



Water quality assessment of rivers using diatom metrics across Mediterranean Europe: A methods intercalibration exercise



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HIGHLIGHTS

- Four diatom methods were compared using the Intercalibration Common Metric (ICM).
- The ICM correlated well with the national metrics and responded to nutrients.
- Upper class boundaries were adjusted and translated to national systems.
- Diatom assemblages for Good and Moderate quality classes were defined.
- Diatom patterns were affected by different taxonomic conventions but not by season.

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ABSTRACT

The European Water Framework Directive establishes a framework for the protection of water resources. However, common water management tools demand common understanding of assessment methods, so quality goals are equally met. Intercalibration of methods ensures the comparability of biological elements across similar geographical areas. Many aspects can influence the outcome of intercalibration: data sampling, treatment methods, taxonomic reliability of databases, choice of metrics for ecological quality status classification, and criteria for selecting reference sites. This study describes the potentials and constraints of the intercalibration of indices using diatoms for assessment of Mediterranean rivers. Harmonisation of diatom taxonomy and nomenclature was based on a previous ring test which took place at the European level. Four diatom indices (Indice de Polluosensibilité Spécifique—IPS, Indice Biologique Diatomées—IBD 2007, Intercalibration Common Metric Italy—ICMi and Slovenian Ecological Status assessment system) were intercalibrated using data from six European Mediterranean countries (Cyprus, France, Italy, Portugal, Slovenia and Spain). Boundaries between High/Good and Good/Moderate quality classes were harmonised by means of the Intercalibration Common Metric (ICM). Comparability between countries was assured through boundary bias and class agreement. The national boundaries were adjusted when they deviated more than a quarter of a class equivalent (0.25) from the global mean. All national methods correlated well with the ICM, which was sensitive to water quality (negatively correlated to nutrients). *Achnanthydium minutissimum* sensu lato was the most discriminative species of Good ecological status class. *Planorhynchium frequentissimum*, *Gomphonema parvulum* and *Nitzschia palea* were the most contributive to Moderate ecological status class. Some taxa were discriminative for both Good and Moderate ecological status classes due to low indication and ecological discriminative power but also due to differences in taxonomy between countries. This intercalibration exercise allowed establishment of common water quality goals across Mediterranean Europe, which is substantiated with the ICM.

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1. Introduction

The comparability of biological methods across geographic areas in Europe was never an issue before the publication of the Water Framework Directive (WFD—European Commission, 2000). The Biological Quality Elements (BQE) used in the WFD (phytoplankton, phytobenthos including the aquatic flora, invertebrates, and fish) were the basis of this approach. Class boundaries using these BQE should be established by taking into consideration characteristics such as taxonomic composition and abundance. The ultimate goal was to derive the 'ecological status', with specific objectives through programmes of measures defined in the River Basin Management Plans (Koller-Kreimel and Chovanec, 1999). The European Union partner countries, therefore, are framed by a single legislative framework that sets uniform standards in water policy throughout the European Union. However, this goal was slowed down by the evidence that implementation could not be straightforward and European-wide, and that it was necessary to establish common quality goals at the ecoregion level. For more than a decade, the different partner countries developed their respective assessment systems and, even under a common perspective of assessment, methodological approaches followed different pathways. Intercalibration of methods and procedures was, therefore, necessary to address common river management goals (Birk et al., 2013). This objective was already foreseen in the WFD description by means of intercalibration exercises (IC) that could assure comparable classifications within the different ecoregions, where comparable levels of ecosystem alteration could be attained when classifications were similar.

The use of aquatic communities for water quality evaluation is at the base of the BQE use, and is not recent (Kolkwitz and Marsson, 1908). Among the different biological elements used in the WFD, the use of diatom assemblages in routine monitoring of the ecological status of water bodies has been widely applied in many European countries as good proxies for phytobenthos. Diatom indices are the most common tool to summarise the information provided by the diatom assemblages. Most of the indices used in Europe are based on Zelinka and Marvan's (1961) approach, which considers the weighted averages of taxa sensibility to pollution (i.e. nutrients, organic degradation), as well as pH and salinity. Among them, the IPS (Cemagref, 1982), the TDI (Kelly and Whitton, 1995), and the TI (Rott et al., 1999) are some of the most commonly used. Because diatom species respond to environmental changes (Ponader and Potapova, 2007; Prygiel et al., 1996), indices routinely used demand taxonomic identification to be done at the species level. These requirements of fine taxonomy, together with the frequent nomenclatural changes, complicate the reliable comparison of quality results based on diatoms, and are an additional reason for intercalibration.

The WFD follows a reference approach (Hughes et al., 1986; Reynoldson et al., 1997) where the ecological status classification of a given water body is presented as a deviation of the biological community from the same biological element but in unaltered (pretended pristine) condition. However, reference conditions can be defined in different ways, and this also affects the class boundaries and its comparison (Pardo et al., 2012; Stoddard et al., 2006). Reference conditions in the Mediterranean region are particularly difficult to establish, not only due to the long history of human disturbances (Feio et al., 2014—in this issue; Hooke, 2006) but also due to the relatively unpredictable seasonal and multi-year variations in water availability that further introduce difficulties when comparing results (Dodkins et al., 2012; Feio et al., 2014—in this issue).

The comparison between different systems of ecological classification is also influenced by differences in sampling and sample processing, as well as in the criteria for site selection, and the choice of parameters for non-biological data (also contributing to quality classifications) including hydromorphological, physical and chemical parameters. As a result, classifications are embedded in ecological noise and sampling variability and therefore "inferences regarding biological condition are

influenced by a variety of individual and combined decisions regarding data collection, treatment and summary" (Cao and Hawkins, 2011), and likewise affecting the comparability of results. In the Mediterranean ecoregion, five common river types were proposed based on catchment size, geology and hydrological regime (ECOSTAT, 2004), but the biological classification does not completely match the abiotic one, adding an additional obstacle in the comparison of the partner countries' results. This paper summarises the results of the intercalibration process carried out in order to constrain the listed limitations, and to provide a common framework for the successful comparison of diatom assessments of river quality across the Mediterranean European region.

