
ELSEVIER
Published in 36 issues each year
Volumes 476–477 • 1 APRIL 2014 • ISSN 0048-9697
Special Section:
Intercalibration of Ecological Quality in European Mediterranean Rivers
Science OF THE Total Environment
Anthroposphere
Biosphere
Atmosphere
TOTAL ENVIRONMENT
Hydrosphere
Lithosphere

Science of the Total Environment 476–477 (2014) 757–767

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Comparability of river quality assessment using macrophytes: A multi-step procedure to overcome biogeographical differences

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HIGHLIGHTS

- A novel approach for WFD-intercalibration with two options was successfully applied.
- Data was the first wide field-based assessment for Mediterranean river macrophytes.
- Macrophyte-based metrics for Mediterranean rivers rely in scoring-indicator species.
- Forthcoming biomonitoring must incorporate ecological accuracy of macrophyte metrics.

ARTICLE INFO

Article history:
Received 28 February 2013
Received in revised form 1 October 2013
Accepted 3 October 2013
Available online 14 November 2013

Keywords:
Ecological quality
Intercalibration
Macrophytes
Mediterranean
Metrics
Water Framework Directive

ABSTRACT

This paper exposes a new methodological approach to solve the problem of intercalibrating river quality national methods when a common metric is lacking and most of the countries share the same Water Framework Directive (WFD) assessment method. We provide recommendations for similar works in future concerning the assessment of ecological accuracy and highlight the importance of a good common ground to make feasible the scientific work beyond the intercalibration.

The approach herein presented was applied to highly seasonal rivers of the Mediterranean Geographical Intercalibration Group for the Biological Quality Element Macrophytes. The Mediterranean Group of river macrophytes involved seven countries and two assessment methods with similar acquisition data and assessment concept: the Macrophyte Biological Index for Rivers (IBMR) for Cyprus, France, Greece, Italy, Portugal and Spain, and the River Macrophyte Index (RMI) for Slovenia. Database included 318 sites of which 78 were considered as benchmarks. The boundary harmonization was performed for common WFD-assessment methods (all countries except Slovenia) using the median of the Good/Moderate and High/Good boundaries of all countries. Then, whenever possible, the Slovenian method, RMI was computed for the entire database. The IBMR was also computed for the Slovenian sites and was regressed against RMI in order to check the relatedness of methods ($R^2 = 0.45$; $p < 0.00001$) and to convert RMI boundaries into the IBMR scale. The boundary bias of RMI was computed using direct comparison of classification and the median boundary values following boundary harmonization. The average absolute class differences after harmonization is 26% and the percentage of classifications differing by half of a quality class is also small (16.4%). This multi-step approach to the intercalibration was endorsed by the WFD Regulatory Committee.

1. Introduction

Macrophyte assemblages are undoubtedly key-elements of freshwaters and act as primary "ecosystem engineers" of fluvial systems (Gurnell et al., 2012). In fact, it is difficult to find scientific literature

Abbreviations: EU, European Union; EQR, Ecological Quality Ratio; GIG, Geographic Intercalibration Group; IC, Intercalibration Exercise; MedGIG, Mediterranean Geographic Intercalibration Group; RM, Mediterranean river types; WFD, Water Framework Directive.

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http://dx.doi.org/10.1016/j.scitotenv.2013.10.021

devoted to river plants that do not bring up the role of macrophytes in structuring and creating habitats, in the air–water–sediments exchanges, in the regulation of water temperature and light, and in sustaining other aquatic communities, such as fish, periphyton and invertebrates. There is also an enormous amount of evidence of the steady responses of diversity and abundance of river plants to abiotic factors (Haslam, 1987a; Bornette and Puijalon, 2010), and especially to nutrient enrichment (e.g., Demars and Harper, 1998), sedimentation (e.g., Jones et al., 2012) and hydrological alterations (e.g., Biggs, 1996; Franklin et al., 2008). These plant communities have the capability of incorporating the effects of successive anthropic disturbances over long periods of time, frequently years, which can be advantageous for the assessment of ecological status of rivers.

Numerous assessment systems were developed worldwide in the last decades using different components of the macrophyte assemblages, such as the vegetation structure, the species diversity or the relative position of macrophyte species in pressure gradients (Dodkins et al., 2012a). However, and surprisingly, until the advent of the Water Framework Directive (WFD; European Commission, 2000), macrophytes have been disregarded in the bioassessment of ecological water quality, in detriment of other biological quality elements, notably the phytobenthos, fish and benthic invertebrates. Despite the difficulties to overpass this lack of biomonitoring tradition in Europe, it turns out that most of the existing macrophyte-based methods were not compliant with the WFD requirements (but see e.g., Haslam, 1982), as they were generally not reference-based or specific to water types (Hering et al., 2010). Presently, only 15 different macrophyte-based national methods are being officially used for national WFD monitoring programs (Birk et al., 2010). Official methods designate the methods that are being used in the European wide harmonization of the classification of the ecological status — the so-called WFD Intercalibration Exercise (hereafter IC). Most of these national methods were developed recently (e.g., LEAFPACS for UK — Willby et al., 2009; RMI for Slovenia — Kuhar et al., 2011), and some have been tested for transferability in similar biogeographical regions and accepted as methods for other EU countries. This was the case of the Biological Macrophyte Index for Rivers, IBMR (Haury et al., 2006), originally developed for France and now applied in for the IC of seven EU countries (Birk and Willby, 2010). Nonetheless, a comparability of results was not done so far, and uncertainties were not fully assessed in most of assessment methods. Whereas Staniszewski et al. (2006) observed that IBMR has a notably low uncertainty in inter-surveyor sampling differences, temporal variation and influence of external effects such as shading other types of uncertainty such as considerations on sampling errors (precision), causality (meaning) and predictability (reliability) in macrophyte metrics have been overlooked (Demars, 2013). This is not a specific weakness of macrophyte metrics, but a larger problem of many bioindicators throughout various types of ecosystems (Moss, 2008), including other Biological Quality Elements of the WFD (diatoms, macroinvertebrates, fish). In addition, the lack of long-term biotic and environmental data at large spatial scales has likely hampered the accuracy studies including the knowledge of ecological responses of indices to single and multiple pressures.

The IC is a legal obligation that requires active developments in a relatively strict timeframe, aiming to achieve a coherent implementation of the WFD between EU countries by ensuring the comparability of the classification results of the biological assessment systems (European Communities, 2011). In particular, the IC is set to harmonize the boundaries between the classes of good and moderate status and high and good status from the member states' assessment methods and to confirm if these classes are consistent with the normative definitions of the WFD. Given this, a number of straightforward feasibility checks were settled in the IC framework to give a supportive guidance to the integration of different views. As the implementation of assessment methods followed different approaches in different countries, there are three methodological pathways, or options, for the intercalibration described in the Guidance Document for the common implementation strategy for the WFD (European Communities, 2011). The choice of the most suitable IC option depends on the similarities of the assessment methods of participating MS, including the conceptual basis of methods, the numerical evaluations, and of the sampling procedures. Option 1 is the simplest option; the boundaries are compared directly between countries that are using the same data acquisition and same numerical evaluation. Option 2 requires the use of a common metric to ensure comparability of national assessment systems, since countries use different data sampling and different assessment methods. The common metrics are ecologically meaningful biological measures produced during the IC exercise (as in Birk and Hering, 2009) or existing assessment methods (and parts of methods) that respond to pressures being intercalibrated (as in Buffagni et al., 2005). Finally, IC Option 3 – direct comparison – compares pair-wise differences of the different national assessment methods, usually at the sampling site level, requiring a sufficiently large and consistent international database.

