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Benefits and limitations of an intercalibration of phytoplankton assessment methods based on the Mediterranean GIG reservoir experience

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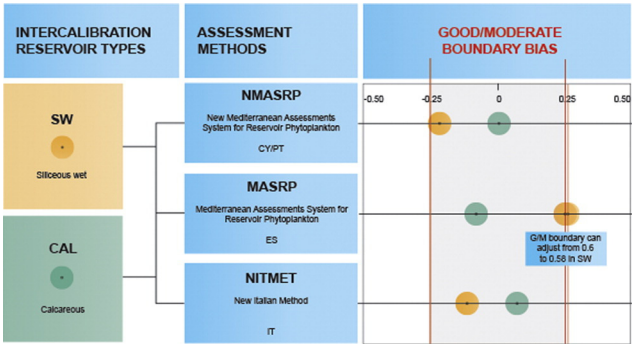
HIGHLIGHTS

- Alkalinity and climate are confirmed sources for type pressure-response variation.
- A screening process of pressure and biological parameters defined unimpacted sites.
- Three methods were compared using median values of every other method involved.
- Only the Spanish method in siliceous wet reservoirs was stricter than the rest.
- Cyanobacteria overcome populations when closer to the Good/Moderate boundary.

GRAPHICAL ABSTRACT



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ABSTRACT

The status of European legislation regarding inland water quality after the enactment of the Water Framework Directive (WFD) originated scientific effort to develop reliable methods, primarily based on biological parameters. An important aspect of the process was to ensure that quality assessment was comparable between the different Member States. The Intercalibration process (IC), required in the WFD ensures the unbiased application of the norm. The presented results were developed in the context of the 2nd IC phase. An overview of the reservoir

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type definition of the Lake Mediterranean Geographical Intercalibration Group, where four types were considered divided by both alkalinity and climate, together with the results for selection of Maximum Ecological Potential sites (MEP) are presented. MEP reservoirs were selected based on pressure and biological variables. Three phytoplankton-based assessment methods were intercalibrated using data from Mediterranean countries. The Mediterranean Assessment System for Reservoirs Phytoplankton (Spain), the New Mediterranean Assessment System for Reservoirs Phytoplankton (Portugal and Cyprus) and the New Italian Method (Italy) were applied. These three methods were compared through option 3 of the Intercalibration Guide. The similarity of the assessments was quantified, and the Good/Moderate (GM) boundaries assessed. All three methods stood as comparable at the GM boundary except for the MASRP in siliceous wet reservoirs, which was slightly stricter. Finally, the main taxonomic groups represented in the phytoplankton community at MEP conditions were identified, as well as their main changes with an increasing trophic status. MEP sites are dominated by chrysophytes in siliceous wet reservoirs and by the diatoms *Cyclotella* and *Achnanthes* in calcareous ones. Cyanobacteria take over the community in both calcareous and siliceous wet reservoirs as eutrophication increases. In summary, the relevance and reliability of the quality assessment methods compared were confirmed both from an ecological perspective and a health risk management point of view.

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1. Introduction

Reservoirs hold approximately 6000 km^3 of freshwater, more or less 13% of the world's surface runoff (Avakian, 1990). Within the Mediterranean ecoregion, due to the uneven distribution of water both spatially and temporally (diCastri and Mooney, 1973), the impoundment of surface runoff through a reservoir network is necessary to provide a stable availability of the resource, and more common than in countries of higher and more regular precipitation (Kondolf and Batalla, 2005). Due to the importance of these water bodies in the most arid climates of Europe, reservoir ecological integrity is a key aspect to a sustainable use of water.

The enforcement of legal tools to protect and ensure the quality of water has been tackled in many countries. Such is the case, for example, of the Water Pollution Prevention and Control Law in China or the US Clean Water Act, where the prevention of ecosystem deterioration is paramount. In Europe, the legal tool developed with a similar purpose is the Water Framework Directive (WFD) (European Commission, 2000). It states, in Annex V, that the ecological quality assessment methods used in each country have to be intercalibrated (European Commission, 2000). This step is essential to ensure the comparability of a diverse array of results branching from different quality assessment methods (Phillips, 2014), which in turn is paramount to produce comparable management goals (Birk et al., 2013). The comparability of bioassessment methods used over broad geographical ranges is a major challenge for legal frameworks in charge of guaranteeing quality for aquatic ecosystems. Many efforts have attempted to study different aspects affecting comparability, such as data collection, treatment and summary procedures, which may jeopardize the comparability of the assessments (Cao and Hawkins, 2011) and others have developed tools to assess and improve comparability (Diamond et al., 2012). The intercalibration process (IC), required by the WFD, is a tool developed for this purpose. In the case of heavily modified water bodies such as reservoirs, it attempts to ensure that the Good/Moderate (GM) threshold established by different methods applied in the member states (MS) refers to a comparable ecosystem quality level (Poikane et al., 2011, 2015) across different water body types and Ecoregions. The importance of this ecological quality boundary relies on the objectives of the WFD, which requires active restoration plans for sites below this level of quality, i.e. anything beyond "slight changes in the values of the relevant biological quality elements as compared to the values found at maximum ecological potential" (European Commission, 2000). The IC is being carried out by Geographical Intercalibration Groups (GIG), one of which was the Lake Mediterranean GIG (L-M GIG). GIGs are responsible for the IC of each biological quality element (phytoplankton, macrophytes, macroinvertebrates, etc.) of their water body types. The water body types were initially established in ECOSTAT (European Commission, 2004) and subsequently revised by each GIG. Some IC results have already been delivered and published in documents both official (European Commission, 2013) and scientific (Poikane et al., 2015; Kelly et al., 2014; Almeida, 2014).

The basic approach of the WFD quality classification system refers to deviations from natural, undisturbed conditions (Poikane et al., 2011). Varied definitions of undisturbed conditions have been investigated (Reynoldson, 1997; Carvalho et al., 2008; Wolfram et al., 2009). Since reservoirs are artificial or heavily modified water bodies, a certain departure from lake natural conditions is assumed. As a consequence, instead of using natural reference sites, the quality goals are defined based on determining sites of maximum ecological potential (MEP) (Borja and Elliott, 2007). MEP is defined as "the best approximation to a natural aquatic ecosystem that could be achieved given the hydromorphological characteristics that cannot be changed without significant adverse effects on the specified use or the wider environment" (European Commission, 2003b). Typically, deviations from natural, undisturbed conditions in lakes refer to an increase in the trophic status; therefore, MEP also pays particular attention to it.