2. Methodology

2.1. Sample collection and processing

The European Mediterranean countries Cyprus, France, Italy, Portugal, Slovenia, and Spain (Fig. 1) provided data for intercalibration (Table 1). Participating countries collected their samples according to standard methods (EN, 2003; Kelly et al., 1998), adapted to the specific requirements in each country.

Diatoms were used as proxies for phytobenthos (Kelly et al., 2008) and most countries (except Slovenia that used a multi-habitat sampling methodology) based their approach on epilithic diatoms. About three quarters of the samples were collected in spring/summer, the seasons when effects on the biota are the most visible because of lower flows and associated higher concentration of dissolved materials. Diatom identification followed standard floras, mainly Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b). Counting of the diatom cells followed standard procedures (EN, 2004) with a minimum of 400 valves identified and counted.

Diatom data were harmonised by screening for inconsistencies and merging synonyms. This was the case of the taxa: *Achnanthes lanceolata* (Brebisson) Grunow and its synonym *Planothidium lanceolatum* (Brebisson ex Kützing) Lange-Bertalot, and *Navicula pupula* Kützing and its synonym *Sellaphora pupula* (Kützing) Mereschkowsky, among others. Harmonisation of taxonomic issues also used the criteria from a previous European ring test (Kahlert et al., 2012), mostly based on expert criteria. Environmental data were also harmonised between countries, and sites with missing values or non-comparable variables (e.g. alkalinity and hardness) were eliminated from the dataset.

2.2. Datasets

Three datasets were prepared for the intercalibration exercise. The first one was a biological dataset with diatom taxa list and relative abundance per sample. Another included site information (i.e. geographical localisation, identification of site/sample) and environmental data (hydromorphological, physical, and chemical data). The third one included the environmental pressures affecting the sites.

Each site was allocated to one of the five river types defined for Mediterranean Europe (ECOSTAT, 2004) which were based on catchment area, geology and hydrological regime. The river type including very large rivers (catchment area > 1000 km²) could not be intercalibrated due to insufficient number of reference sites. The four river types which were intercalibrated were described as follows:

Type 1—small rivers (<100 km²), siliceous geology, highly seasonal hydrological regime;

Type 2—medium size rivers (100–1000 km²), siliceous geology, highly seasonal hydrological regime;

Type 3—small and medium rivers (<1000 km²), non-siliceous, highly seasonal regime;

Type 4—small and medium rivers (<1000 km²), temporary hydrological regime.

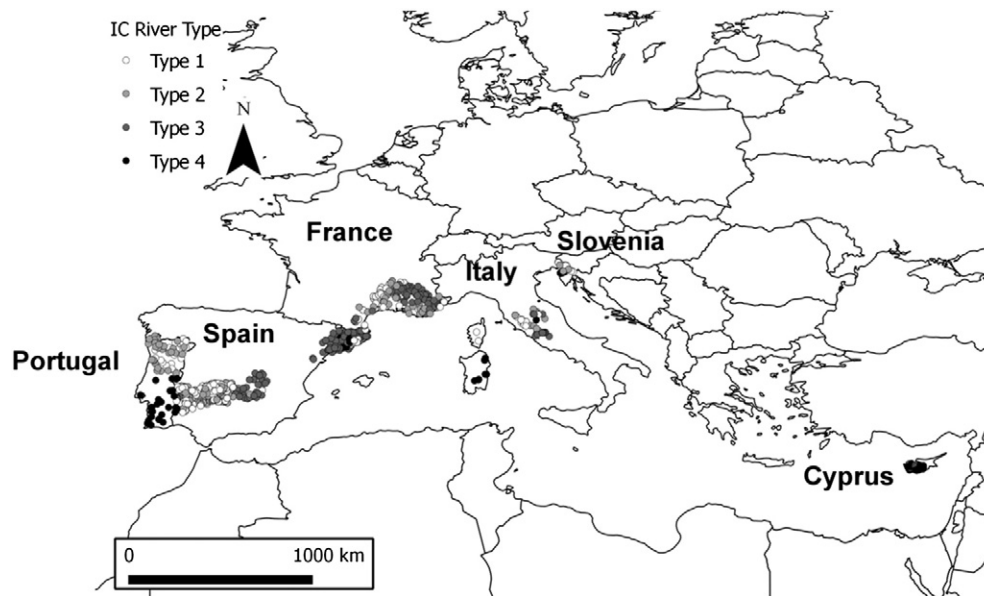


Fig. 1. Geographic distribution of sites (1031) provided by 6 European countries from the Mediterranean region for the Water Framework Directive (WFD) Intercalibration Exercise of diatom assessment methods according to the typology established for rivers in this region.

Countries included more than one national type in each of these four types, and were engulfed by the wide range that characterised the intercalibration types.

2.3. Assessment methods

Several diatom indices were used as surrogates of the bioindication value of diatom assemblages (Table 2). The OMNIDIA software (version 5.3—Lecoine et al., 1993) was used to calculate the diatom indices. The quality indices used addressed nutrient and organic contamination (Table 2).

Reference sites were screened from the entire number of samples (1031) by adopting the criteria defined in Feio et al. (2014—in this issue) in order to create a common dataset for reference sites. A final number of 205 least disturbed sites were selected (Table 1). The potential correlation of the Intercalibration Common Metric (ICM) values to pressures was assumed to be low and non-significant in the least disturbed sites; the reverse could imply that the least disturbed sites were in fact affected by environmental pressures. The response of the ICM calculated for all sites, including the least disturbed, to individual pressures was also estimated by means of Spearman correlations.

