Water Framework Directive Intercalibration Technical Report: Mediterranean Lake Phytoplankton ecological assessment methods

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Introduction

The European Water Framework Directive (WFD) requires the national classifications of good ecological status to be harmonised through an intercalibration exercise. In this exercise, significant differences in status classification among Member States are harmonized by comparing and, if necessary, adjusting the good status boundaries of the national assessment methods.

Intercalibration is performed for rivers, lakes, coastal and transitional waters, focusing on selected types of water bodies (intercalibration types), anthropogenic pressures and Biological Quality Elements. Intercalibration exercises were carried out in Geographical Intercalibration Groups - larger geographical units including Member States with similar water body types - and followed the procedure described in the WFD Common Implementation Strategy Guidance document on the intercalibration process (European Commission, 2011).

In a first phase, the intercalibration exercise started in 2003 and extended until 2008. The results from this exercise were agreed on by Member States and then published in a Commission Decision, consequently becoming legally binding (EC, 2008). A second intercalibration phase extended from 2009 to 2012, and the results from this exercise were agreed on by Member States and laid down in a new Commission Decision (EC, 2013) repealing the previous decision. Member States should apply the results of the intercalibration exercise to their national classification systems in order to set the boundaries between high and good status and between good and moderate status for all their national types.

Annex 1 to this Decision sets out the results of the intercalibration exercise for which intercalibration is successfully achieved, within the limits of what is technically feasible at this point in time. The Technical report on the Water Framework Directive intercalibration describes in detail how the intercalibration exercise has been carried out for the water categories and biological quality elements included in that Annex.

The Technical report is organized in volumes according to the water category (rivers, lakes, coastal and transitional waters), Biological Quality Element and Geographical Intercalibration group. This volume addresses the intercalibration of the Lake Mediterranean Phytoplankton ecological assessment methods.

Contents

Annexes

1. Introduction

In the Mediterranean Lake Phytoplankton Geographical Intercalibration Group (GIG):

- Initially, 6 Member States (Cyprus, France, Italy, Portugal, Romania and Spain) submitted 7 assessment methods (as Italy has one method for reservoir assessment and one – for lake assessment);
- After evaluation, only 4 countries participated in the intercalibration (CY, IT, PT and ES) because: (1) FR and RO withdrew their methods from the IC and (2) lake intercalibration was not feasible (as only 1 country - Italy - was left after FR withdrew their method);
- All methods address eutrophication pressure and follow a similar assessment principle (including biomass metrics and composition metrics);
- Intercalibration "Option 3" was used - direct comparison of assessment methods using a common dataset via application of all assessment methods to all data;
- The comparability analysis show that methods give a closely similar assessment (in agreement to comparability criteria defined in the IC Guidance), so only one slight boundary adjustment was needed (as ES boundary was too precautionary for LM5/7 type)
- The final results include EQRs of CY, IT, PT and ES lake phytoplankton assessment systems for 2 common intercalibration lake types: LM5/7 and LM8.

2. Description of national assessment methods

In the Mediterranean Phytoplankton GIG, initially six countries started the intercalibration with finalised phytoplankton assessment methods (Table 2.1, for detailed descriptions see Annex A).

Table 2.1 Overview of the national phytoplankton assessment methods

Member State	Method	Status of national method
Cyprus (Reservoirs)	New Mediterranean Assessment System for Reservoirs Phytoplankton (NMASRP)	Finalized and formally agreed
France (Lakes and reservoirs)	Lake phytoplankton index (IPLAC)	Intercalibratable finalized method
Italy (Reservoirs)	New Italian Method (NITMET)	Finalized and formally agreed
Italy (Lakes)	Italian Method for Lakes (ITMET)	Finalized and formally agreed
Portugal (Reservoirs)	Reservoirs Biological Quality Assessment Method – Phytoplankton (New Mediterranean Assessment System for Reservoirs Phytoplankton: NMASRP)	Finalized and formally agreed
Romania (Reservoirs)	Romanian Assessment System for Reservoirs-Phytoplankton	Finalized and formally agreed
Spain (Reservoirs).	Mediterranean Assessment System for Reservoirs Phytoplankton (MASRP).	Finalized and formally agreed