Indirect comparisons via IC common metrics were used for most of biological quality elements and water bodies both in the first IC phase, 2004–2008 (Buffagni et al., 2005; Birk et al., 2006) and in the second phase, 2009–2012 (Kelly et al., 2009; Bennett et al., 2011). However, some difficulties were reported in using robust common metrics, namely due to low relations of national indices to common metrics, and to the scarcity of reference sites to standardize the common metrics (Birk and Hering, 2009; Hering et al., 2010).

The intercalibration exercise is undertaken within Geographical Intercalibration Groups (GIGs) rather than the ecoregions defined in Annex XI of the Water Framework Directive (European Communities, 2011). GIGs aggregate countries or parts of countries sharing common intercalibration types. The Mediterranean Geographical Intercalibration Group (MedGIG) is a geographically homogeneous region that share five Mediterranean river types (Annex I, European Communities, 2011) and includes South European countries, Cyprus, France, Greece, Italy, Malta, Portugal, Slovenia and Spain. Regardless the numerous studies done before the WFD publication relating river macrophytes' sensibility to stressors in this region (e.g. Haslam, 1987b; Papastergiadou and Babalonas, 1993; Ferreira, 1994; Romero and Onaindia, 1995), there was limited data availability for the IC and a poor advancement of national assessment systems. This has dictated an unsuccessful IC in the first phase. Indeed, a debate arose at its end whether South-European river macrophytes could reliably indicate human pressure, or if they could be compared across so diverse Mediterranean landscapes (European Commission, 2007).

For these reasons, a thorough first screening was performed over the existing and currently used national assessment methods. Most of these did not go beyond the intercalibration feasibility check (Aguiar et al., 2009a). Four different reasons were documented: i) they addressed different types of pressures at different habitats and spatial scales, namely the Riparian Vegetation Index (Aguiar et al., 2009b) and the Riparian Habitat Quality (Munné et al., 2003), ii) they followed different protocols of sampling and data processing, namely the IVAM (Moreno et al., 2008) and the IM (Suarez et al., 2005), iii) they lacked near-natural reference conditions (see Dodkins et al., 2012b), and iv) they had poor coverage of the impact–pressure relationships notably the Mean Trophic Rank (MTR; Holmes et al., 1999), due to large biogeographical differences between the country where the method was developed (UK) and the Mediterranean region.

Ultimately, two assessment methods surmounted the screening phase and were accepted for intercalibration in highly seasonal Mediterranean rivers — the Macrophyte Biological Index for Rivers (IBMR; Haury et al., 2006) for Cyprus, France, Greece, Italy, Portugal and Spain, and the River Macrophyte Index (RMI; Kuhar et al., 2011) for Slovenia.

This work presents a new methodological procedure for the intercalibration of the national methods, by sequentially applying Option 1 (for most of the countries) and the direct comparison (for the remaining metric). The combined approach outlined here is a novel contribution for the intercalibration of national systems and can be applied within other Geographical Intercalibration Groups and quality elements, to overcome biogeographical and intrinsic differences. The paper concludes with a discussion of the relevancy of macrophytes for the classification of the ecological quality in Mediterranean rivers, and a closer look on the difficulties of the intercalibration procedure.

2. Material and methods

2.1. Sampling procedures

Macrophytes are large plants of freshwaters which are easily seen with the naked eye, including aquatic vascular plants, bryophytes, stoneworts (Characeae) and macro-algal growths. Data was originated from national monitoring programs and R&D projects such as the Karatheodoris Project for Greece. Sampling methods were based on the European standards EN14184:2003 (Comité Européen de Normalisation, 2003) and EN14996:2006 (Comité Européen de Normalisation, 2006), and followed national sampling protocols. One-shot surveys were done in spring–summer season (April to September) between 2004 and 2011 according to each country.

Survey area includes the in-stream part that is under water most of the time, although it may be exposed temporarily under conditions of dry-water flow or for longer periods under certain natural (climatic, geological) conditions. Sampling involves wading into the water where conditions allow and following a zigzag pattern upstream along the length of the reach, which are usually 100 m long sections of the river channel. Sites spanned about 100 m of river length, with a minimum sampling area of 50 m^2. Specimens of bryophytes were collected from all the existing microhabitats within the study reach (e.g., woody debris, rocks) in the channel and banks, and on trees at no more than 0.5 m above ground level. Cover abundance was recorded for each taxa. Vascular plants and bryophytes were identified at the species level, whereas macroalgae were generally identified at genus level. Water samples were collected in each sampling site for chemical analysis. On-site water quality measurements were made with field probes, and included for most of countries the conductivity, pH, water temperature and dissolved oxygen. Field records also include estimation of some abiotic parameters, such as channel width. Appendix A presents a description of all abiotic parameters of the common database, including range and/or units (if applicable).

2.2. Building a common dataset

Site identification, environmental, pressure, biological and classification data were submitted by each country under the same agreed frame. An overview of the harmonization procedure is presented in Fig. 1. Data addressed highly seasonal rivers belonging to three common intercalibration river types (or Mediterranean river types, Table 1). Temporary rivers were not considered in the present study due to the low number of monitoring and reference sites in the common dataset.

A preliminary screening of sampling sites included the assessment of the Mediterranean character of the sites using climate data, resulting in a total of 318 sites across the seven countries (Fig. 2). A validation of the sites' allocation to the river types made at national level was also performed. This validation was made using the mean altitude above the sea level, catchment area and from qualitative information concerning hydrological features that were included in the dataset (see Appendix A). In addition, an analysis of similarities, ANOSIM — a routine procedure available in the PRIMER software (PRIMER 6, PRIMER-E Ltd., Clarke and Gorley, 2005) was performed with biological data to test for significant differences between river types. ANOSIM is a non-parametric test, applied to the rank similarity matrix and analog to the ANOVA, analysis of variance (Clarke and Warwick, 2001). The values of the Global R higher than 0.75 reveal a clear segregation of types, whereas values lower than 0.25 expose no differences between groups of sites.

All countries delivered taxa lists with similar taxonomic precision (species level for most of the taxa), codes and synonymy were defined at the coordination level. Nomenclature followed Flora Europaea for vascular plants and Paton (1999) and Smith (2006) for bryophytes. The Characeae identification was based mainly in Wood and Imahori (1964) and in Krause (1997).