2.4. Ecological Status assessment and class boundaries' establishment

The Ecological Status (ES) was defined into 5 quality classes of increasing degradation, from High—H, Good—G, Moderate—M, Poor—P

Table 1

Number of samples provided by countries for each type of river in the Mediterranean region. Data delivered included diatom assemblage data, and site data on geographical, physical and chemical variables. Empty cells refer to the absence of a type for the country. In brackets the number of least disturbed sites selected for the intercalibration exercise.

Country	Mediterranean River typology				Total
	1	2	3	4	
Cyprus	–	–	26 (11)	34 (6)	60 (17)
France	56 (34)	44 (8)	92 (39)	–	192 (81)
Italy	18 (3)	23 (1)	30 (2)	13 (4)	84 (10)
Portugal	30 (8)	30 (5)	–	30 (3)	90 (16)
Slovenia	15 (3)	14 (3)	–	10 (2)	39 (8)
Spain	156 (28)	163 (13)	235 (30)	12 (2)	566 (73)
Total	275 (76)	274 (30)	383 (82)	99 (17)	1031 (205)

to Bad—B, based on a value that represents the deviation from the least disturbed conditions (EQR—ecological quality ratio). The EQRs were calculated as ratios between the value of the assessment method for a site and the value for the same assessment method for least disturbed sites of the same typology (using the river types and the common reference conditions dataset). An EQR close to zero refers to a site with a biological community which greatly deviates from the one found at reference sites (least disturbed in our case). Each partner country defined class boundaries considering the guidelines for boundary setting (European Commission, 2011), with some specificities. Some countries (Cyprus and Portugal) set their H/G boundary as the 25th percentile of values at reference sites while the rest (France, Italy, Slovenia and Spain) derived their H/G boundary from metric variability at reference sites. In this case the range below H/G boundary was divided in 4 equal classes: $G/M = H/G \times 0.75$; $M/P = H/G \times 0.50$; and $P/B = H/G \times 0.25$. France increased 1 point to the national metric (IBD) for all national types, and then the values were checked for significant decreases in sensitive species from High to Poor status. Italy set up the G/M boundary taking into account the decrease or absence of sensitive species and the spread of tolerant species to eutrophication and organic pollution, and an equidistant division for the remaining class boundaries (M/P and P/B) was set.

A non-metric multi-dimensional scaling (MDS—using Primer 6, Primer-E Ltd, Plymouth, UK) was performed on the entire diatom dataset in order to check how well the diatom assemblages discriminated among quality classes. The possible influence of other factors (i.e.: country, season) in diatom assemblage patterns was discarded by using, analyses of similarity (ANOSIM—Primer 6) on the entire diatom data matrix, and separately on the reference diatom dataset. The description of the biological assemblages representing the “borderline” conditions between Good and Moderate status was obtained by means of SIMPER analysis (Bray—Curtis coefficient; up to 90% of contribution to average similarity; Primer 6).

2.5. The intercalibration process

The diatom metrics in the Mediterranean region were intercalibrated following option 2 (European Commission, 2011; Birk et al., 2013). This option allowed the EQR values of the national methods to be placed on a common metric scale for comparison. This option was selected because diatom intercalibration was constrained by differences in data

Table 2

Diatom assessment methods adopted by European Mediterranean countries and pressures addressed by each method.

Country	Method	Pressure
Cyprus, Portugal, Spain	IPS—Indice de Polluosensibilité Sécifique (Coste in Cemagref, 1982)	Eutrophication, organic matter, acidification, salinity
France	IBD 2007—Indice Biologique Diatomées (Coste et al., 2009)	Eutrophication, general degradation,
	AFNOR NF-T-90-354, December 2007	pollution by organic matter, acidification
Italy	ICMi—Intercalibration Common Metric Index (Mancini and Sollazzo, 2009)	Eutrophication, general degradation,
		pollution by organic matter
Slovenia	Ecological status assessment system for rivers using phytobenthos and macrophytes in Slovenia; Phytobenthos. Based on the Saprobic and Trophic index by Rott et al. (1997, 1999)	Eutrophication and pollution by organic matter

acquisition (epilithic versus multi-habitat sampling, sampling not exclusive in riffle areas, etc.) and the different numerical evaluation (4 different indices were used in the Mediterranean area). Therefore, the intercalibration proceeded using a common metric scale, the Intercalibration Common Metric (ICM).

The ICM (Kelly et al., 2009) is an index that results from the combination of two widely applied diatom indices, the IPS (Coste, in Cemagref, 1982) and Rott's Trophic Index—TI (Rott et al., 1999). The IPS accounts for general water quality estimates, low values corresponding to high pressure levels and low EQRs. The TI measures nutrient load and was adjusted so that high values represented high EQR values. The ICM is thus defined as:

$$\text{ICM} = (\text{EQR}_{\text{IPS}} + \text{EQR}_{\text{TI}}) / 2$$

where $\text{EQR}_{\text{IPS}} = \text{Observed value} / \text{Reference value}^*$ (*Reference value = median IPS value of reference sites for a national dataset) and $\text{EQR}_{\text{TI}} = (4 - \text{observed value}) / (4 - \text{reference value}^{**})$ (**Reference value = median TI value of reference sites for a national dataset).

However, another requisite for intercalibration to proceed was that there should be a significant correlation between the ICM and the national indices, in addition to the ICM response to pressures. Pearson correlation was calculated to estimate the relation between partner country indices and the ICM, with the criterion that Pearson's correlation coefficient (r) should be equal or higher than 0.5. Finally, linear regression between values of national indices and the corresponding Intercalibration Common Metric (ICM) values were calculated. Regression characteristic criteria were established as that: the relationship should be significant; the slope should vary between 0.5 and 1.5; and the observed minimum r^2 that should at least be half of the observed maximum r^2 . Regression equations were then used to translate the national boundary positions to the common metric scale (ICM). River Type 4 from Spain and Slovenia had a low number of sites, and therefore the global median of the indices composing the ICM (IPS and TI) was used to calculate the ICM values, instead of the national data.