The WFD established that both biomass and composition metrics together with bloom sensitive parameters should be applied for assessing the ecological water quality of lakes using phytoplankton. Chlorophyll-a (chl-a) and total phytoplankton biovolume (BV) are typical biomass metrics used for eutrophication assessment (Poikane et al., 2015). Phytoplankton communities (Reynolds, 1980; Hörnström, 1981; Watson et al., 1997; Willén, 2000) and different indexes based on phytoplankton composition (Catalan and Ventura, 2003; Padisak et al., 2006; Salmaso et al., 2006; Mischke et al., 2008; Wolfram et al., 2008; Marchetto et al., 2009; Morabito and Carvalho, 2012; Phillips et al., 2013) have been applied to assess eutrophication and related pressures. The methods developed in the countries involved in the L-M GIG mostly share parameters such as chl-a concentration and BV, and use different metrics based on phytoplankton composition (de Hoyos et al., 2014; Poikane et al., 2015).

Here we describe the procedure applied to compare several national phytoplankton-based assessment methods used in Mediterranean countries for reservoirs, which was conducted during the 2nd phase of the IC (2008–2011). The procedure included, as necessary steps previous to the comparison, a typological study of the common reservoir types (based on hydromorphological, chemical and climatic variables (European Commission, 2004; Poikane, 2009)), and a study of MEP sites together with the characteristic communities for each reservoir type at different pressure levels.

In this study, we aimed for checking if it was possible to develop a simple water body classification which is applicable over large territories and defined by a few key factors, assessing if different assessment methods converge into similar results when defined under common basic rules and explore if, whereas high ecological quality conditions are characterized by differentiated taxonomic groups in the defined

types, the key standard quality boundary (GM) and decaying conditions have more in common.

2. Materials & methods

2.1. Data

The dataset compiled for this project included 179 reservoirs belonging to 7 different countries (Cyprus (CY), 7; Spain (ES), 122; France (FR), 6; Greece (GR), 1; Italy (IT), 15; Portugal (PT), 18; Romania (RO), 10) (Table 1; Fig. 1). A total of 365 reservoir-year records were used for the most part of the analyses, and only included summer samples. The database contained 15,442 phytoplankton records (cell counts and biovolume calculations), morphological water body characteristics, climatic variables (mean annual temperature and annual precipitation in the catchments), pressures, mainly total phosphorus (TP), CORINE land-cover derived variables and population density (PD) and 63,742 physicochemical records (Table 1).

A considerable amount of the total data comes from Spanish reservoirs. This bias in the origin of data is partly rooted on the size of the countries and their abundance of reservoirs. For example, even though Cyprus or the Mediterranean regions of France contribute with a low number of reservoirs to the database, they represented most of their existing water bodies. This was not the case in Greece, but this country finally did not participate in the IC process. Most phytoplankton data from PT and RO were either lacking biovolume or being estimated using a method alternative to the common Utermöhl method (Utermöhl, 1958). For this reason, data from these countries were not considered in the method comparability assessment (Section 3.3) and in the community composition analysis (part 3.4).

All the treatment of the data and calculations together with its compilation was done in the Department of Aquatic Environment of the Centre for Hydrographic Studies (CEH) in Spain.

2.2. Type analysis

Common IC types had to be defined for the comparison of the national assessment methods. Based on the types originally defined by the ECOSTAT (European Commission, 2004), a basic four group classification was agreed by the MSs participating in the L-M GIG (Poikane, 2009): Siliceous arid (SA), siliceous wet (SW), calcareous arid (CA) and calcareous wet (CW) reservoirs. The alkalinity threshold was set at 1 meq L^{-1}, while the wet and arid classification depended on mean annual temperature (threshold at 15 °C) and annual precipitation (800 mm) (Table 2). Few reservoirs had low mean annual temperatures together with low precipitation or vice versa, but all the observed cases were from the Spanish dataset. To determine the wet or arid status of these "ambiguous" reservoirs, the national classification, which uses a humidity index parameter, was used. All the reservoirs considered had a mean depth >15 m, a surface area >0.5 km^2 and a catchment area <20,000 km^2 (Poikane, 2009).

The details of the contribution of each participating country to the four different types are presented in Table 3.

As the classification was reached by consensus, the first question to address was whether the division in four reservoir types was ecologically meaningful, i.e. if the biological parameters differed among the types, given a similar pressure level. We first compared the pressure levels across groups for the different pressures: Artificial land use (ALU%), intensive agricultural land use (IA%), natural and semi-natural land use (NASN%), PD and TP. These pressures have been selected for their potential impact on phytoplankton communities since, theoretically, they all affect nutrient inputs, and consequently are directly (or inversely in the case of NASN%) proportional to anthropogenically induced eutrophication of water bodies. TP summarizes all the other pressures considered. Once fairly homogeneous pressures were confirmed, a Kruskal–Wallis rank sum (KW) (S-PLUS 7.0 software) test was performed for determining whether any of the pressure response parameters considered (total cell abundance, BV (when possible) and chl-a) was significantly different among any of the groups. Once differing parameters were identified, a pair by pair Wilcoxon rank sum (WRS) test (S-PLUS 7.0 software) was performed to define on which groups the differences were.

Finally, two further analyses were performed: a canonical correspondence analysis (CCA) and a discriminant analysis (DA) (CANOCO software), based on species abundance and environmental variables (temperature, precipitation, latitude, altitude, mean depth, TP, CORINE landcover variables and PD, in the case of the CCA), with the aim of defining the phytoplankton compositional bases of the differential responses.

2.3. MEP sites

The selection of MEP sites was one of the main steps in the IC (Wolfram et al., 2009; Poikane et al., 2011). The selection was based on the IC Guidance Document (European Commission, 2000), using CORINE landcover parameters (i.e., ALU%, IA% and NASN%), PD and TP. Two threshold values were selected for each variable: a "Reference limit" and a "Rejection limit" (Table 4). The limits were based on proposed pressure screening criteria for selection of reference sites (European Commission, 2003a), criteria and thresholds used by the L-M GIG MSs in the first IC round, and criteria and thresholds used in other GIGs. The reservoirs selected as MEP should not have had more than two pressures above the reference limits, and none above the rejection limits. Furthermore, three more features were considered in this step: exploitation of fish population by fishery (strong extractive fishing activity can alter the ecological equilibrium in several ways, including phytoplankton grazer depletion, or top-down control) (Jeppesen et al., 2000), recreational activities (strong recreational use of reservoirs can subject a water body to many different effects, including turbulence due to boat propellers, allochtonous species introductions, increased presence of heterotrophic taxons in bathing areas and nutrient level increase among other confounding factors) and presence/absence of zebra mussel, *Dreissena polymorpha* (which is known to potentially have strong effects on water turbidity through filter grazing activity (MacIsaac, 1996)). The first two were classified according to four possible levels (none, low, medium and strong). The absence of zebra mussel was compulsory for MEP sites, while none of the other two features could be classified as "strong". The managers of the different water bodies provided the data.