During the first round of the IC, boundaries for some metrics (chlorophyll-a, biovolume, percentage of cyanobacteria, IGA and MedPTI) were agreed in the Med GIG. With these metrics two methods were set up:

- MASRP used in Cyprus, Portugal and Spain (metrics - chlorophyll-a, biovolume, percentage of cyanobacteria and IGA);
- ITMET used in Italy (metrics - chlorophyll-a, biovolume, percentage of cyanobacteria and MedPTI).

In the second round of the IC a new MASRP (NMASRP) and a new ITMET (NITMET) have been developed (see Annex A for method description and Annex D for boundary setting procedure):

- NMASRP used in Cyprus and Portugal (metrics - chlorophyll-a, biovolume, biomass of cyanobacteria and IGA);
- NITMET used in Italy (metrics - chlorophyll-a, biovolume, biomass of cyanobacteria and MedPTI).

The main difference among the old and new methods is that in NMASRP and NITMET the metric percentage of cyanobacteria was replaced by biomass of cyanobacteria, bloom metric recommended in the WISER project (Deliverable D3.1-2: Report on phytoplankton bloom metrics).

Important:

- FR and RO withdrew their methods in the late stage of the IC;
- Because of this lake IC was not possible (as there was only 1 lake method - IT);
- In consequence, only CY, IT (reservoirs), PT and ES participated in the final intercalibration. Greece has not developed their method yet.

2.1. Methods and required BQE parameters

All MS have developed full BQE methods (see Table 2.2).

- Parameters concerning frequency and intensity of algal blooms are missing in methods of Spain, France, Italy (method for lakes) and Romania, although chlorophyll-a, biovolume and specially percentage of cyanobacteria may be sensitive to bloom situations (Annex C);
- Boundaries for the bloom metric developed in the WISER project and based on abundance (biovolume) of Cyanobacteria are included in NMASRP (Portugal and Cyprus) and NITMET (Italy for reservoirs), described in this Milestone (Annex C).

Some Mediterranean countries´ methods (France, Romania and Spain) lack a specific methodology to cover frequency and intensity of algal blooms. There is no clear definition of a bloom but if we agree that a bloom is a situation with high biomass and dominance of few species, it could be considered that the biomass metrics (chlorophyll-a and/or biovolume) and composition metrics (especially the percentage of cyanobacteria) could detect blooms in an indirect way. Annex C supports that metrics already incorporated in the assessment methods of these countries reflect cyanobacterial blooms.

Therefore all methods (also methods missing bloom metrics) are considered WFD compliant.

Table 2.2 Overview of the metrics included in the national phytoplankton assessment methods

MS	Full BQE	Biomass metrics	Taxonomic composition metrics	Bloom metrics	Combination rule of metrics
CY	Yes	Chl-a concentration Total biovolume	IGA index	Biovolume of Cyanobacteria	Arithmetic average of normalized EQRs
IT res	Yes	Chl-a concentration Total biovolume	MedPTI index	Biovolume of Cyanobacteria	Arithmetic average of normalized EQRs
IT lakes	Yes	Chl-a concentration Total biovolume	MedPTI index % Cyanobacteria	Metric not considered	Arithmetic average of normalized EQRs
FR	Yes	MBA biomass metric based on Chl-a	MCS specific composition metric	Metric not considered	Weighted average of the normalized EQR of the metrics
PT	Yes	Chl-a concentration Total biovolume	IGA index	Biovolume of Cyanobacteria	Arithmetic average of normalized EQRs
RO	Yes	Chl-a concentration Total biovolume	Total taxa number % Cyanobacteria from the total nº of cells Shannon-Wiener diversity index	Metric not considered	Weighted average of the metrics
ES	Yes	Chl-a concentration Total biovolume	% Cyanobacteria IGA index	Metric not considered	Arithmetic average of normalized EQRs