2.6. Boundary comparison and harmonisation

The quality gradient was divided in five classes or bands (from High to Bad quality), and boundary bias and class agreement were used as measures of class comparability across partner countries. The global mean boundary (including all countries) was calculated in ICM units, and the difference between the global mean and each national boundary was also calculated. National boundaries should not deviate more than a quarter of class equivalents (0.25) from the global mean (European Commission, 2011). Class boundaries that did not comply with this deviation were adjusted. The quarter of band width was calculated using the high maximum (HM) EQR value (the maximum EQR found for each country translated into the ICM). An exception was Type 4 for Cyprus, where the high maximum value for boundary harmonisation was the mean of the high maximum values of the other countries. The common boundary for the Mediterranean countries (H/G and G/M) was attained by averaging the types' boundaries within each country first, and then calculating the global mean value

considering one boundary for each country. This approach assured the same weight to the process by the different countries.

Class agreement referred to the coincidence between national methods to report the same quality class for a given site. Class agreement was computed for paired combinations of national methods for sites classified as High (H), Good (G) and Moderate (M). Class agreement was considered acceptable if the overall class difference for all countries remained lower than 1 class. A piecewise transformation of the ICM EQR values was performed using the formula: $\text{MinT} + ((X - \text{Min}) * 0.2) / (\text{Max} - \text{Min})$, being

MinT	minimum of the new transformed class (0.6 for G and 0.8 for H);
X	ICM—EQR value;
Min	theoretical index minimum,
Max	theoretical index maximum.

Class agreement was finally 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).

3. Results

3.1. Least disturbed conditions and diatom types

From a total of 1031 samples analysed, 205 were considered representative of least disturbed (Table 1) and, therefore, used as part of the reference sites in subsequent analyses. As previously reported in Feio et al. (2014—in this issue) the least disturbed sites showed poor segregation between Types 1, 2 and 3. Following this evidence, the data of these former types were pooled together as a single type (Types 1–2–3), while Type 4 (temporary rivers) was treated separately. This separation was also according to the differences in reference criteria between these types (Feio et al., 2014—in this issue). Non-significant Spearman rank correlations characterised the relationship between diatom data and the environmental pressures in the least disturbed sites (Table 3). However, nitrate concentration in Types 1–2–3 was related with diatom data in the least disturbed sites, even though, with a very low correlation coefficient ($\rho = -0.1738$).

3.2. Assessment of methods' performance

The ICM was responsive to nutrient enrichment, especially ammonia, total phosphorus and phosphates (Table 3) when considering the entire quality gradient.

All national assessment methods for all types were significantly correlated with the ICM (Pearson's correlation coefficient varied 0.82 and 0.97), and the regression equations were highly significant and complied with the established requirements (Table 4). The regression lines of French and Spanish methods for Types 1–2–3 with the ICM are shown in Fig. 2 as examples.

The boundary between Good and Moderate water quality (G/M) separates good from disturbed conditions. The MDS plot (Fig. 3) arranged the diatom assemblages in a gradient of decreasing quality

Table 3

Intercalibration Common Metric (ICM) Spearman rank correlation coefficient (ρ), probability (p) and number of samples correlated for each pressure variable (based on least disturbed sites and on the entire database). Bold data indicates significant/highest correlations; $p < 0.05^*$; $p < 0.01^{**}$; $p < 0.001^{***}$.

Pressure variables	Least disturbed sites ICM (ρ , n)		Entire database ICM (ρ , n)
	Types 1–2–3	Type 4	
General morphology (class)	−0.07796, 190	−0.2832, 17	−0.112 ^{***} , 1007
General hydrology (class)	0.0662, 190	0.2797, 17	−0.123 ^{***} , 1014
Riparian vegetation (class)	0.09340, 84	−0.5455, 8	−0.323 ^{***} , 979
O ₂ (% saturation)	−0.02231, 154	0.3143, 15	0.100 ^{**} , 916
N-NH ₄ ⁺ (mg l ^{−1})	0.0272, 130	−0.2487, 15	−0.519 ^{***} , 829
N-NO ₃ [−] (mg l ^{−1})	−0.1738 [*] , 145	0.0052, 14	−0.254 ^{***} , 858
P-total (mg l ^{−1})	−0.0009, 81	0.0000, 13	−0.428 ^{***} , 377
P-PO ₄ ^{3−} (mg l ^{−1})	0.0311, 142	−0.1022, 14	−0.608 ^{***} , 803
BOD ₅ (mg l ^{−1})	0.0408, 97	−0.1986, 12	−0.221 ^{***} , 391
Land use (%)	−0.0180, 172	−0.1055, 17	−0.220 ^{***} , 1042
Agriculture (%)	−0.1154, 172	0.0016, 17	−0.242 ^{***} , 1042
Urbanisation (%)	–	–	−0.005, 1033

from the right (High ecological status) to the left (Poor/Bad ecological status), and showed the overall ability of diatoms to discriminate between different levels of impairment. However, intermediate quality classes were mixed up, interfering with the description of diatom assemblages from these particular quality classes. ANOSIM results showed significant differences in diatom assemblage composition among quality classes as predicted, but also showed differences between countries and period when considering the entire diatom database (Table 5). ANOSIM analyses were also performed for the diatom assemblages of least disturbed sites (Table 5), and confirmed that diatom assemblages were in fact conditioned by the country, but not by season (non-significant global R).