Biological parameters were further filtered for sites affected by hidden pressures not considered in the pressure screening. The variables studied were chl-a and BV, wherever possible. If any given reservoir's median value for the metric was above the 75th percentile of all the

Table 1
Number of reservoirs (and reservoir-years) with biological, physicochemical and pressure data for water bodies included from each country and totals for the L-M GIG. Res., reservoir; res-y, reservoir-year.

Data			
	Biological	Physicochemical	Pressure
	Res./res-y	Res./res-y	Res./data available
CY	7/19	7/19	7/7
FR	6/7	6/7	6/6
GR	1/2	1/2	1/1
IT	15/29	15/29	15/14
PT	18/20	18/20	18/18
RO	10/30	10/30	10/10
ES	122/258	122/258	122/118
L-M GIG	179/365	179/365	179/174

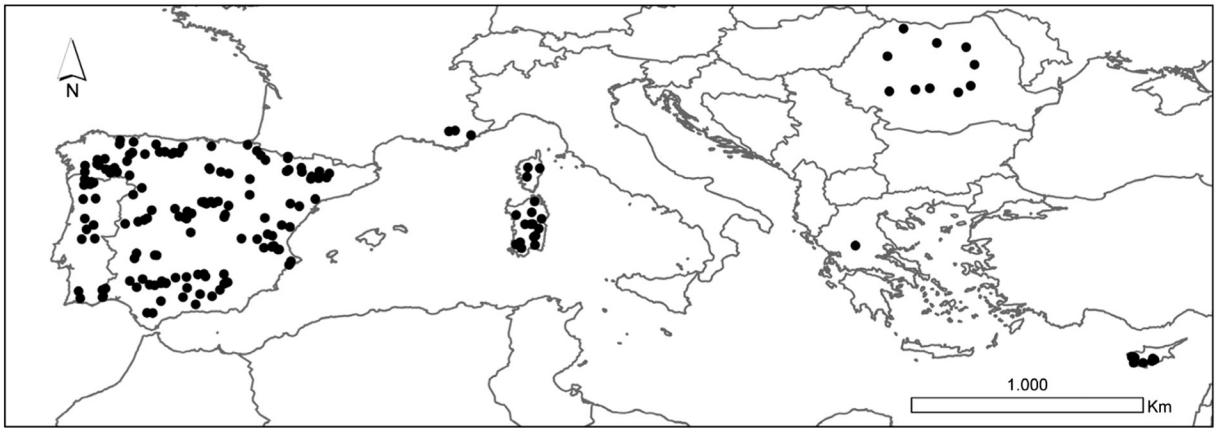

Fig. 1. Map showing the distribution of the reservoirs in the database. During this exercise Romania worked within the L-M GIG.

data of the reservoirs that went through the pressure screening criteria, the reservoir was excluded.

2.4. National assessment methods

Phytoplankton ecological quality assessment methods have to consider both biomass and composition parameters. Bloom sensitive metrics should also be a component of the methods (European Commission, 2000). The three methods intercalibrated were the MASRP, the NMASRP and the NITMET (de Hoyos et al., 2014) (Table 5). Some methods developed in the MSs were not intercalibrated: the French *Indice Phytoplanctonique Lacustre* (IPLAC), which was withdrawn due to some issues regarding one of its component metrics; the Romanian Assessment System for Reservoir Phytoplankton (ROMET), which showed a below-threshold response to pressures within the common dataset and the Greek method which was under development during the IC process. Chl-a concentration and BV were the common biomass terms for the three methods compared. However the methods differed in the composition metrics applied. The methods used combinations of the Mediterranean Phytoplankton Trophic Index (MedPTI) (Marchetto et al., 2009), total cyanobacteria biovolume (CyaBV), the Índex des Grups Algals (IGA) (Catalan and Ventura, 2003) and total cyanobacteria percentage biovolume (Cya%) (de Hoyos et al., 2014).

2.5. Intercalibration

A common methodology was developed for comparison and harmonization of ecological assessments in the second phase of the IC, (European Commission, 2013) which established that: 1) all the assessment methods should be compliant with the WFD normative definitions (i.e. they should meet the requirements set in the Directive); 2) the comparison should be feasible (e.g. the methods address the same pressure); and 3) the requirement of benchmarking should be studied, that is, dissimilarities in the methods due to biogeographical and methodological differences are identified and corrected (Poikane et al., 2015).

Table 2
Reservoir classification of the common IC types tested. Climatic factors and alkalinity are the characteristics considered.

Reservoir type	Mean depth	Alkalinity	Mean annual temperature	Annual precipitation
CA	>15 m	>1 meq L^{-1}	>15 °C	<800 mm
CW			<15 °C	>800 mm
SA		<1 meq L^{-1}	>15 °C	<800 mm
SW			<15 °C	>800 mm

In the L-M GIG, the comparison between the methods was done following option 3 of the IC Guide (European Commission, 2013; Birk et al., 2013; Poikane et al., 2015) as the type of samples used for the three methods are comparable. In the comparison we used an IC template sheet (EXCEL – MS OFFICE software) with slight modifications that include using, in the setting of a pseudo-common metric (PCM), the median instead of the mean of all the methods other than the one being compared.

Continuous benchmarking was selected to adjust biogeographical differences, as recommended when a limited number of MEP sites are available (Birk et al., 2013). A generalized linear model approach was applied using all reservoirs (SPSS software). This model type allows the inclusion of categorical values (e.g., member state) in the analysis, as well as data distributions other than Normal (Birk et al., 2013; Kelly et al., 2014) in order to overcome the limitation imposed by the low MEP site number.

Each method applied was linearly regressed with the PCM and TP for SW and CAL reservoirs, respectively (de Hoyos et al., 2014). The relationship with TP was assessed within the compliance check of the WFD. There were some requirements to ensure the comparability of the methods and their boundaries: 1) the three assessment methods must be positively and significantly correlated with the PCM ($p << 0.01$ and Pearson coefficient >0.5); 2) the slope of the best fit models must be between 0.5 and 1.5; and 3) from all the best fit models considered, the maximum R^2 coefficient value should not be more than twice as high as the minimum R^2 coefficient.

The final step of the assessment methods comparison required an analysis of the relative positions of the different GM boundaries. The boundary bias is a measure of the difference in Ecological Quality Ratio (EQR) units between the boundaries of the different methods. The tolerable variation between the boundaries was of half a class; therefore all GM boundaries had to fall within the same 0.1 EQR units (since one quality class was 0.2 units wide).

Table 3
Distribution of IC reservoir types per country.