Synonyms for bryophytes were checked in the Nomenclatural Database of French Flora (http://www.tela-botanica.org/site:eflore), for vascular plants in the International Plant Name Index (http://www.ipni.org/) and for macroalgae in the AlgaeBase (http://www.algaebase.org/). Codes were assigned using the seven character principle of the database of the Comité Europeéne de Normalizacion (CEN), allowing contributing to the enlargement of the CEN plant database and facilitating the IBMR calculations. Codes were assigned as following: GEN.SPE, where GEN = first three letters of genus and SPE = first three letters of species. GEN.SPX was used when a taxon was identified only to genus level.

The common dataset was harmonized for the cover scales, since it enclosed three different measures of cover for the seven countries. A second issue concerned the harmonization of the lists of species, considering the different degree of colonization of the fluvial bed by plants of terrestrial origin, typical of these systems. For the purpose of quality assessment, which represents a response of a biological community to pressure exerted upon the aquatic ecosystem, it is clear that only species reactive to aquatic pressures should be considered.

All countries assigned a floristic group for each species (algae, bryophyte moss, bryophyte liverwort, pteridophyte, spermatophyte, lichen, heterotrophic organisms — fungi & bacteria) and an *aquaticity* level (C. Chauvin in Birk et al., 2007). The aquaticity is a qualitative expression of the taxa affinity to water, and ranges from 1 — exclusively aquatic species to 8 — species of brackish water or salty marshes (Appendix B). For data treatment, only species with aquaticity level 5 or lower were accepted in order to achieve comparable biological information.

Finally, a feasibility check was also performed to evaluate if the national systems were set according to WFD-compliance criteria: i) methods should be set in line with the boundary setting procedure and classify the ecological status of water bodies in five quality classes; ii) all relevant parameters of the biological quality element should be covered (composition and abundance), and iii) similar anthropogenic pressure(s) should be addressed (Fig. 1).

2.3. National assessment methods

Two indices (henceforth metrics) were considered intercalibration: the IBMR and the RMI, whereas four national methods did not go beyond the feasibility check. Both metrics use the concept of positive, negative or neutral relationships of indicator taxa with specific pressures.

The IBMR was first described in Haury et al. (2006) and can be calculated using the following formula:

$$\mathrm{IBMR} = \frac{\sum_{i=1}^{N} (CS_i \cdot E_i \cdot K_i)}{\sum_{i=1}^{N} (E_i \cdot K_i)},$$

where K_i = abundance of the i-th taxon (translated in 5 classes), CS_i = indicator value for the i-th taxon (0–20), E_i = stenoecy coefficient of the i-th taxon (1–3).

RMI was recently described in Kuhar et al. (2011) and can be calculated using the following equation:

$$\mathrm{RMI} = \frac{\sum_{i=1}^{n_A} Q_{Ai} + \frac{1}{2}\sum_{i=1}^{n_{AB}} Q_{ABi} - \frac{1}{2}\sum_{i=1}^{n_{BC}} Q_{BCi} - \sum_{i=1}^{n_C} Q_{Ci}}{\sum_{i=1}^{n_S} Q_{Si}},$$

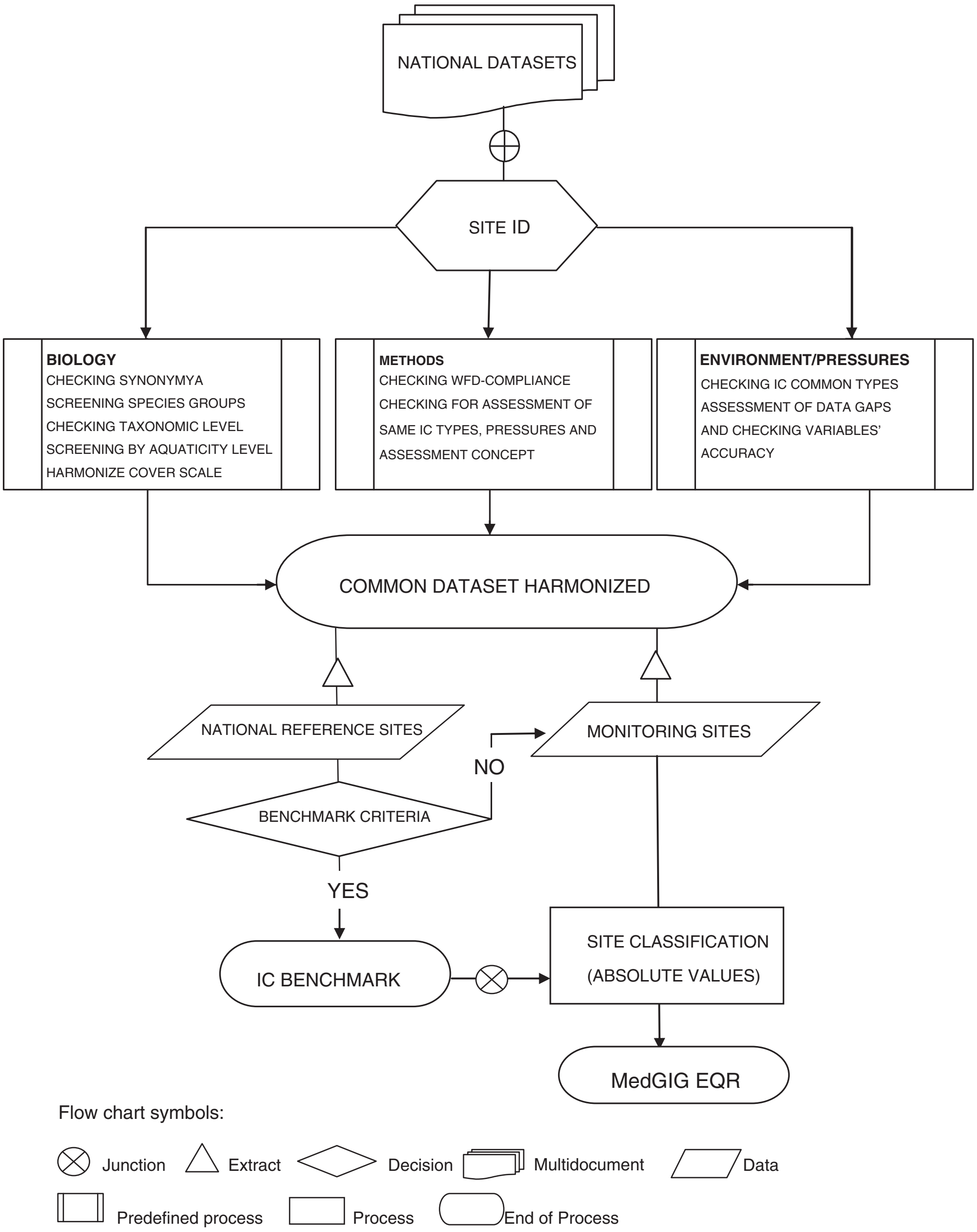

Fig. 1. Overview of harmonization procedures of common dataset, feasibility check of WFD-compliance for national methods and benchmark standardization. EQR — Ecological Quality Ratio; MedGIG — Mediterranean Geographical Intercalibration Group.

where Q_{Ai} = abundance of the taxa *i* from the group *A*, Q_{ABi} = abundance of the taxa *i* from the group *AB*, Q_{BCi} = abundance of the taxa *i* from the group *BC*, Q_{Ci} = abundance of the taxa *i* from the group *C*, Q_{Si} = abundance of taxa *i* from all groups (groups *A*, *AB*, *B*, *BC*, *C*; taxa from the group *ABC* are not considered), n_A = total number of taxa in group *A*, n_{AB} = total number of taxa in group *AB*, n_{BC} = total number of taxa in group *BC*, n_C = total number of taxa in group C, n_S = total number of taxa in all groups (groups *A*, *AB*, *B*, *BC*, *C*; taxa from the group *ABC* are not considered).