The average dissimilarity between sites of Good (G) and Moderate (M) ecological status was 84% (SIMPER analysis of Types 1–2–3). The taxa that most contributed to this dissimilarity were *Achnanthes minutissimum* (average abundance 14.1%), *Amphora pediculus* (average abundance 5.7%) and *Cocconeis placentula* (average abundance 6.7%), which were more abundant in sites with Good ecological status. *Nitzschia inconspicua* (average abundance 7.2%), *Planothidium frequentissimum* (5.5%), *Mayamaea permitis* (4.3%) and *Nitzschia palea* (4.6%) contributed more to sites with moderate ecological status (Fig. 4a).

Similarly, Type 4 (temporary rivers) also showed a large dissimilarity between sites of Good and Moderate ecological status (86%). The most discriminative species for the sites of Good ecological status was also *A. minutissimum* (average abundance 19.5%), where *C. placentula* var. *lineata* (9.9%), *N. inconspicua* (5.7%), *Cocconeis euglypta* (6%), and *Encyonopsis minuta* (3.5%) were also abundant. The most abundant taxa in Moderate ecological status sites were *P. frequentissimum* (average abundance 7.2%), *A. pediculus* (7.9%), *Gomphonema parvulum* (5.5%), *Navicula veneta* (5.2%) and *Rhoicosphenia abbreviata* (4.2%) (Fig. 4b).

Table 4

Statistics of linear regression for diatom assessment methods' EQRs and the Intercalibration Common Metric (ICM). $p < 0.001$ for all regressions.

Method	Type	Linear regression	
		equation	r ²
CY/IPS	3	ICM = 1.293 IPS − 0.2931	0.9402
CY/IPS	4	ICM = 0.857 IPS + 0.034	0.8352
FR/IBD 2007	1, 2, 3	ICM = 0.970 IBD − 0.035	0.8591
IT/ICMi	1, 2, 3	ICM = 1.016 ICMi − 0.005	0.6643
IT/ICMi	4	ICM = 1.065 ICMi + 0.005	0.9678
PT/IPS	1, 2	ICM = 1.093 IPS − 0.066	0.9150
PT/IPS	4	ICM = 1.096 IPS − 0.063	0.9231
SI	1, 2	ICM = 0.751 SESAR + 0.454	0.7384
SI	4	ICM = 0.979 SESAR + 0.157	0.9326
SP/IPS	1, 2, 3	ICM = 0.841 IPS + 0.003	0.8802
SP/IPS	4	ICM = 1.021 IPS − 0.013	0.9409

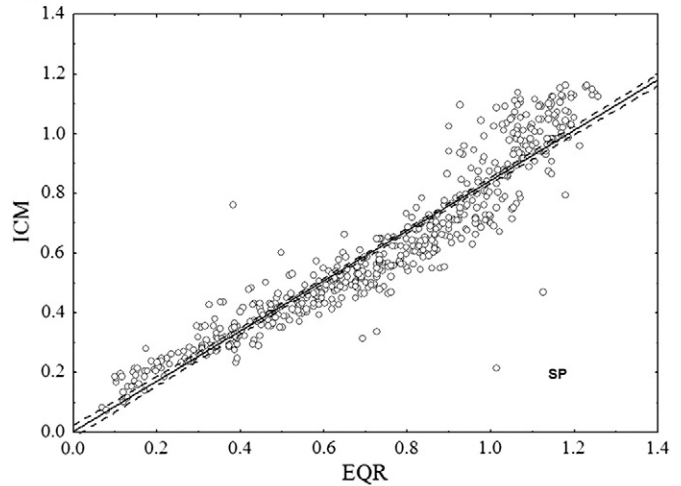
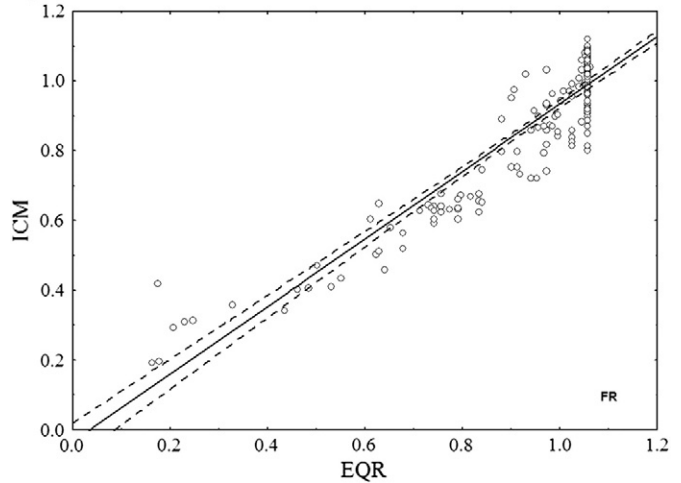
a) ICM = 0.841 IPS + 0.003**b) ICM = 0.970 IBD - 0.035**

Fig. 2. Linear regression between national methods (in EQR values) and the Intercalibration Common Metric (ICM) for Types 1–2–3. a) Spanish assessment method—IPS; b) French method—IBD 2007.

3.3. Boundary comparison and harmonisation

Upper boundaries (High/Good and Good/Moderate) of the partner states were translated to ICM through regression equations. The original

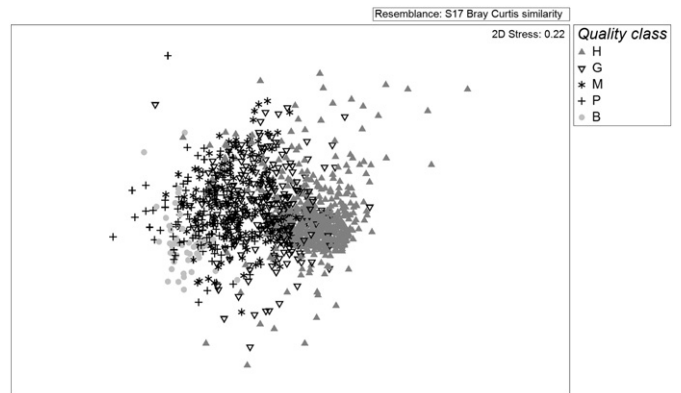


Fig. 3. Non-metric multi-dimensional scaling (MDS) of diatom assemblages from the entire quality gradient according to quality class (H—High; G—Good; M—Moderate; P—Poor; B—Bad).