Country	IC reservoir types			
	SA	SW	CA	CW
CY	–	–	7	–
FR	–	2	–	4
GR	–	1	–	–
IT	5	6	–	4
PT	4	14	–	–
RO	–	7	–	3
ES	19	35	44	24
L-M GIG	28	65	51	35

Table 4
Rejection and reference limits for the five selected pressures. Artificial land use (ALU%), intensive agricultural land use (IA%), natural and semi-natural land use (NASN%), population density (PD) measured as inhabitants per km^2 and total phosphorus (TP).

	ALU (%)	IA (%)	NASN (%)	PD (inh km^{-2})	TP (μg P L^{-1})
Rejection limit	<4	<20	>70	<30	<30
Reference limit	<1	<10	>80	<10	<12

2.6. Community description at MEP and GM sites

To assess how the phytoplankton species varied along the pressure gradient and to describe the transition from MEP sites to GM sites and below, we evaluated, for each species, the biovolume weighted average of the MASRP (selected arbitrarily from the three available methods) values (BWAM) in the reservoirs where the species was appearing (Eq. (1)),

$$BWAM_i = \frac{\sum(BV_{ij} * MASRP_j)}{\sum(BV_{ij})} \quad (1)$$

where BV_{ij} was the species (i) biovolume in reservoir-year (j) and $MASRP_j$ was the MASRP value for reservoir-year (j).

High values (≫0.6) for a species indicated a tendency to appear in localities classified as good ecological status. Low values (≪0.6) indicate a preference for moderate or below conditions. Species around GM transition showed BWAM around 0.6. Variance of BWAM estimation and number of observations of the species in the database were used to screen the species: high variance would indicate tolerance of a broad range of conditions independent of BWAM values, while low number of observations warned about spurious results. Species selected with these criteria were grouped taxonomically and, transformed to percentage biovolume of the total mean biovolume per reservoir-year, plotted against the NMASRP scores. The variation of the indicator groups was investigated.

3. Results

3.1. Type analysis

The initial step was to check if a fairly equivalent pressure level along all reservoir types existed (SA, SW, CA and CW). There were no differences in three of the five variables considered (Table 6).

Therefore, given the fairly homogeneous set of pressures among reservoir types, we could proceed with the study of phytoplankton parameters. The KW test indicated no difference in abundance ($p = 0.0792$) and BV ($p = 0.0566$), but different chl-a variance between reservoir types ($p = 0.0029$). In particular, the WRS tests showed significant differences in chl-a between CA and SA ($p = 0.0036$), CA and SW ($p = 0.0052$), CW and SA ($p = 0.0146$) and CW and SW ($p = 0.0203$), whereas differences between CA and CW ($p = 0.7145$) and SA and SW ($p = 0.8095$) were not significant. In summary, chl-a distributions were not equivalent between calcareous and siliceous reservoirs, but there were no differences within them according to climate.

The species abundance showed a certain distribution following an alkalinity gradient in the CCA, where the CA and SW reservoirs were, respectively, more represented at the extremes (Fig. 2A). The DA indicated that SW type phytoplankton composition was easily distinguishable from the other groups (Fig. 2B). Thereafter, CA can be differentiated from the CW and SA groups (Fig. 2B) and, the latter, only differentiates according to Axis 3 (Fig. 2C). We decided to complete the intercalibration considering only SW and calcareous (CAL, an amalgamate of CW and CA) as the intercalibration of SA type was not possible because there were not enough data available, and CAL were considered in a unique group in the 1st IC phase. The characteristics of the two final intercalibrated types are summarized in Table 7.

Table 5
Metrics of the methods applied in the countries participating in the Mediterranean GIG. Chl-a, chlorophyll-a concentration; BV, total phytoplankton biovolume; MBA, algal biomass metric; MCS, specific composition metric; CyaBV, biovolume of cyanobacterial; CyaCell%, percentage of cyanobacterial cells; TotTaxNum, total number of taxa; SW index, Shannon–Wiener diversity index; Cya%, percentage of cyanobacterial biovolume from total. * This metric was developed within the WISER project as a suitable bloom sensitive metric. ** The assemblage Index (Padisak et al., 2006) was on the process of modification to assign water body specific Q values to Greek water bodies.

Country	Method	Component metrics	
		Abundance	Composition Tolerance
CY	NMASRP	Chl-a, BV	CyaBV*, IGA index
GR	N/A	–	Mod. Assemblage index**
FR	IPLAC	MBA	MCS index
IT	NITMET	Chl-a, BV	CyaBV*, MedPTI index
PT	NMASRP	Chl-a, BV	CyaBV*, IGA index
RO	N/A	Chl-a, BV	CyaCell%, TotTaxNum, SW index
ES	MASRP	Chl-a, BV	Cya%, IGA

Table 6
Comparison of pressure distribution among the four reservoir types (CA, CW, SA and SW); Total Phosphorus (TP), Artificial land use (ALU), intensive agricultural land use (IA), natural and semi-natural land use (NASN) and population density (PD).

		Normality check		Variance comparison		Assessment
Pressures	Units	Normal	Levene (p)	ANOVA	KW	Difference among types?
TP	(μg L^{-1})	+	0.197	0.408	N/A	No
ALU	(%)	−	0.016	N/A	0.846	No
IA	(%)	−	0.020	N/A	0.000	Yes
NASN	(%)	+	0.156	0.000	N/A	Yes
PD	(inh km^{-2})	+	0.561	0.836	N/A	No

3.2. MEP sites

A total of 29 MEP reservoirs were selected through pressure screening (Table 4) and the phytoplankton parameter confirmation process (described in Section 2.3). Additionally, several reservoirs reported as MEP by the MSs, but with pressures above the MEP thresholds (Table 4), were included whenever the biological criteria were met (Table 8).

MEP sites showed phytoplankton biomass levels lower than the rest of the reservoirs in their type (Fig. 3). On the other hand, CAL reservoirs were less sensitive to pressures than their low alkalinity counterparts (Fig. 3), as they showed lower chl-a levels than siliceous reservoirs and there was homogeneity of the pressures among types (Section 3.1, Table 6).

3.3. Method comparability

Three different methods (NITMET, NMASRP and MASRP) used in four countries (CY, IT, PT and ES) (Table 5) were intercalibrated (see Section 2.4). The IC of these methods was performed for two reservoir types: CAL and SW (Section 3.1). CAL reservoirs were intercalibrated using a data set that comprised 77 reservoirs and 186 reservoir-year observations from CY, IT and ES. SW reservoirs were intercalibrated using 38 reservoirs and 71 reservoir-year observations from ES and IT. Not all the data initially available (Table 1) could be used at this step since some MSs withdrew from the process (see Section 2.4) and PT had no BV data (see Section 2.1).

Only after confirmation of both the compliance of the methods with the WFD and the feasibility of the comparison between them (see Section 2.5), was benchmark standardization addressed. The existence of systematic discrepancies such as methodological, typological or biogeographical differences poses a problem for transboundary databases.