Table 1
General characteristics of common intercalibration river types and countries sharing each type. Original thresholds (WFD, Annex V) were modified following an overall approach of biological elements of the Mediterranean Geographical Intercalibration Group.

River type	Characteristics	Countries
Type 1	Small rivers — catchment <100 km2; mixed geology (except non-siliceous); highly seasonal	France, Italy, Portugal, Slovenia, Spain
Type 2	Medium-sized rivers — catchment 100–1000 km2; mixed geology (except non-siliceous); highly seasonal	France, Greece, Italy, Portugal, Slovenia, Spain
Type 3	Small and medium sized rivers — non-siliceous streams (calcareous, ophiolite); highly seasonal	Cyprus, France, Greece, Italy, Spain

2.4. Intercalibration procedure — a two-step approach

According to the WFD, water bodies have to be assessed against type-specific near-natural reference conditions and the respective assessment results have to be expressed as deviating values, Ecological Quality Ratios (EQR). However, different reference condition settings were used by the different countries; therefore, common benchmark criteria (Appendix C) were applied to the national reference data to obtain a selection of benchmark sites. Feio et al. (2013) presents the overall methodological approach. Classification values of monitoring sites

Fig. 2. Map with the location of sites, including the distribution of benchmark sites. PT, Portugal; SP, Spain; FR, France; IT, Italy; SI, Slovenia, GR, Greece; and CY, Cyprus.

were converted to EQR by dividing the absolute values of each site by the median of the IC benchmark values of each country (see flowchart of Fig. 1).

The choice of the appropriate intercalibration option depends on the extent of the comparability of the approaches of the national assessment methods. Exception to Slovenia, all MedGIG countries shared the same method — the IBMR. Using the biological harmonized dataset the IBMR was computed for Slovenian sites and their national method (RMI) was also calculated for the entire database. However, for around one-third of the sites the RMI could not be adequately calculated, due to a small number of bio-indicator species of the ranking system in the floristic lists.

To surpass the problem of biased results, we performed a two-step procedure for the IC, which is illustrated in the flowchart of Fig. 3. IC Option 1 was performed for countries that shared a common method (IBMR), followed by a boundary harmonization using Option 3 to check the comparability between the Slovenian method (RMI) and the IBMR. This multi-step approach to the intercalibration was endorsed by the WFD Regulatory Committee (European Commission, 2012).

The evaluation of the IC performance was done using boundary bias criteria: boundary bias should be less than a quarter of the width of a class. We used the median of the boundaries to compute the boundary bias. We changed iteratively the values of the country boundaries that did not meet the criteria until the median of the boundaries was included within the quarter of the class (High or Good). Then, the IBMR was regressed against RMI in order to check the relatedness of metrics and to convert RMI boundaries into the IBMR scale. Criteria are the following: slope term of linear regression must lie between 0.5 and 1.5, regression must be significant at $p \leq 0.05$ and the coefficient of determination has to be lower than 0.3. The boundary bias of RMI (after conversion to the IC EQR IBMR scale using the regression equation) was computed using the median values after Option 1 boundary harmonization. If the RMI boundaries did not meet the criteria (boundary bias lower than a quarter of a class), these were moved until the median of the boundaries was included within a quarter of a class. We converted the harmonized boundaries at the IC EQR IBMR scale into RMI values using the inverse function of the regression equation.

The average absolute class differences were computed for all pair combination of national methods, as a measure of the confidence that two or more national methods will classify any given site the same.

To reinforce the comparability process, only sites that were classified as High, Good or Moderate according to both national methods were used to compute the average absolute class differences. First, a piecewise transformation of the IC EQR IBMR values was performed using the formula:

$$\frac{MinT-((X-Min)\cdot 0.2)}{(Max-Min)},$$

where $MinT$ = minimum of the new transformed class (0.6 for G and 0.8 for H), X = index value, Min = theoretical index minimum, and Max = theoretical index maximum.

The average absolute class differences were computed as the mean absolute difference between the index values after piecewise transformation divided by 0.2 (the width of each class after piecewise transformation). Average absolute class differences should be less than 1 (meaning that the mean differences should be less than the width of 1 class). The percentage of classifications differing by half of a quality class was also calculated.

3. Results

3.1. Building a common dataset

The database included 318 sampling sites from the seven participating countries distributed into three common river types.

Table 2 provides summary data on figures of sites, including the national references and benchmarks and Fig. 1 illustrates the location of sites, including the benchmark sites, i.e., the national reference sites filtered with the MedGIG criteria. Reference conditions setting at national

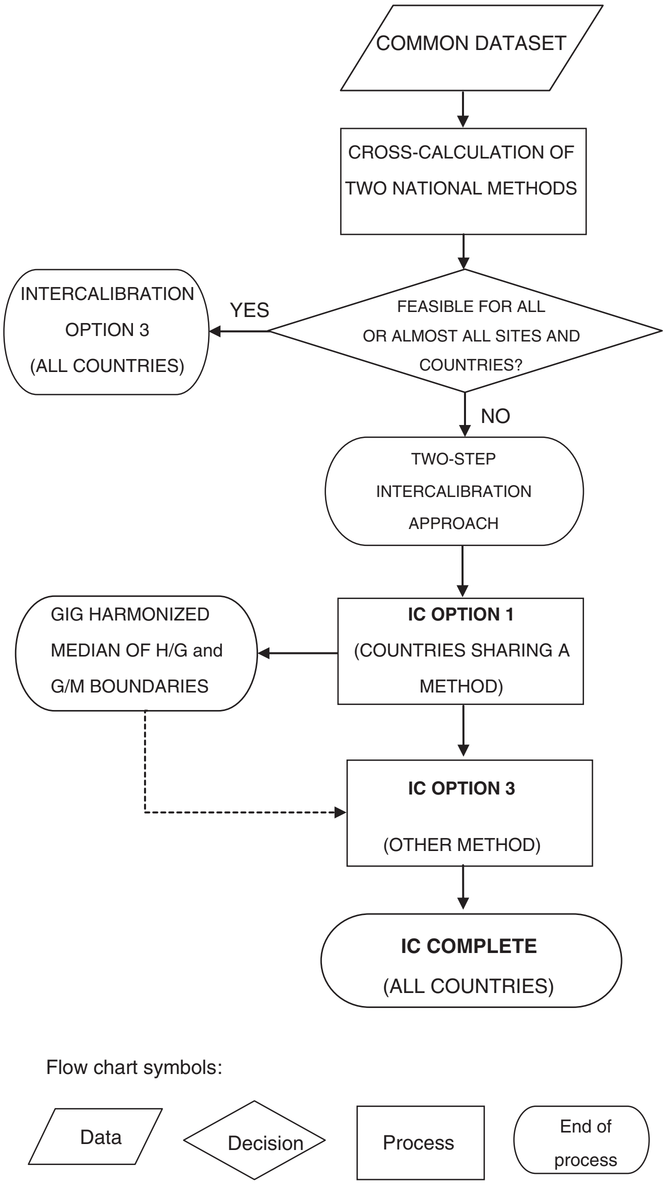

Fig. 3. Overview of the two-step IC procedure for MedGIG river macrophytes. H/G — High/Good; G/M — Good/Moderate.