Table 5

ANOSIM results for entire diatom database and least disturbed diatom database from six Mediterranean countries (Cyprus, France, Italy, Portugal, Slovenia and Spain); quality class—High, Good, Moderate, Poor, Bad; season—spring, summer, autumn, winter; $p < 0.001^{***}$; $p < 0.05^{**}$. n—number of samples.

Variable	Entire diatom database		Least disturbed diatom database
	Types 1–2–3	Type 4	
	Global R, n		
Country	0.164 ^{***} , 977	0.330 ^{***} , 95	0.282 ^{***} , 205
Quality class	0.419 ^{***} , 977	0.286 ^{***} , 95	–
Season	0.066 ^{***} , 977	0.219 ^{**} , 95	0.03, 205

boundaries translated into ICM values \pm quarter of band width, and the common ICM boundary (dashed line) are presented in Fig. 5 for Types 1–2–3 and in Fig. 6 for Type 4.

Four national types (SP—Type 1, SP—Type 2, SP—Type 3, and PT—Type 6) needed to adjust their original boundaries to fit within the ICM boundary range (Table 6).

Class difference between Cyprus and the other countries ranged from 0.253 to 0.402 (average 0.317), that of France between 0.139 and 0.329 (average 0.257), that of Italy between 0.192 and 0.386 (average 0.247), that of Portugal between 0.139 and 0.319 (average 0.247), that of Slovenia between 0.209 and 0.416 (average 0.328), and finally Spain class difference values varied between 0.176 and 0.550 (average 0.314).

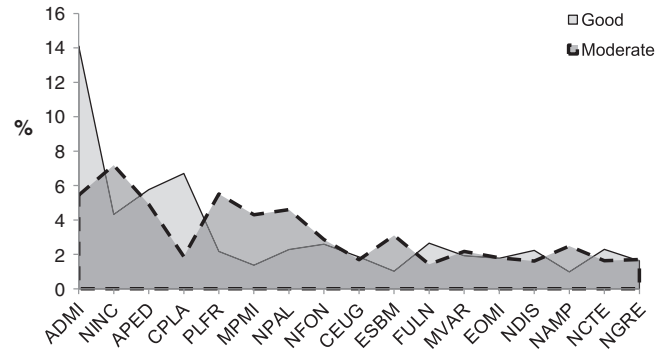
4. Discussion

The comparison of diatom assessment methods in six Mediterranean countries was facilitated by the adoption, at the national level, of European standards for sampling, sample treatment and analysis of diatoms (EN, 2003, 2004; Kelly et al., 1998). Variability in the identification process at the species level required *a posteriori* harmonisation of the diatom data (process which has been confirmed to be important for attaining higher similarity between identifications—Kahlert et al., 2009), but the robustness of the diatom indices (adopted by the Mediterranean countries) and that of the hybrid ICM probably accounted for minor taxonomic differences within the dataset. Despite these results, it is still necessary to update knowledge on taxonomic improvements, so that difficult/problematic and ecologically relevant species complexes can be included in the implementation of water management legislation. The intercalibrated diatom indices accounted well for the responses of diatom communities to environmental pressures, in the way described elsewhere for diatoms (Chessman et al., 2007; Kelly, 2003; Rimet et al., 2004; Rimet, 2012).

The segregation of river types according to diatom assemblages was not consistent with the pre-established abiotic types (Feio et al., 2014—in this issue). The disagreement between river ecotypes and biotic groupings was also the case in the Central Baltic ecoregion of Europe (Van de Bund, 2009). However, the establishment of common abiotic thresholds helped setting up common boundaries applicable to biological basis. Kelly et al. (2009) referred to the lack of rigour of the reference screening process (variable criteria in the selection of the reference sites). Rigour in the selection of sites among those intentionally proposed as reference in the Mediterranean dataset did not assure the existence of pristine conditions in several river types. The Spearman rank correlations between the ICM and the environmental conditions of the least disturbed sites showed significant correlation for nitrate, indicating that Mediterranean rivers and streams are affected by diffuse pollution (Sabater et al., 2008; Ros et al., 2009).

The high correlation between the diatom metric and nutrients is intrinsic to diatoms and widely reported (Rimet et al., 2005; Weckström and Juggins, 2005; Potapova and Charles, 2007). However, based on previous studies (Potapova, 1996; Tison et al., 2005; Feio et al., 2009), we know that diatoms can also be useful for detection of other stressors

a) Types 1, 2 and 3



b) Type 4

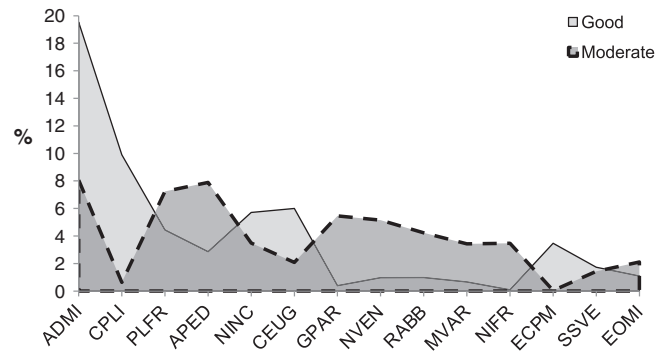


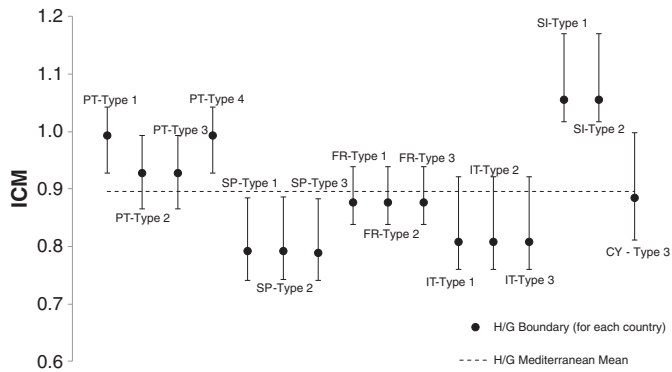
Fig. 4. Average abundance (%) of diatom taxa that contributed the most (up to about 50%) to differentiate sites classified with Good and Moderate ecological status for rivers of the Mediterranean region. a) Types 1–2–3; b) Type 4 (temporary rivers).¹