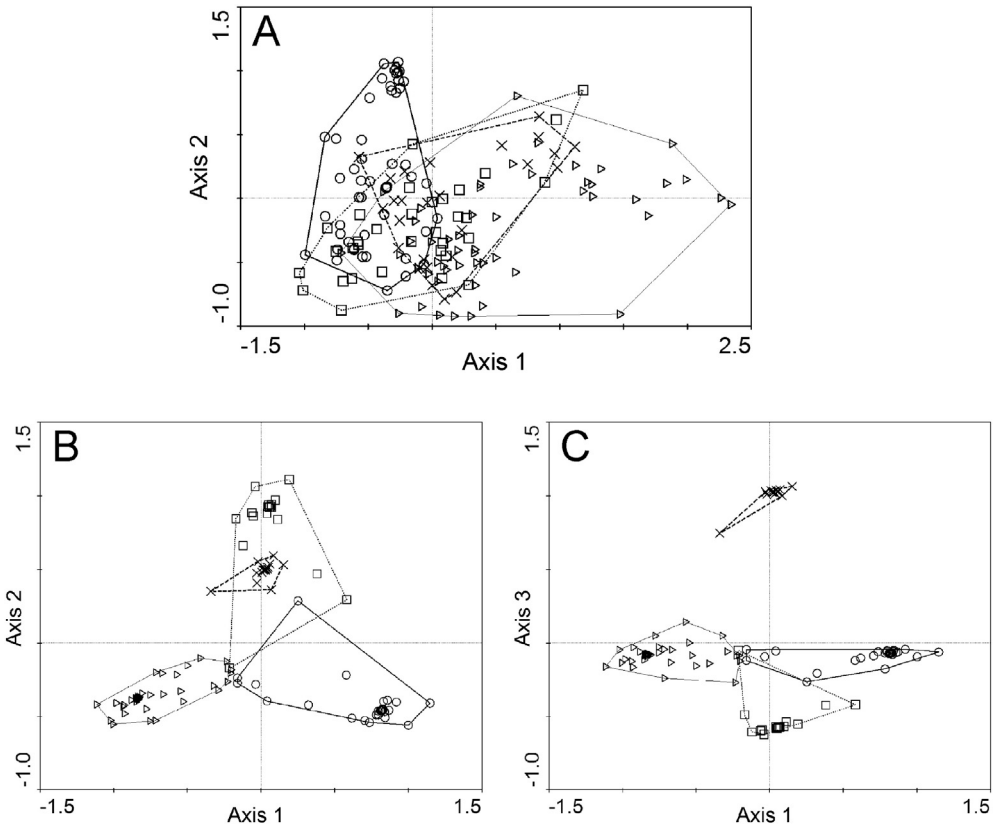

Fig. 2. A: Canonical correspondence analysis (CCA) ordination of the phytoplankton species abundance showing the distribution of the reservoir types. B and C: Discriminant analysis (DA) trying to classify phytoplankton samples according to the four reservoir types. CA reservoirs represented by circles and a thick solid line; CW reservoirs by squares and a dotted line; SA reservoirs by crosses and a dashed line; and SW reservoirs by triangles and a fine solid line.

A study of such differences is therefore essential, and corrections to the data must be done if necessary. Despite the uneven contribution of data by the different countries to the dataset, they were sufficient to perform the "continuous benchmarking" (Birk et al., 2013). Minimal offset values (variations in assessment results due to the above-mentioned sources of variation between countries) were established for CAL reservoirs, while no differences were found when applying the same method to SW reservoirs.

The three methods were positively and significantly correlated with the PCM ($p << 0.01$ and Pearson coefficient >0.5). The slope of the models was between 0.5 and 1.5 in all cases and the maximum R^2 coefficient value was never above twice the value of the minimum R^2 coefficient (Fig. 4, Table 9). These results were in agreement with the requirements of the IC (see Section 2.5) and implied that the relation of each individual method with the PCM was good enough to compare their GM boundaries.

The boundary bias was estimated for the SW reservoirs and found to fall within the limits for NITMET and NMASRP methods (0.25 class equivalent units above and below the median GM boundary). The boundary of the MASRP was slightly stricter than the other two (Fig. 5A). By moving the GM MASRP boundary down to 0.583, bias falls within the limits (Fig. 5B). In the case of CAL reservoirs, all GM boundaries were sufficiently comparable; hence the boundaries required no adjustments, remaining at 0.6 (Fig. 5C).

3.4. Community trends along the pressure gradient

In both types of reservoir phytoplankton compositional changes were apparent across the GM boundary. However, the more indicative taxa and groups were not the same for the two groups.

3.4.1. Siliceous wet reservoirs

In the SW reservoirs, when quality was still high, near the MEP community type, the phytoplankton community was composed mainly of chrysophytes, good quality indicator diatoms and chlorococcales. The genera *Dinobryon*, *Pseudopedinella* and *Ochromonas*, from the Chrysophytes, *Ankyra*, *Sphaerocystis* and *Coenochloris* from the Chlorococcales and *Asterionella*, *Nitzschia* and *Discostella* from the diatoms were typical in sites above the GM boundary, and peaked at MEP sites. Some species such as *Crucigenia tetrapedia*, *Monoraphidium minutum* (chlorococcales) and *Ulnaria ulna* (diatoms) were also representative of good quality sites. They steadily decreased towards the GM boundary, and almost disappeared at that point. In parallel to this change in the community, cyanobacterial presence, which started at low values, increased in the community at approximately the same point where the other groups tended to disappear. The genera *Anabaena*, *Woronichinia* and

Table 7
Definition, according to alkalinity and the considered climatic factors, of the reservoir types that were finally intercalibrated.

	Mean depth	Alkalinity	Mean annual temp.	Annual precipitation
CAL	>15 m	>1 meq L^{-1}	–	–
SW		<1 meq L^{-1}	<15 °C	>800 mm

Table 8
Number of Maximum Ecological Potential (MEP) reservoirs per country and reservoir type.