2.2. Sampling and data processing

Sampling procedure and the sample analysis methodologies are clearly specified by most of the countries and produce representative information about water body quality and ecological status in space and time

All countries use similar sampling strategies and data processing techniques:

- CYPRUS: Two samplings between June and September**,** integrated sample of the euphotic zone;
- FRANCE: 3 samplings between May and October, one sampling between February and March (not used in assessment), integrated sample of the euphotic zone;
- ITALY: 6 sampling per year: 4 samplings between April and October, one sampling at the end of the autumn and one between January and March (not used in assessment), integrated sample of the euphotic zone;
- PORTUGAL: 3 sampling during the growing season, 3 sampling during autumn, winter and spring (not used in assessment), integrated sample of the euphotic zone;
- ROMANIA: 4 samples from May to September, integrated sample of the euphotic zone;
- SPAIN: 2 sampling dates between June and September**,** integrated sample of the euphotic zone;

- All methods use Spectrometric determination for chlorophyll-a analysis and Utermöhl method for biovolume analysis.

2.3. National reference conditions

All countries have set national reference conditions based on near-natural reference sites in combination with other approaches (Table 2.3).

Table 2.3 Overview of the methodologies used to derive the reference conditions for the national phytoplankton assessment methods

MS	Methodology used to derive the reference conditions
CY	Reference Conditions were derived within MedGIG using "reference sites" from all MS.
IT	Reference conditions were derived from existing near-natural reference sites within the Italian dataset used for the implementation of MedPTI. For the rest of the metrics, Reference Conditions were derived within MedGIG using "reference sites" from all MS.
FR	For chl-a (MBA): RC are site specific, based on a mathematical model between chl-a and mean depth, For composition metric (MCS): RC are specific per macro lake type, based on ref sites (median value of MCS on ref site per macro lake type) or site specific (when not enough ref sites in macro lake type), based on a mathematical model (regression with TP)
PT	Reference Conditions were derived within MedGIG using "reference sites" from all MS.
RO	Existing near-natural reference sites. Least disturbed sites. Expert knowledge.
ES	Reference Conditions were derived within MedGIG using "reference sites" from all MS.

2.4. National boundary setting

Table 2.4 Overview of the methodologies used to derive the reference conditions for the national phytoplankton assessment methods

MS	Methodology used to set class boundaries
CY, PT	Two parallel approaches were applied, one based on the response of each metric throughout the trophic gradient and the other based on equidistant division of pressure gradient (Annex D)
IT	For MedPTI, GM boundary established using discontinuities in the relationship of anthropogenic pressure and the biological response (TP-MedPTI), other boundaries based on equidistant division of EQR gradient. For the rest of the metrics, two parallel approaches were applied, one based on the response of each metric throughout the trophic gradient and the other based on equidistant division of pressure gradient (Annex D)
RO	H/G boundary 75th percentile for %Cyanobacteria, no of taxa, biomass and chlorophyll-a and 25th percentile for diversity index of data from vegetation period and each type. G/M boundary 50th percentile for %Cyanobacteria, no of taxa, diversity index biomass and chlorophyll-a of data from vegetation period and each type
ES	GM Class boundary was set (1) the 95th percentile of the distribution of summer average values for Chlorophyll-a and Total biovolume, (2) the 90th percentile of the distribution of summer average values for Percentage of Cyanobacteria, IGA index. Boundary setting was validated by exploring the indexes response to the pressure gradient

2.5. Pressures-response relationships

In reservoirs, a test to study the relationship between the assessment methods and the pressures was carried out. There was a significant correlation between the methods and the eutrophication pressure measured as total phosphorus (Annex E, Table E.5).