¹ADMI—*Achnantheidium minutissimum* (Kützing) Czarnecki; NINC—*Nitzschia inconspicua* Grunow; APED—*Amphora pediculus* (Kützing) Grunow; CPLA—*Cocconeis placentula* Ehrenberg; PLFR—*Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot; MPMI—*Mayamaea permissis* (Hustedt) Bruder and Medlin; NPAL—*Nitzschia palea* (Kützing) W. Smith; NFON—*Nitzschia fonticola* Grunow in Cleve and Möller; CEUG—*Cocconeis euglypta* Ehrenberg; ESBM—*Eolimma subminuscula* (Manguin) Moser, Lange-Bertalot and Metzeltin; FULN—*Fragilaria ulna* (Nitsch.) Lange-Bertalot; MVAR—*Melosira varians* Agardh; EOMI—*Eolimma minima* (Grunow) Lange-Bertalot; NDIS—*Nitzschia dissipata* (Kützing) Grunow; NAMP—*Nitzschia amphibia* Grunow; NCTE—*Navicula cryptotenella* Lange-Bertalot; NGRE—*Navicula gregaria* Donkin; CPLI—*Cocconeis placentula* Ehrenberg var. *lineata* (Ehrenberg) Van Heurck; GPAR—*Gomphonema parvulum* (Kützing) Kützing; NVEN—*Navicula veneta* Kützing; RABB—*Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot; NIFR—*Nitzschia frustulum* (Kützing) Grunow; ECPM—*Encyonopsis minuta* Krammer and Reichardt; SSVE—*Staurisira venter* (Ehrenberg) Cleve and Möller.

besides nutrients, suggesting that assessment methods must be established using other pressures in their construction, such as those related to morphological and hydrological alterations, and land use (see Almeida and Feio, 2012).

The ICM proved robust and adequate for Intercalibration purposes, and reliably reflected national water quality. An analogous result was also shown in a diatom taxonomic ring-test where seventeen analysts identified and counted diatoms in nine samples from seven countries. They showed that differences in taxa lists were large but, were not the major source of variation of the ICM (Kahlert et al., 2012). They concluded that different taxonomic conventions between countries did not affect the reliability of ICM results. However, it's also true that different taxonomic approaches can certainly make datasets “noisier” and more difficult to interpret. It was clear during the intercalibration that diatom assemblages also reflected differences in national diatom identification conventions. It is obvious that the response of diatom assemblages to the factor “quality class” could be improved with more data. We were also able to rule out the possibility that diatom assemblages could be mostly responding to the factor “season”. This will allow Mediterranean countries to be able to proceed with monitoring programmes and

a) Types 1, 2 and 3 - original boundaries



b) Types 1, 2 and 3 - original boundaries

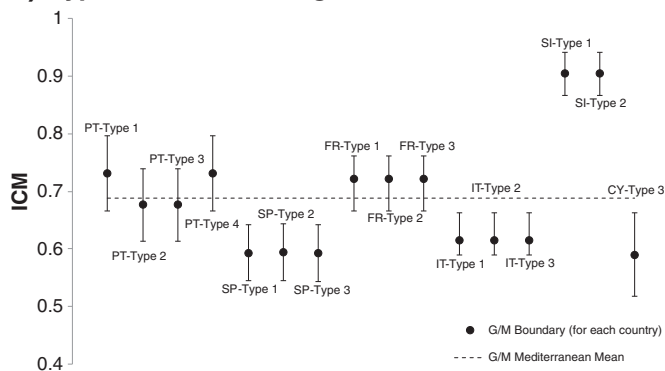
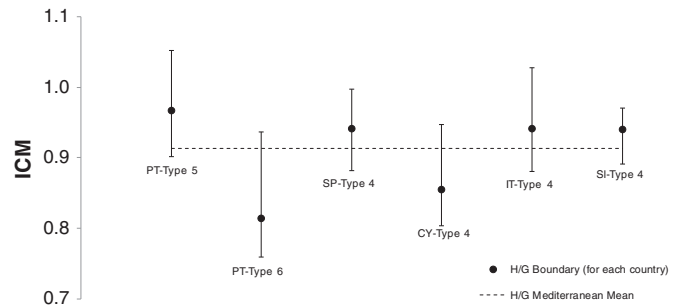


Fig. 5. Original boundaries translated into the Intercalibration Common Metric (ICM) for Types 1–2–3 pooled together and distance to the common Mediterranean boundary (dashed line). a) High/Good (H/G) boundary. b) Good/Moderate (G/M) boundary. Deviations represent a quarter (0.25) of class width.

environmental impact studies during the whole year, and still be confident that diatoms will be responding more to quality status than to variations due to the seasonal cycle.