	CY	FR	GR	IT	PT	RO	ES	MEP reservoirs	Total reservoirs
CAL	2	–	–	–	–	–	19	21 (24.4%)	86
SW	–	–	1	–	3	3	12	19 (29.2%)	65
SA	–	–	–	–	3	–	4	7 (25.0%)	28

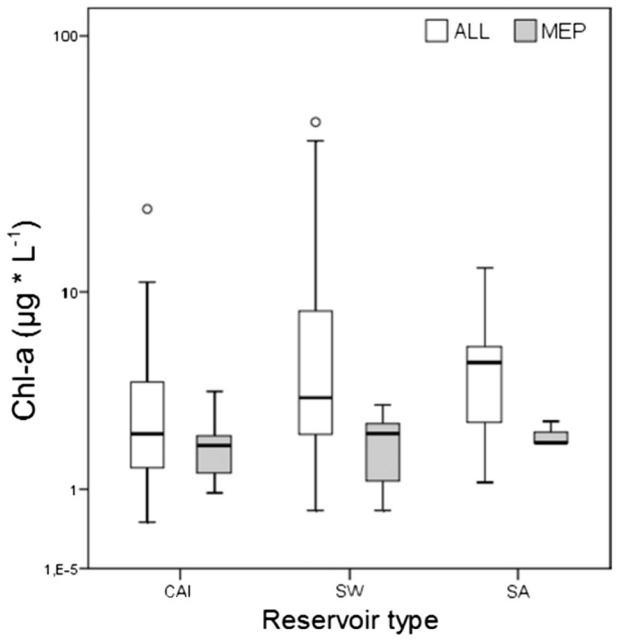

Fig. 3. Box-plot diagram presenting chl-a per reservoir type, separating MEP reservoirs from the rest. Each box represents the median, the 25th and 75th quartiles, extreme values (whiskers) and outliers (single dots).

Table 9
Correlation of PCM and TP with the intercalibrated methods according to reservoirs. Model equations are shown in the related figures.

		Pseudo-common metric			Total phosphorus	
		Slope	Pearson	R^2	Pearson	p-value
CAL	MASRP	0.955	0.959	0.9192	−0.46	<0.001
	NMASRP	0.8784	0.983	0.9663	−0.39	<0.001
	NITMET	1.018	0.941	0.8851	−0.42	<0.001
SW	MASRP	0.909	0.969	0.938	−0.57	<0.001
	NMASRP	1.0101	0.978	0.9599	−0.66	<0.001
	NITMET	0.9777	0.970	0.9432	−0.7	<0.001

Aphanizomenon were the main representatives of this change in the community (Fig. 6).

3.4.2. Calcareous reservoirs

The genera *Cyclotella and Achnanthes*, together with species such as *Ulnaria acus* and *Ulnaria ulna* were typical from high quality communities, and peaked at MEP sites. They steadily declined towards the GM boundary, and below this limit, in parallel to the increase of Cyanobacteria (*Anabaena*, *Microcystis* and *Aphanizomenon*) and Chlorococcales (*Coelastrum*, *Scenedesmus* and *Pediastrum*) (Fig. 7).

4. Discussion

Response to pressures in reservoirs depends on the bedrock substrate of the catchments and related major water chemistry (Margalef, 1975). Consequently, the WFD required the MSs to establish a typology

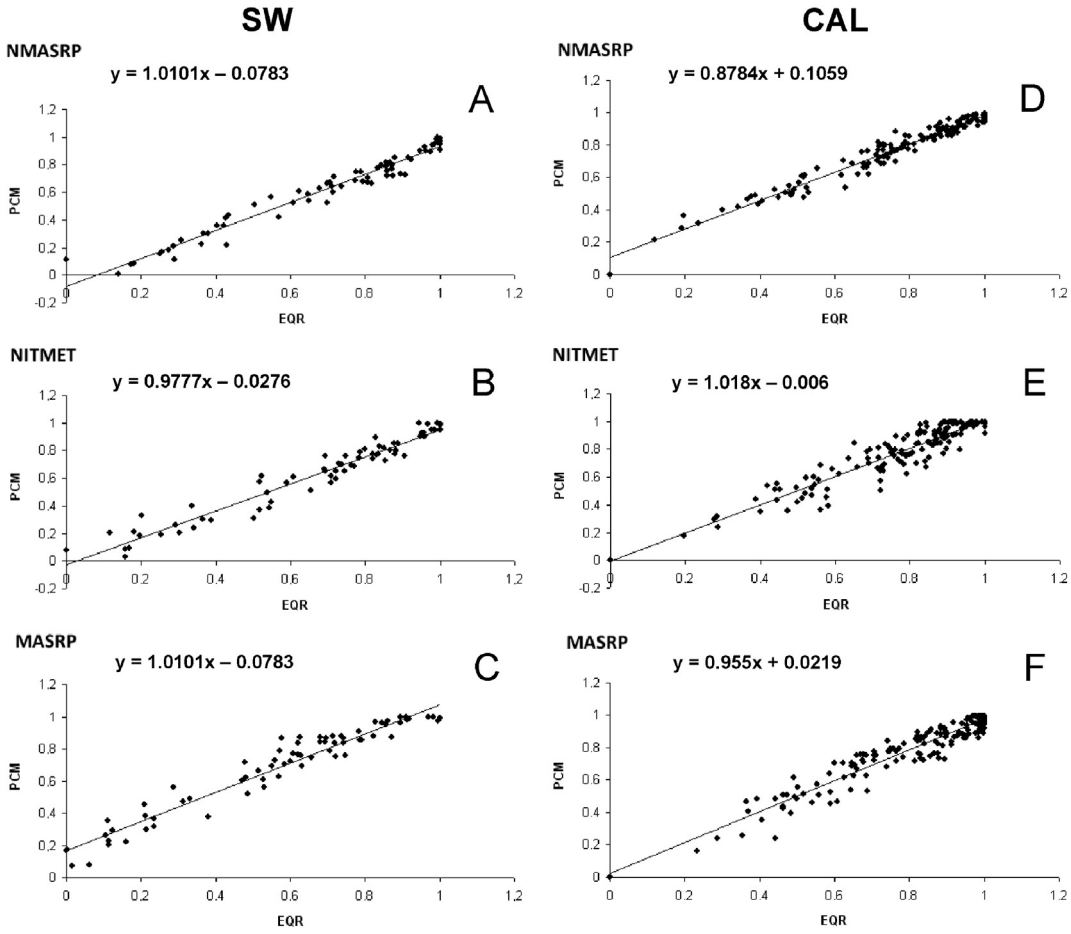

Fig. 4. Linear regression between the three methods and the pseudo-common metric (PCM) on siliceous wet reservoirs (A, B and C) and calcareous reservoirs (D, E and F). The model equation is shown. PCM — pseudo-common metric; EQR — ecological quality ratio.