Table 2.5 Relationships between MS assessment methods and eutrophication pressure (total phosphorus concentration)

Member State	Metrics tested	Siliceous wet	Calcareous *
CY	NMASRP	R 0.66 (P<<0.01)	R 0.39 (P<<0.01)
FR	IPLAC	R 0.65 (P<<0.001)	R 0.19 (P =0.064)
IT	NITMET	R 0.70 (P<<0.01)	R 0.42 (P<<0.01)
IT	ITMET	R 0.69 (P<<0.001)	R 0.45 (P<<0.01)
PT	NMASRP	R 0.66 (P<<0.01)	R 0.39 (P<<0.01)
RO	ROMET	R 0.29 (P =0.03)	R 0.41 (P <<0.001)
ES	MASRP	R 0.57 (P<<0.001)	R 0.46 (P<<0.01)

* Jucar RBD abd Guadalquivir RBD are excluded form anbalysis (see Annex E)

In lakes, the correlation between assessment methods and pressures was also studied (Annex E), but no significant correlation was revealed with total phosphorus (only with % of artificial land use in lake catchment).

3. Results of WFD compliance checking

All MS methods are considered WFD compliant (Table 3.1.) with some considerations for Romanian method (see explanations below). FR and RO withdrew their methods in the late stage of the IC.

All the national assessment methods participating in the intercalibration are compliant with the WFD requirements. Although there are some doubts about the Romanian method due to two main reasons:

- The boundary setting was done based on statistical division (G/M boundary 50th percentile of all data), not identifying ecological changes of BQE to the pressure, as required in the Intercalibration Guidance;
- The correlations between some of the metrics and the pressure are opposite to the expected: Diversity index and Total number of taxa are expected to decrease as the pressure increases, as is indicated in the boundaries of the metrics, but they have a positive correlation with NH4 (see Annex C.2).

In a very advanced state of the intercalibration process, FR and RO decided not to participate in it for different reasons:

- The position of Romania about its method is that it is compatible with the WFD requirements but not comparable with other methods in the GIG (see Annex B);
- France decided to revise its method (IPLAC) and not participate in the final intercalibration process. It will re-enter at a later stage with finalised method.

Table 3.1 List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	CY, PT: Only above good" and "below good" ecological potential, as the ecological potential for HMWB should be expressed as above/below this boundary. FR, IT, ES: Ecological status is classified by one of five classes. ROMANIA: Ecological potential is classified in 3 classes (High Ecological Potential, Good and Moderate).
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	See Table and text above (National boundary setting)
3. **All relevant parameters** indicative of the biological quality element are covered (see Table 1 in the IC Guidance).	See Table and text above (Methods and required BQE parameters)
4. Assessment is adapted to intercalibration common types	All countries: yes
5. The water body is assessed against type-specific near-natural reference conditions	See Table and text above (National reference condition setting)
6. Assessment results are expressed as **EQRs**	All countries: yes
7. Sampling procedure allows for **representative** information about ecological status **in space and time**	See Table and text above (Sampling and data processing)
8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	All data covered in all the countries. It is not clear if the actual metrics under consideration cover bloom situations.
9. Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes, the WISER list and REBECCA codes are used in all countries (Cyprus, France, Italy, Portugal, Spain, Romania and Greece).
10. Other criteria	Methods for chlorophyll-a analysis, cell counting and biovolume calculation (see the list below)

Due to these position, the Romanian method and French method (together with the reservoirs submitted) were excluded from the results of the comparisons although these countries (methods and reservoirs) are included in the rest of the analysis.

Therefore, for reservoirs, only 3 methods (4 countries) were intercalibrated:

- Mediterranean Assessment System for Reservoirs Phytoplankton (MASRP) used in Spain;
- New Mediterranean Assessment System for Reservoirs Phytoplankton (NMASRP) – Portugal and Cyprus;
- Italian assessment method for reservoirs (NITMET).

For lakes, only two methods were initially ready for intercalibration: the Italian assessment method for Mediterranean lakes and the Lake phytoplankton index (IPLAC) from FR. The data were very scarce (see Chapter 5). Since France left the IC, there are not IC results for lakes in the Lake Mediterranean GIG.

4. Results IC Feasibility checking

There were 5 initial types of the second round of IC (see table below).