The WFD legal obligation for defining the diatom assemblages that distinguish the Good/Moderate ecological status classes is highly demanding, since sites with some degradation usually meet the appropriate abiotic conditions for the development of stable and diverse diatom assemblages, including species with wide ecological ranges. It is clear from the MDS plot (Fig. 3) that sites classified with Good and Moderate ecological status have communities almost completely mixed up, evidencing a similar diatom assemblage structure. Most diatoms shown in Fig. 4 are ubiquitous and non-specific indicators of water quality and can, therefore, be present in diverse ecological situations. Some of the diatoms which discriminated the Good from Moderate ecological status classes were present in both quality classes but with different relative abundances. Nonetheless, in the Moderate quality class less sensitive taxa were more abundant (i.e.: *G. parvulum*, *N. palea*), while *A. minutissimum* was the most discriminative taxon in the Good quality class. *A. minutissimum* is frequently designated as a species complex and widely distributed usually pointing to oligotrophic and oligosaprobic environments and good water quality (Feio et al., 2007; Leclercq and Maquet, 1987; Sládeček, 1986). Ponader and Potapova (2007) refer that this taxon is mostly associated with low nutrient and ionic content. *A. pediculus* was more abundant in the Good quality class for Types 1–2–3, but more abundant in the Moderate quality class for the temporary rivers (Type 4). This species has been considered sensitive according to the IPS, but of moderate sensitivity to nutrients according to the TI (Coste in Cemagref, 1982; Rott et al., 1999), so it can contribute to both water quality classes depending on the available dataset. A similar situation occurred with *N. inconspicua* (less sensitive

a) Type 4 - original boundaries



b) Type 4 - original boundaries

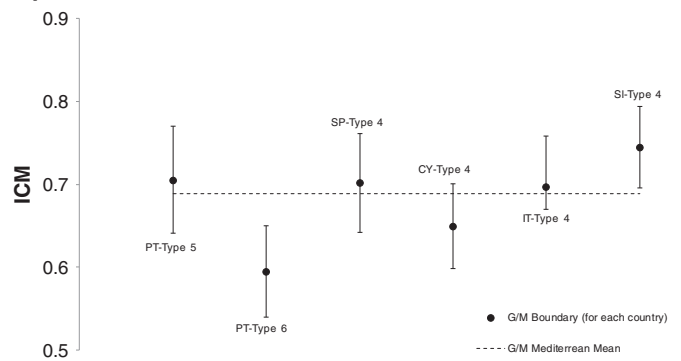


Fig. 6. Original boundaries translated into the Intercalibration Common Metric (ICM) for Type 4 (temporary rivers) and distance to the common Mediterranean boundary (dashed line). a) High/Good (H/G) boundary. b) Good/Moderate (G/M) boundary. Deviations represent a quarter (0.25) of class width.

to pollution according to the IPS—Coste in Cemagref, 1982; and TI—Rott et al., 1999) but dominating in the Moderate quality class for Types 1–2–3 and in the Good quality class in temporary rivers (Type 4). These apparent discrepancies can be explained, due to intrinsic autoecology (low indicator value of these two species) making it possible for them to be present in both Good and Moderate quality classes.

Table 6

Boundary values for High/Good (H/G) and Good/Moderate (G/M) quality classes in Ecological Quality Ratio (EQR) of partner countries' assessment methods according to typology and code adopted for each type. Values in brackets refer to adjusted boundaries after the intercalibration process.

Country	Code	Boundary	
		H/G	G/M
Cyprus	CY–Type 3	0.910	0.683
	CY–Type 4	0.958	0.718
France	FR–Type 1	0.940	0.780
	FR–Type 2	0.940	0.780
	FR–Type 3	0.940	0.780
Italy	IT–Type 1	0.800	0.610
	IT–Type 2	0.800	0.610
	IT–Type 3	0.800	0.610
	IT–Type 4	0.880	0.650
Portugal	PT–Type 1	0.970	0.730
	PT–Type 2	0.910	0.680
	PT–Type 3	0.910	0.680
	PT–Type 4	0.970	0.730
	PT–Type 5	0.940	0.700
	PT–Type 6	0.800	0.600 (0.651)
Slovenia	SI–Type 1	0.800	0.600
	SI–Type 2	0.800	0.600
	SI–Type 4	0.800	0.600
Spain	SP–Type 1	0.937	0.701 (0.727)
	SP–Type 2	0.938	0.702 (0.727)
	SP–Type 3	0.935	0.701 (0.727)
	SP–Type 4	0.935	0.700

Boundary bias and class agreement criteria were fulfilled, so it can be concluded that the national assessment methods intercalibrated are sufficiently comparable. Boundary harmonisation between Mediterranean countries needed adjustments in a few cases. The high maximum value (HM) was used to establish the boundary band width for each partner country. This was the maximum EQR translated into ICM values. The intercalibration process and consequently the need for boundaries' adjustments was, therefore, very dependent on this HM value. The highest index value measured can fluctuate over the years and with increasing datasets, so countries with larger datasets are naturally favoured since the possibility to attain higher EQR values is larger than for countries with smaller datasets.

5. Conclusion

Diatom assessment methods were compared, national benchmark sites were checked, and the translation of adjusted boundaries to national systems was completed during the intercalibration exercise of Mediterranean European countries. Intercalibration of diatom assessment methods was attained despite the difficulties encountered during the process, including the taxonomic inconsistencies and different data acquisition due to the robustness of these methods. Constraints increased when establishing the diatom assemblages for Good/Moderate classes, highlighting the need for continued improvement in diatom taxonomic and ecological knowledge and refinement of methodological issues (sampling, treatment and study of diatom slides). A final constraint was the lack of real pristine sites, and in particular for temporary rivers (Type 4).

The intercalibration procedure faced the difficulty of attributing biotic identity to the abiotic types, and small databases of some partner countries conditioned the highest classification scores used for boundary width establishment. Despite these constraints, the intercalibration exercise allowed for the definition of common boundaries for all partner countries and, therefore, contributed to uniformise the standards of water policy in the Mediterranean ecoregion of the European Union. Nevertheless, in temporary rivers, a better understanding of temporal and spatial variability of diatom communities and its effects in assessment methods should be attained.

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