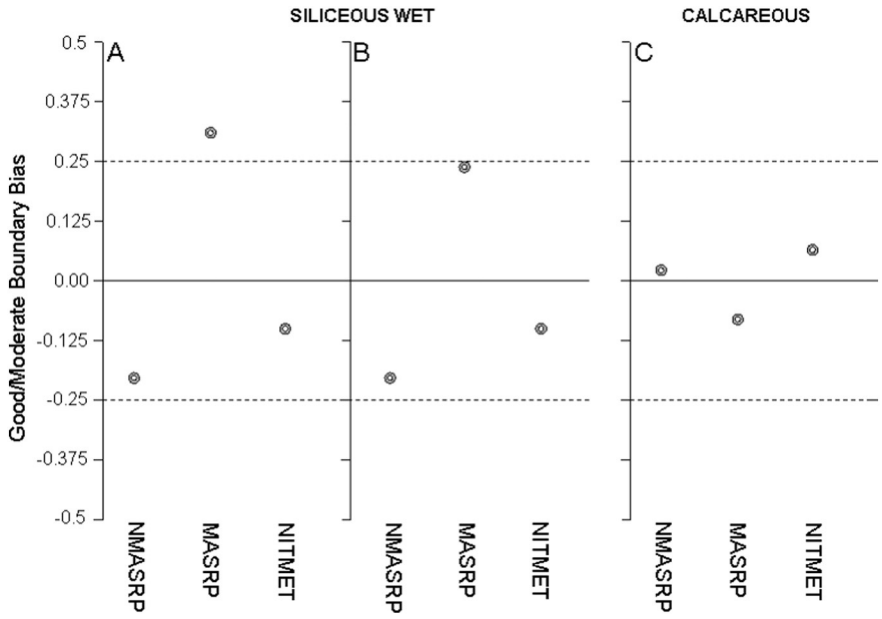

Fig. 5. Good/Moderate (GM) boundary bias for the intercalibrated methods and SW and CAL reservoirs, respectively. Bias is indicated by Class equivalent units of the GM boundary of each method to the global median when the GM Ecological Quality Ratio (EQR) boundary for each method at SW reservoirs lies at A) 0.6. The bias should be reduced to a minimum of 0.25 class equivalent units by moving the GM (EQR) boundaries. B) When the boundary for the GM (EQR) of the MASRP is moved to 0.583 boundary bias is contained within the required limits. C) Bias when the boundaries of the methods in CAL reservoirs are located at 0.6.

of water bodies (European Commission, 2000). In addition, the IC exercise required the establishment of a "Common IC typology" for each ecoregion, which was established by a European Working Group (European Commission, 2004). Any typological classification is, by definition, a simplification of the natural variation observed in water bodies, which can be revised according to new knowledge or new purposes. Accordingly, the types developed for the Mediterranean ecoregion were later modified by the L-M GIG, introducing climatic aspects to the initial classification (Poikane, 2009). Other IC groups have also proceeded by applying variations to the typology established by the ECOSTAT with the aim of improving the IC exercise outcome (Kelly et al., 2009). The results obtained in our typological analysis supported the additions made by the L-M GIG to the ECOSTAT typology. The division according to climatic variables of the siliceous reservoirs was biologically better supported than for calcareous reservoirs. The common IC types finally considered (CAL, SA and SW) were coincident with the national typologies in the four countries in which IC was possible. Unfortunately, the scarcity of data for SA reservoirs prevented a robust comparison of the assessment methods in this type.

The non-impacted site as a target of quality for all other sites is the basis on which the WFD operates. This was translated in the concept of EQR, where the actual quality of a site is always measured in comparison to its non-impacted counterpart. A variety of methods have been applied to establish reference conditions (Poikāne et al., 2010), including the use of historical and paleolimnological studies (Wolfram et al., 2009). In the case of the L-M GIG, the criteria for the classification of MEP reservoirs were common for all participating MSs. This common approach was particularly important in reservoirs, since in these heavily modified water bodies the concept of a "non-impacted site" was not applicable, and therefore, a common view on what can potentially be a MEP site was helpful. Even though the exclusive use of pressure criteria for selection of reference (or MEP) sites was recommended in some cases (see Poikāne et al. (2010)), so as not to fall in circular reasoning by using biological criteria, other documents recommended the

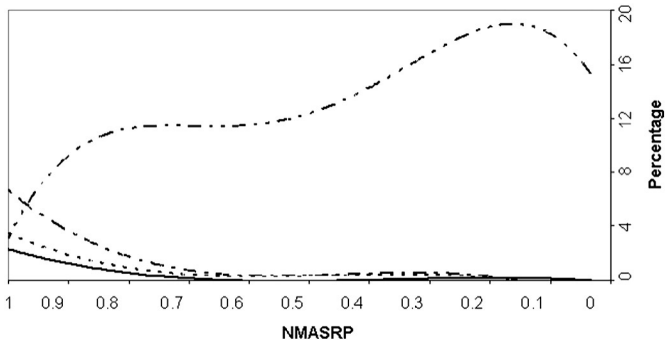

Fig. 6. Polynomial adjustments on scatter-plot betwen NMASRP and the mean percentage contribution of different taxonomic groups to the mean total biovolume per reservoir-year. Chrysophytes (_ _ _ _) including *Dinobryon*, *Pseudopedinella*, *Ochromonas* and *Chrysochromulina*; Cyanobacteria (_._) including *Aphanizomenon*, *Woronichinia* and *Anabaena*; Bacillariophytes (_._) including *Asterionella*, *Nitzschia*, *Discostella* and the species *Ulnaria ulna*; Chlorococcales (—) including *Ankyra*, *Sphaerocystis* and *Coenochloris* and the species *Crucigenia tetrapedia*.

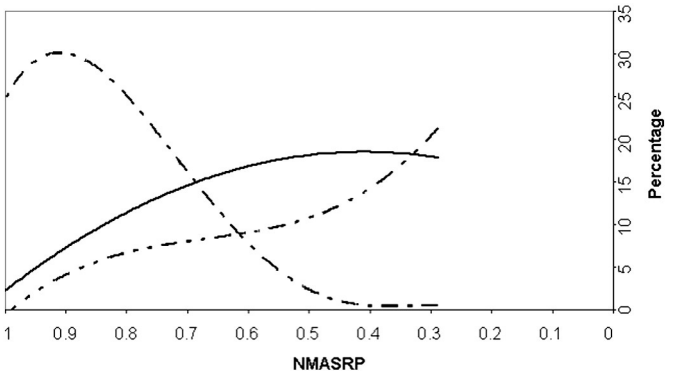

Fig. 7. Polynomial adjustments on scatter-plot between NMASRP and the mean percentage contribution of different groups to the mean total biovolume per reservoir year. Bacillariophytes (_._) including *Cyclotella* and *Achnanthes*, and the species *Ulnaria ulna* and *Ulnaria acus*; Chlorococcals (—) include *Coelastrum*, *Scenedesmus* and *Pediastrum*; Cyanobacteria (_._) include *Anabaena*, *Microcystis* and *Aphanizomenon* among others.

inclusion of this second type of criteria in the selection process (European Comission, 2011). This was the case for the L-M GIG, and it served as a safe-line when dealing with pressures that may have eluded the available pressure data set.