Table 4.1 Description of initial common intercalibration water body types and the MS sharing each type

Common IC type	Type characteristics	MS sharing IC common type
Reservoirs Siliceous Wet	Reservoirs deep (> 15 m depth), large (> 0,5 km^2), with catchment area < 20.000 km2, siliceous (< 1 meq/l), situated in “wet areas” (annual mean temperature below 15 ºC and annual precipitation higher than 800 mm, where only one of two criterion fulfilled, national typology considered)	GR, FR, IT, PT, RO, ES : Yes. CY: No.
Reservoirs Siliceous Arid	Reservoirs deep (> 15 m depth), large (> 0,5 km^2), with catchment area < 20.000 km2, siliceous (< 1 meq/l), and situated in “arid areas” (annual mean temperature above 15 ºC and annual precipitation lower than 800 mm, where only one of two criterion fulfilled, national typology considered)	IT, Pt, RO, ES: Yes. CY, GR, FR: No.
Reservoirs Calcareous Wet	Reservoirs deep (> 15 m depth), large (> 0,5 km^2), with catchment area smaller than 20.000 km2, calcareous (> 1 meq/l) and situated in “wet areas” (annual mean temperature below 15 ºC and annual precipitation higher than 800 mm, where only one of two criterion fulfilled, national typology considered)	FR, IT, RO, ES: Yes. CY, GR, PT: No.
Reservoirs Calcareous Arid	Reservoirs deep (> 15 m depth), large (> 0,5 km^2), with catchment area < 20.000 km2, calcareous (> 1 meq/l) and situated in “arid areas” (annual mean temperature above 15 ºC and annual precipitation lower than 800 mm, where only one of two criterion fulfilled, national typology considered)	CY, ES: Yes. GR, FR, IT, PT, RO: No.
Calcareous lakes	Shallow lakes (mean depth 3 – 15 m) or deep (> 15 m), calcareous (> 1 meq/l) at altitude from 0 to 1000 m .	GR, FR, IT, ES: Yes. CY, PT, RO: No.

Lately, in the boundary setting procedure for the metrics of the NMASRP (Annex D) and in the comparison of the boundaries of this method with the rest of the Mediterranean methods (Chapter 7), only 2 types (calcareous and siliceous wet) were considered, although it is recommended to maintain the four types in the national typologies.

Correspondence between the IC typology of reservoirs and national typologies can be problematic:

- Cyprus, Italy, Romania and Spain have considered (as the IC typology does) geology (siliceous and calcareous) as a descriptor in their typologies;
- Portugal and Spain have also considered the climate (wet and arid);
- In the rest of the countries there are cases in which two IC types correspond to the same national types (in Italy, siliceous wet and siliceous arid correspond to the national type ME5);
- The French typology doesn’t follow system A, so one IC type corresponds to different reservoirs or lakes from several French types or similarly, one French type corresponds to different reservoirs or lakes from several IC types.

Table 4.2 Description of final common intercalibration water body types and the MS sharing each type

Type	Lake characterization	Altitude (m)	Annual mean precipitation (mm) and T (ºC)	Mean depth (m)	Area (km^2)	Catchment (km^2)	Alkalinity (meq/l)
L-M5/7	Reservoirs, deep, large, siliceous, "wet" areas	<1000	>800 and/or<15	>15	0.5-50	< 20 000	<1
L-M8	Reservoirs, deep, large, calcareous	<1000	-	>15	0.5-50	< 20 000	>1

Type L-M5/7: Greece, France, Italy, Portugal, Romania, Spain

Type L-M8: Cyprus, France, Italy, Romania, Spain

In some cases, although the IC typology and national typology are based on the same descriptors, the type of a single reservoir is different in both classifications because the country considers different parameters than those considered in IC and, as a result, there are reservoirs classified in different groups in both typologies. Examples:

- The intercalibration typology separates wet and arid reservoirs based on precipitation and temperature and in the Spanish typology the difference between these two groups is based on evapotranspiration;
- The intercalibration typology separates calcareous and siliceous reservoirs based on alkalinity and in the Romanian typology the difference between these two groups is based on basin geology.

