted stations, as to the median and not to the maximum and minimum values of box-plots analysis (EPA 1998) (Figure IIIb).

- The coefficient of variation of each metric/index had to have a small value [not higher than one (EPA 1998)].

- The Spearman's correlation (SPSS 22.0) among metrics was checked (a high correlation (<│0.25│) did not provide additional information for the final assessment of the ecological status).

- According to the above results four metrics (% ERTC, SemiHES, Shannon, Evenness) and the indices: Star ICMi and HESY were finally used to form polymetric or biotic indices.

- Apart from Star ICMi, HESY, and Star ICMi with HES, AHES and SemiHES instead of ASPT-2 the following multimetric indices were tested:

POLYMETRIC1	EPTC% *0.3	SemiHES*0.4	Shannon*0.15	Evenness*0.15
POL2	SemiHES*0.4	EPT%*0.3	Shannon*0.20	1-Chiron*0.10
POL3	EPT%*0.3	SemiHES*0.4	Shannon*0.15	Evenness*0.15

- The quality thresholds of the multimetric/biotic indices were calculated according to Buffagni et al. (2005). Box plots to the normalized values of the three mentioned above polymetric indices as well as EQR HESY, EQR STAR_ICMi. EQR_STARICMi_with HES, AHES, and SemiHES were carried out. The value of 25% of the reference values (i.e. the peak value of the lower quartile) was the boundary between the high and good water quality. The range between the zero value and high-good quality limit (i.e. higher value of the lower quartile - 0) was divided into four equal parts (quarters). The lower value of the first quarter (from the top) was the boundary between good and moderate quality.

- Comparison of the above indices (biotic/multimetric) was done through discrimination analysis (DA) (using the SPSS 22.0) in all IC river types. DA was applied to benthic macroinvertebrates as independent values and quality class as grouping in order to find out how many samples have shifted from moderate to high and good quality (major importance for the WFD) before the intercalibration exercise. According to the results in HESY2 no shift occurred in three out of five river types (RM1, 2, 5) in the high quality while only 5.6-5.7 % shifted from moderate to good in RM-1,-2 and RM-4 river types (Table 8).