At the start of the IC process in 2004, official methodologies were not developed in any of the participating countries in the L-M GIG. The IC exercise has served as a catalyst for a cooperative development of common methods, analyses and sampling protocols for phytoplankton (e.g. sampling of the euphotic layer) (Poikane, 2009; de Hoyos et al., 2014). The quality boundaries established for most of the metrics by the MSs were agreed within the L-M GIG framework and the assessment methods adopted in the respective countries were developed cooperatively during the first and second IC phases (Poikane, 2009; de Hoyos et al., 2014). Consequently, the similarity of the assessment methods that were finally intercalibrated made the comparison step easier. This has not been the case in other GIGs, in which methods were well established in some MSs at the start of the IC process, and not always based on similar principles (Birk et al., 2012; Poikane et al., 2014). In the L-M GIG, the only differences found between the intercalibrated assessment methods were in the composition metrics. Italy used the Med PTI (Marchetto et al., 2009) instead of the IGA (Catalan and Ventura, 2003), which is used in the other three countries. Spain used percentage of cyanobacteria biovolume instead of cyanobacteria biovolume, which was used in the other three countries (de Hoyos et al., 2014).

The component metrics of the intercalibrated methods of the L-M GIG were divided in two groups: biomass and composition, as required by the WFD (European Commission, 2000). Biomass parameters were chl-a and BV. BV was widely used throughout phytoplankton assessment systems in Europe, and chl-a was used in every MS (Poikane et al., 2015). Composition parameters could also be divided into two groups: those based solely on the appearance of cyanobacteria (which are considered as bloom sensitive metrics) (Mischke et al., 2012), which are applied in many European phytoplankton assessment methods (Poikane et al., 2015), and those considering a broader range of taxa within the phytoplanktonic community. This last group includes the Med PTI, which is based on genera and species (Marchetto et al., 2009) and the IGA, which is based on broader taxonomic groups and cell organization (Catalan and Ventura, 2003). Most composition indexes used in European MSs are based on TP optima of the species or genus (Poikane et al., 2015). Composition indexes that use coarser taxonomic groups can be less sensitive to differences in ecological preferences than indexes based on species, but they are more robust to identification mistakes and, therefore, results are more comparable among laboratories and agencies. This issue has been discussed in other publications (Kelly et al., 2014).

The relationship of the intercalibrated national assessment methods with TP (as a proxy for pressure) was significant and high in all three cases (Pearson correlation coefficient ranging from −0.39 to −0.7). The range of correlation was similar to those found in the assessment comparisons of other MSs (Phillips et al., 2014; Kelly et al., 2014).

The obtained results showed that all the assessment methods performed very similarly in all the considered reservoir types. According to the IC methodology, the GM boundaries did not require an adjustment. The only case of a slight shift in the GM threshold is for the MASRP in SW reservoirs, where it seemed to perform slightly more strictly than the other two. All the assessment methods that were not intercalibrated (see Section 2.4) will have to be subject to this process as soon as the methods are finalized, in order to ensure the comparability of the GM boundary. The remaining GIGs have produced results for phytoplankton assessment methods. In the case of the Alpine GIG, where many of the component metrics of the methods were agreed upon during developmental stages (Wolfram et al., 2009), the intercalibrated methods showed acceptable bias in both the GM and the High/Good boundaries in all countries (Wolfram et al., 2014). The Northern GIG, similarly, shows acceptable levels of bias at the key boundaries, after some adjustments to the combination rules of the methods of several countries (Lyche-Solheim et al., 2014; Poikane et al., 2015). In the case of the Central-Baltic GIG, and after the accommodation of boundary biases within the required thresholds, all countries except two were able to achieve comparable boundaries (Phillips et al., 2014).

Describing the typical phytoplanktonic communities at MEP sites and their shift along a trophic gradient (and around the GM threshold) offered criteria for management purposes. This became especially relevant seeing the generalized tendency of cyanobacteria taxa to increase across the trophic gradient in all types of water bodies (Willén, 2000; Lyche-Solheim et al., 2008; Carvalho et al., 2013; Järvinen et al., 2012 & Poikane et al., 2014), since they pose a risk to human activities and health (Carvalho et al., 2013; WHO., 2003; WHO, 2006; Codd et al., 2005). Several of the cyanobacteria genera observed to gain importance towards and beyond the GM boundary in Mediterranean reservoirs are known as toxin producers, such as *Anabaena*, *Aphanizomenon* and *Microcystis* (Chorus and Bartram, 1999), and *Woronichinia* (Bober et al., 2011). The dominance in high ecological quality sites of chrysophytes and diatoms for SW reservoirs agreed with the reported dominance of these same taxonomic groups in northern European lowland siliceous lakes (Järvinen et al., 2012). The reduction of chrysophytes with increasing eutrophic conditions has also been observed in other water bodies (Willén, 2000; Lyche-Solheim et al., 2008). In CAL reservoirs, the main descriptors of Mediterranean water bodies were the diatom genera *Cyclotella* and *Achnanthes*. *Cyclotella* has been considered as an indicator for reference conditions in high alkalinity lowland central Baltic lakes (Järvinen et al., 2012). Moreover, the decline in *Cyclotella* towards lower quality classes was parallel to that observed in Alpine lakes (Wolfram et al., 2009).

The IC exercise of phytoplankton in reservoirs was particularly relevant due to the relative importance of this biological quality element in these water bodies. Due to hydrological factors, such as the marked level fluctuations intrinsic to reservoir management, other assessment methods based on phytobenthos, macrophytes or macroinvertebrates were scarcely applicable as opposed to natural lakes. On the other hand, if the focus was on hydromorphological pressures rather than on eutrophication, the use of other BQEs seems to overrun the indicator properties of phytoplanktonic communities, particularly in natural lakes.

5. Conclusions

In the reservoir phytoplankton L-M GIG experience, the selection of common IC types, the identification of MEP water bodies, the development of sampling protocols and the design of the assessment methods were tackled from a collaborative point of view, which has ultimately maximized the comparability of the results, and eased the IC process. The methods compared, belonging to four different countries, were extremely similar in their assessment results for the two IC types considered and only a slight strictness of the MASRP GM boundary compared to the NMASRP's and NITMET's was observed in SW reservoirs. The successfully intercalibrated methods addressed in this paper are, additionally, tracking steep community gradients of pressure sensitive and potentially hazardous phytoplanktonic communities through the application of the GM boundary.

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Environment Agency (former Portuguese Water Institute) (Portugal), Aristotle University of Thessaloniki (Greece), the Ministerul Mediului şi Pădurilor (MMP) and the National Administration "Apele Române" (Romania) and the Rhône Méditerranée Corse Water Agency (France). Additionally, the close collaboration and feedback provided by Vasiliki Tsiaoussi and Otilia Mihail were essential for the intercalibration process.

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