level vary greatly between countries, and included the use of multivariate procedures (e.g., Greece, Cyprus), joint analysis of pressures and biological data (e.g., Italy), spatial analysis, historical data and expert judgment of abiotic variables (e.g., Portugal, Spain, France). From the 120 national reference sites, 35% of reference sites were not accepted as benchmarks, mostly due to high nitrate and phosphate contents.

Table 2
Total number of sites provided for the common database of highly-seasonal rivers by country. Number of national reference sites and number of benchmark sites.

Country	Sites	National reference sites	Benchmark sites
Cyprus	14	3	3
France	37	27	16
Greece	32	14	10
Italy	70	17	15
Portugal	60	20	13
Slovenia	21	0	0
Spain	84	39	21
Sum	318	120	78

Rates of reference sites' approval ranged from around 54% (Spain; n = 39) to 100% (Cyprus; n = 3), with Italy (92%; n = 17) and Greece (71.4%; n = 14) having high rates of acceptance. Abiotic screening of sites identified one site without Mediterranean characteristics, which was removed, and allowed to detect inconsistencies in national allocation of common intercalibration types (Table 1). Given this, we attempted to validate this abiotic classification of sites with biological data using an analysis of similarities (ANOSIM). The ANOSIM revealed a very poor segregation between river types, both for all sites (Global R of ANOSIM = 0.218; significance level = 0.1%; partial R of ANOSIM type 1 vs. type 2 = 0.121; type 1 vs. type 3 = 0.178; type 2 vs. type 3 = 0.277) and for benchmark sites (Global R of ANOSIM = 0.275; significance level = 0.1%). We thus performed the data treatment as a single river type, hereafter called highly seasonal rivers.

From the 736 species gathered for the common database sites, around 40% were eliminated (woody riparian species, brackish water or salty marsh species, terrestrial ruderals and non-hygrophyte grasses). Though synonymy occurred in less than 2% of the species, it could not be neglected due to some frequent and abundant species, such as *Rorippa nasturtium-aquaticum* (L.) Hayek/*Nasturtium officinale* L. or *Rhynchostegium riparioides* (Hedw.) Card/*Platyhypnidium riparioides* (Hedw.) Dixon.

3.2. Floristic overview

Vascular plants represent around half of the final species list (458 taxa), of which 62 taxa are helophytes and 49 are hydrophytes (truly aquatic species), followed by bryophytes (144 mosses and 20 liverworts) and macroalgae (62 taxa). Single-occurrences of species sum more than one-third of the species, and only 64 taxa occurred in more than 5% of the sampling sites. A similar pattern occurred in benchmark sites with 38% of the 214 taxa occurring in only one site. Table 3 presents the most frequent species and the respective constancy values in the overall database and in benchmark sites. *Cladophora* Kützing sp., *Spirogyra* Link sp., and *P. riparioides* (Hedw.) Dixon were the commonest aquatic macrophytes in the overall database, and benchmark sites were frequently colonized by *P. riparioides*, *Lemanea* sp. (*L.* gr. *fluviatilis*), *Spirogyra* sp., *Fontinalis antipyretica* Hedw., *Cratoneuron filicinum* (Hedw.) Spruce, and *Cladophora* sp. Some nitrophilous species were less frequent and abundant in benchmark sites, such as *Apium nodiflorum* L., *Lemna minor* L., *Sparganium erectum* L., *Polygonum hydropiper* L. and *R. nasturtium-aquaticum* (L.) Hayek, and invasive alien species were also less common (e.g., *Bidens frondosa* L.). The macrophyte-based typology and characteristic species of each group are described in detail by Feio et al. (2013).

Average species richness in the 318 sites was 12.6 ± 7.6, whereas in benchmark sites number of species averaged 9.4 ± 5.8.

3.3. Feasibility checks for WFD-compliance

After the preliminary screening and general standardization of the database the feasibility checks for WFD-compliance of national metrics were performed (see the "Checking Methods" box in the workflow of Fig. 1). All countries gave information on the response to pressures of the assessment methods at national level. This was done either by reporting to WISER Project, "Water Bodies in Europe — integrative systems to assess ecological status and recovery" (Birk et al., 2010; http://www.wiser.eu/results/method-database/), namely France, Italy and Slovenia, or by means of national reports for those countries that did not have assessment methods at the beginning of the 2nd phase of the IC, namely Cyprus, Greece, Portugal and Spain. Slovenia reported a significant correlation of the biological metric with eutrophication and land-use (Kuhar et al., 2011), and the remaining countries reported significant responses to multiple pressures mainly eutrophication, land use, general degradation and hydromorphological alterations. These pressure impact relationships with the metrics were significant ($p < 0.0001$) and ranged from r = 0.8 for Slovenia and Cyprus to r =

Table 3
Most frequent species of the common dataset and respective constancy. Constancy, C = (p × 100) / N, where p is the number of surveys containing the analyzed species and N the total number of surveys. Groups of macrophytes and aquaticity level are shown for each species. Aquaticity level followed expert judgment of macrophyte experts from the seven participating countries. Alg — Algae; Hyg — Hygrophytes; Hel — helophytes; Hyd — hydrophytes; Brm — Bryophyte moss.

Species	Group	Aquaticity	Constancy (%)	
			Overall	Benchmarks
Cladophora sp. Kützing	Alg	1	30.2	18.4
Platyhypnidium riparioides (Hedw.) Dixon	Brm	1	28.9	46.1
Spirogyra sp. Link	Alg	1	22.3	21.1
Apium nodiflorum (L.) Lag.	Hyd	2	19.2	6.6
Lycopus europaeus L.	Hel	4	18.2	13.2
Lemanea sp. (*L.* gr. *fluviatilis*)	Alg	1	17.6	27.6
Rorippa nasturtium-aquaticum (L.) Hayek	Hel	2	17.6	3.9
Lythrum salicaria L.	Hel	4	17.3	17.1
Agrostis stolonifera L.	Hel	4	17.0	11.8
Veronica anagallis-aquatica L.	Hel	4	17.0	9.2
Oenanthe crocata L.	Hel	2	16.7	14.5
Mentha aquatica L.	Hel	4	16.0	15.7
Oscillatoria Vaucher ex Gomont sp.	Alg	1	15.4	18.4
Fontinalis antipyretica Hedw.	Brm	1	15.1	19.7
Polygonum hydropiper L.	Hel	4	13.5	10.5
Bidens frondosa L.	Hyg	5	12.6	7.9
Lotus pedunculatus Cav.	Hyg	5	12.6	13.2
Cratoneuron filicinum (Hedw.) Spruce	Brm	1	11.6	19.7
Carex elata All.	Hel	4	11.6	14.5
Sparganium erectum L.	Hel	4	11.6	5.3
Vaucheria A.P. de Candolle sp.	Alg	2	11.3	11.8
Cladophora glomerata (L.) Kützing	Alg	1	11.0	10.5
Juncus effusus L.	Hel	5	10.4	10.5
Rumex conglomeratus Murray	Hyg	5	10.4	7.9
Rivularia sp. C. Agardh ex Bornet & Flahault	Alg	1	10.1	13.2
Lemna minor L.	Hyd	1	10.1	1.3

0.5–0.7 for the remaining countries. A degradation gradient using common pressure data from the MedGIG database was obtained from PCA axis scores (Appendix D). The first PCA axis explained 56.6% of the total variation (100% on the first three axes), and was mainly related with the channelization (0.39), habitat alteration (0.32), and percentage of semi-natural areas in the catchment (0.28). The correlation of the PCA axis 1 with the biological metric was 0.55 ($p < 0.000001$).

3.4. Intercalibration procedure and boundary comparison

We first computed the national method of Slovenia for the overall database (see workflow of Fig. 1). The classification results were not conclusive for around 2/3 of the database due to low number of indicator taxa, which specially occurred for the database of France, Cyprus and Spain. Given this, we performed a two-step approach to allow the intercalibration of metrics of all countries (Fig. 3).

Firstly, we used Option 1 to intercalibrate methods of Cyprus, France, Greece, Italy, Portugal and Spain (Fig. 4, step 1). Fig. 4 illustrates the harmonization of High/Good and Good/Moderate boundaries using IC Option 1 from the six countries that share a common assessment method — IBMR. Intercalibration boundaries for High/Good and Good/Moderate were derived from the median of boundary values of each country. Boundary values for Cyprus, France, Italy and Spain were within the limits of the harmonization band for the High/Good boundary, whereas Greece and Portugal were too stringent. Concerning the Good/Moderate harmonization boundary, values for Cyprus, Italy and Portugal were within the limits of the harmonization band, whereas Greece and France were too stringent and Spain was too relaxed in setting their national Good/Moderate boundary. We changed iteratively the values of the boundaries that did not meet the criteria until the median of the boundaries was included within the quarter of the class (High or Good). During the iterative procedure for harmonizing the Good/Moderate boundary, Cyprus becomes too stringent, and the national boundary had to be harmonized (Fig. 4, step 2). Required boundary adjustments, bias and final boundary values, shown as EQR values of national classification systems are reported in Table 4; values were back-transformed to values of the national metrics. Spain agreed to raise the High/Good boundary to comply with the comparability criteria. The countries Cyprus, Greece, Portugal and France can lower the national boundaries, but all except France preferred to maintain the original national values before the harmonization.

For the intercalibration of Slovenian national method, the direct comparison of IBMR (6 countries) and RMI was performed. The feasibility checking confirmed that metrics' relatedness was according to the feasibility criteria. Fig. 5 shows the regression of Ecological Quality Ratios, EQR, of IBMR and EQR values of RMI, calculated with MedGIG benchmark data. Sites values used are from Greece, Italy, Portugal and Slovenia; $n = 103$. Fig. 6 presents the illustrative results of boundary comparison of Slovenian classification with the median of the previous harmonized boundaries of the other MedGIG countries. The Slovenian method was within the limits of the harmonization band for both the High/Good and Good/moderate boundaries.

The average absolute class difference (AACD) is 26%, and 100% of the classification values are within one class. The percentage of classifications (PCHC) differing by half of a quality class is small (16.37%). See Table 5 for AACD values of national methods after boundary harmonization and percentage of classifications differing by half of a quality class for each country. Appendix E presents the AACD and PCHC for all pairs of countries.

3.5. "Borderline" conditions between the good and the moderate ecological status

The common approach to ecological quality classification of MedGIG allowed analyzing the major changes in the floristic communities between the good and the moderate ecological status. We observed an overall decrease of species richness (median in Good sites = 12; Moderate sites = 7), and an increase in cover and frequency of pondweed taxa, such as *Potamogeton pectinatus* L. and *Potamogeton nodosus* Poir., macroalgae (e.g., *Enteromorpha* Link sp., *Cladophora* sp.), and other hydrophytes (for instance *Lemna gibba* L.), and of some emergent species, such as *Schoenoplectus lacustris* (L.) Palla. In opposition, there is a loss and/or decrease in cover of bryophytes (both mosses and liverworts), mainly *P. riparioides*, *F. antipyretica* Hedw., *Fissidens crassipes* Wilson, *Eurhynchium praelongum* (Hedw.) Schimp, *Lunularia* cruciata (L.) Lindb. and *Leptodictyum riparium* (Hedw.) Warnst, and of some amphibious and hygrophyte species (*Lotus pedunculatus* Cav., *Carex elata* All., *Carex pendula* Huds.). Some infrequent species in the database, bryophytes (*Bryum* sp.), isoetids, *Juncus* sp., *Myosotis* sp. were only observed in sites classified in Good ecological status. Some alien invasive species also raise their abundance due to an increase of pressures; this is the case of *Azolla* sp., an aquatic pteridophyte.

4. Discussion

To make a real headway in the intercalibration of Mediterranean river macrophytes, we have particularly tackled four major issues: (i) the achievement of a workable dataset; (ii) the feasibility of assessment methods; (iii) the suitability of the intercalibration options; and finally (iv) the ecological interpretation of the results obtained. Below, we focused our discussion on the use of macrophytes as bioindicators across the Mediterranean Europe, and in the importance of devising new approaches to surpass specific difficulties in the WFD implementation.

River macrophytes are an artificial (multiphyletic) group of macroscopic photosynthetic organisms that colonize a multiplicity of habitats from submersed and emerged rocks to soft substrates, bankside edges,

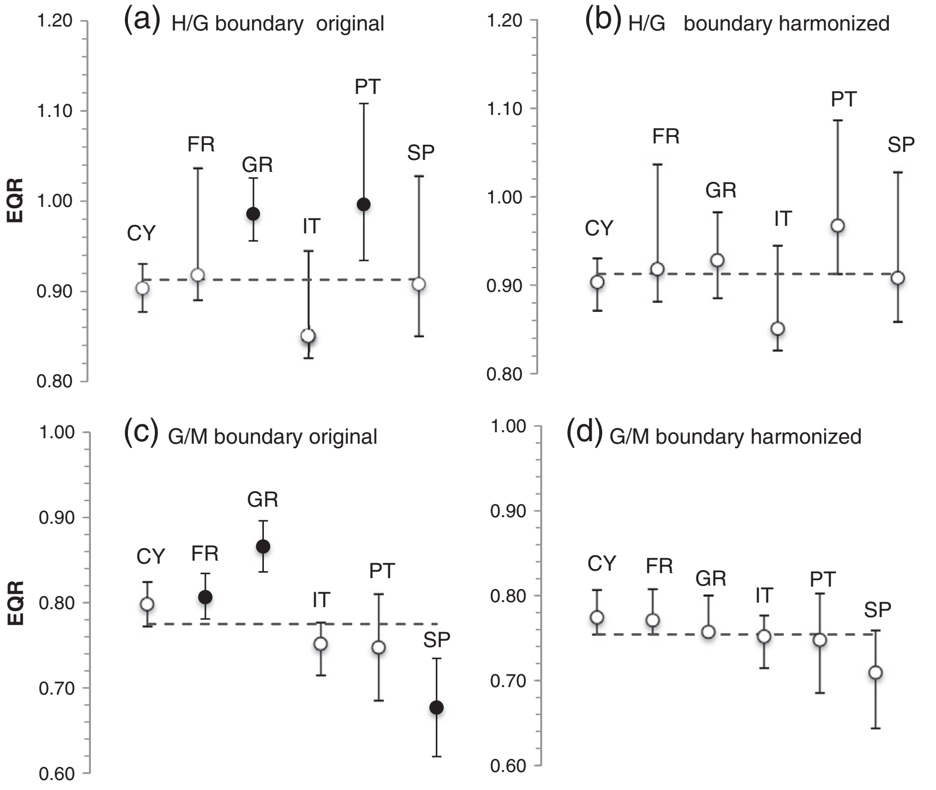

Fig. 4. Illustrative results of boundary comparison using Option 1: (a) High/Good original and (b) harmonized boundaries; (c) Good/Moderate original and (d) harmonized boundaries. Black circles represent the original "non-harmonized" boundary; and an empty circle means a harmonized boundary; range represents a quarter of the H/G class (+) and a quarter of the G/M class (−) for each country. Dashed lines represent the median of the all (MedGIG) country boundaries. H/G — High/Good; G/M — Good/Moderate. CY — Cyprus; GR — Greece; FR — France; IT — Italy; PT — Portugal; SP — Spain.

tree trunks and roots, and that can also occur unattached on the water surface. As a result of the broad ecological tolerance coupled with the high taxonomic diversity and a large variety of life forms, aquatic plants are recognized as having broad plastic responses to multiple stressors (hydrology, nutrient loading, morphological alterations) in an extensive biogeographical context (Santamaría, 2002). However, transnational studies in Europe using field data for macrophytes are relatively recent (Hering et al., 2006; O'Hare et al., 2006; Birk and Willby, 2010), and as far as we know, none were done across the European Mediterranean region. This work constitutes the first field-based assessment of river macrophytes across the broad longitudinal gradient of the EU Mediterranean basin, from the southwestern tip (Portugal) up to Cyprus at the South eastern EU borders. Biological data had the advantage of being relatively recent, expressly collected for the intercalibration exercise or generated for the WFD at national level in accordance with the European standard for sampling allowing us to produce a synoptic view of Mediterranean macrophyte communities.

We detected a high incursion of non-riverine species (40% of original data) in inner banks and channels, along with a relatively low proportion of vascular helophytes and hydrophytes species (ci. 25% of the floristic list), a pattern also observed locally in the region by Dodkins et al. (2012b), among others. In addition, more than one-third of the recorded species were single-occurrences and species constancy across sites and countries was relatively low. Mediterranean regions are characterized

Table 4
National class boundaries and boundaries bias adjustment (adjusted/harmonized boundaries if bias > |0.25|). Proposed adjustments: ↑ boundary to be raised and ↓ boundary should be lowered. Final accepted boundaries.

Country		Original		Harmonized				Final agreed national boundaries	
		H/G	G/M	H/G		G/M		H/G	G/M
Cyprus	Boundary	0.795	0.596			0.550	↓	0.795	0.596
	Bias	−0.090	0.220			0.240			
France	Boundary	0.930	0.790			0.745	↓	0.930	0.745
	Bias	0.040	0.310			0.250			
Greece	Boundary	0.750	0.560	0.660	↓	0.390	↓	0.750	0.560
	Bias	0.610	0.760	0.090		0.250			
Italy	Boundary	0.900	0.800					0.900	0.800
	Bias	−0.170	−0.230						
Portugal	Boundary	0.920	0.690	0.890	↓			0.920	0.690
	Bias	0.340	−0.110	0.250					
Spain	Boundary	0.950	0.710			0.740	↑	0.950	0.740
	Bias	−0.010	−0.420			−0.230			
Slovenia	Boundary	0.800	0.600					0.800	0.600
	Bias	−0.101	−0.125						

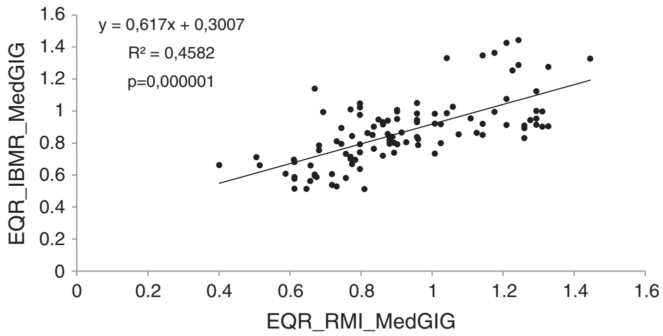

Fig. 5. Scatterplot showing the regression of EQR values of IBMR (EQR_IBMR_MedGIG) and EQR values of RMI (EQR_RMI_MedGIG) calculated with MedGIG benchmarks.

Table 5
Average absolute class differences (AACD) of national methods after boundary harmonization and percentage of classifications differing by half of a quality class (PCHC) (0.1 after piecewise transformation).

Country	AACD	PCHC
Cyprus	0.27	20.18
France	0.23	14.01
Greece	0.34	23.74
Italy	0.26	17.57
Portugal	0.25	13.17
Spain	0.24	12.81
Slovenia	0.22	13.09
Average	0.26	16.37

by moderate to low rainfall, hot dry summers and mild to cold winters, and a relatively predictable seasonal rainfall pattern. This high seasonal flow variation result in a reduced aquatic plant diversity, especially hydrophytes. Despite low number of truly aquatic species and the diverse abundance of macrophyte groups observed along the river longitudinal gradient (Aguiar et al., 2006; Manolaki and Papastergiadou, 2013), the number of indicator taxa per site and the high responsiveness of some aquatic macrophytes to nutrient enrichment and other pressures allow mirroring the ecological quality of rivers in the region.

Some studies have already address biogeographical differences between countries by comparing macrophyte metrics, including indicator values of individual species and correlations to a variety of pressures beyond eutrophication (Schneider, 2007; Szoszkiewicz et al., 2009; Aguiar et al., 2011) and causal–effect relationships (Schneider et al., 2013). It was observed that if an index is to be applied in a different region from where it was developed, some local indicator taxa are probably neglected, and minor differences in ecological status might be overlooked. Portugal, Greece, Cyprus and Spain tried to develop or to apply existing local assessment metrics for the WFD intercalibration, but did not succeed due to diverse reasons: different conceptual methods, sampling or taxonomic precision, different pressures addressed and lack of reference standards for metric validation. IBMR was adopted as the official assessment method for six MedGIG countries, mainly due to the relatively high number of taxa from various groups on the indicator species list, which generally ensured a good confidence in the classification of the ecological quality status. In addition, it was reported in general, a reliable relationship of the metric with the pressure gradients observed with local/national data. However, the relationship between this metric and nutrients was not fully assessed with the MedGIG database for three main reasons: i) different physicochemical variables provided by countries, ii) data were mostly from spot measurements, and iii) existence of gaps at site level. The intercalibration of biological metrics has been often influenced towards the need to find high quantitative relationships (usually using correlation) between the metrics and water chemical variables, whereas attention must be also paid to the causal relationship between multiple stressors and the related ecological responses. This issue has been addressed in recent limnological studies, such as the one of Schneider et al. (2013) which discussed the importance nutrient enrichment of water and sediments and of N-source in explaining the excessive plant growth in oligotrophic rivers and lakes.

IBMR indicator taxa are distributed among various groups - macroalgae (e.g. Characeae), aquatic bryophytes (e.g. *Fontinalis* sp.), truly aquatic macrophytes (e.g., *Potamogeton* sp.) and emergent vascular species (e.g. *Polygonum* sp.). Slovenian metric follows a similar assessment concept, but main indicators are spermatophytes and pteridophytes. This methodological background created a particular deadlock for the intercalibration due to a low level of confidence on the results on cross-calculations for some countries, owing to the low number of indicator species. Thus, the direct comparison for the overall countries would lead to biased results. In addition, a common metric could not be devised. The two-step IC alternative hereby presented was especially suitable, since six of the seven Med GIG countries and 93.4% of the sites were classified using the IBMR, and could be easily intercalibrated using IC option 1. On the other hand, the remaining method could be intercalibrated with the major group by direct comparison, using the median of boundaries previously attained. This innovative strategy can be used in similar situations where single IC options could not be straightforward applied. However, caution must be taken, to avoid excessive pragmatism in taking this approach to other situations, since the iteration procedure using a fixed median (or mean) could be too penalizing for isolated methods.

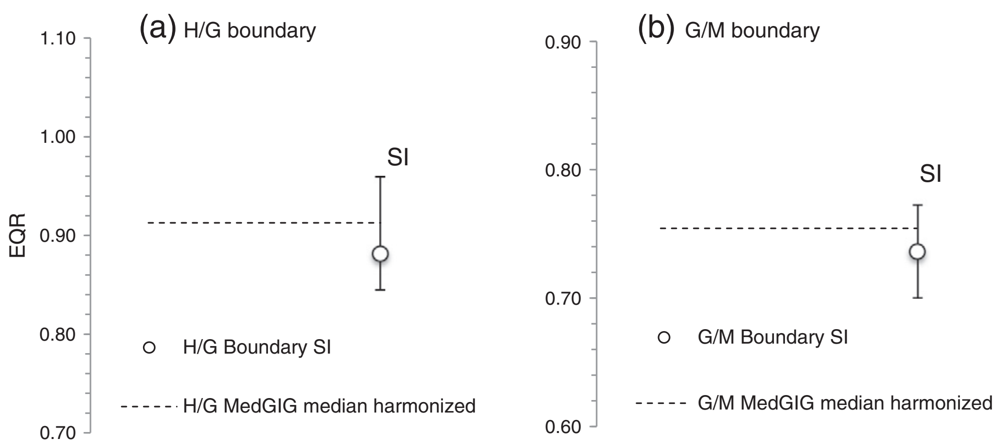

Fig. 6. Illustrative results of boundary comparison for the intercalibration using Option 3 for RMI (Slovenian metric): (a) High/Good original boundaries; (b) Good/Moderate original boundaries, dashed lines represent the median of the MedGIG country harmonized boundaries; range represents a quarter of the H/G class (+) and a quarter of the G/M class (−).

5. Conclusions

Regardless of the successful implementation of the two-step approach for the IC of MedGIG river macrophytes, further work on comparability needs to pay greater attention on index foundations, particularly those related with the ecological accuracy. Our results also highlight the importance of a good common ground to make feasible the scientific work beyond the intercalibration, namely in the:

- harmonization of sampling procedures, including sampling facies, season, taxonomic precision, and macrophyte groups surveyed;
- quantification of the ecological accuracy against specific pressures;
- adequacy of river typologies and monitoring sites;
- harmonization for species synonymy, cover scale, and aquaticity level;
- accomplishment of benchmark criteria;
- assessment of the WFD-compliance and common assessment concept for national methods;
- achievement of common environmental and pressure data, notably in national reference sites.

In spite of the broad-spatial scale and of natural differences between MedGIG countries, including the type and the magnitude of main pressures, floristic lists, proportion of benchmark sites, the common view of the ecological quality using river macrophytes did not diverge substantially, both for the High/Good and Good/Moderate boundaries. The novel two-step IC approach permits the intercalibration of all countries for highly seasonal Mediterranean rivers, something that seemed impracticable at first and with the single available IC options.

Acknowledgments

We thank two anonymous reviewers and the Editorial Commission for comments on the manuscript that much improved the original version. This paper resulted from the work done for the implementation of the EU-Intercalibration of Mediterranean Geographic Intercalibration Group (2nd phase). Authors acknowledge all collaborators on field sampling, floristic identification and office data and technical work; some deserving particular mentioning namely Felisbina Quadrado (INAG IP/Agência Portuguesa do Ambiente IP, Portugal), Cristiana Vieira (Universidade do Porto, Portugal), G. Fyttis, E. Giannouris, K. Kostara, I. Tziortziis and K. Stefanidis (University of Patras, Greece), José Luis Moreno (University of Castilla-La-Mancha, Spain), Irene Carrasco and Ana Lara (Ministry of Environment, Spain), Núria Flor-Arnau (University of Barcelona, Spain) and Urška Kuhar (University of Ljubljana, Slovenia). We are also indebted for the institutions providing financial support, monitoring and environmental data, namely the INAG IP/Agência Portuguesa do Ambiente IP (Portugal), IRSTEA (France), ENEA Saluggia Research Centre (Italy), Water Development Department (Cyprus), University of Ljubljana and Ministry of Agriculture and the Environment of the Republic of Slovenia (Slovenia), University of Patras (Karatheodoris research grants, Greece), Catalan Water Agency, Spanish Ministry of Environment and Confederación Hidrográfica del Ebro (Spain). First author received funding from the Fundação para a Ciência e a Tecnologia (FCT) through a post-doc grant (SFRH/BPD/29333/2006).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.10.021.

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