As a general conclusion, the IC typology fits well with the national typologies:

- **Very well** in the case of Cyprus and Portugal;
- **Quite well** in the case of Italy, Spain and Romania;
- **Not very well** in the case of France;
- In the case of Greece, national typologies are currently under revision during the development of river basin management plans.

4.1. Pressures

Intercalibration is feasible in terms of pressures addressed by the methods as all methods address eutrophication. Nevertheless, in case of Romanian method, relation of two metrics (Diversity index based on number of cells and Number of taxa) to eutrophication is not clear (Annex B).

4.2. Assessment concept

Intercalibration feasible in terms of assessment concepts as all parameters considered in the national assessment methods focus on:

- Phytoplankton community of the pelagic zone of the lake or reservoir;
- Always based in the same part of the water column: 2.5* Secchi depth;
- Except in Italy, all the assessment methods are based on the phytoplankton of the growing season. An analysis of the difference between the Italian method

when calculated with only summer samples and with both winter and summer samples was made (Annex F).

Although the criteria above are considered in the national assessment methods, sometimes historical data is received from the countries. This data does not always fit these criteria (especially regarding the water column and the season covered).

5. Collection of IC dataset

Huge dataset was collected within the Mediterranean Phytoplankton GIG (Table 5.1 and Table 5.2).

Table 5.1 Overview of the Mediterranean GIG phytoplankton IC dataset

Member State	Number of sites or samples or data values		
	Biological data	Physico- chemical data	Pressure data
	Number of sites/site years	Number of sites/site years	Number of sites/data values
Reservoirs			
Cyprus	7/ 19	7/ 19	7/ 7
France	6/7	6/7	6/6
Greece	1/2	1/2	1/1
Italy	15/29	15/29	15/14
Portugal	18/20	18/20	18/18
Rumania	10/30	10/30	10/10
Spain	122/258	122/258	122/118
L-M GIG	**179/365**	**179/365**	**179/174**
Lakes			
France	2/2	2/2	2/2 TP; 2/2 land use
Greece	1/1	1/1	1/1 land use (not TP)
Italy	4/8	4/8	4/4 TP; 4/4 land use
Spain	34/106	34/110	34/33 land use; 34/29 TP
L-M GIG	**41/117**	**41/117**	**41/41**

Table 5.2 Data acceptance criteria used for the data quality control

Data acceptance criteria	Data acceptance checking
Data requirements (compulsory and optional) Reservoir characteristics. Physico-chemical parameters. Biological parameters. Pressures.	Portugal: Biovolume data in 3 reservoirs of a total of 18. Italy: In some reservoirs aggregated date (mean TP and biovolume) was given. Rest of the countries: All data required.
The sampling and analytical methodology Sampling: Accepted one or more samples	Sometimes historical data is filled by the countries and, therefore, does not always fit the criteria considered in the national assessment methods; E.g: • Part of Romanian phytoplankton biovolume data was not analyzed with Utermöhl method.

Data acceptance criteria	Data acceptance checking
taken in the euphotic zone. At least one sample during the growing season required. Analysis: Spectrophotometric measurement of Chlorophyll-a required Utermöhl method required for biovolume calculation.	• Part of the Spanish data set comes from reservoirs sampled just once during the growing season and, in some cases, samples have been taken at different depths. • Part of the Spanish chlorophyll-a data was obtained with a fluoroprobe. • Most of Portuguese data comes from sub-superficial sampling.
Level of taxonomic precision required and taxa lists with codes	Taxa are identified at finest level possible, species, species-group or genus. A Mediterranean taxa list, made in agreement with the WISER project, with an extra synonyms list and with REBECCA codes was used for revising and homogenizing phytoplankton data from all the countries.
The minimum number of sites / samples per intercalibration type	25 lakes per type suggested as minimum. 2 samples/site.
Sufficient covering of all relevant quality classes per type	Preliminarly, there are enough water bodies in each type (calcareous arid reservoirs: 51, calcareous wet reservoirs: 35, siliceous arid reservoirs: 28, siliceous wet reservoirs: 65 and calcareous deep lakes: 36). All relevant quality classes are covered in all types, but there are few high impacted sites
Other aspects where applicable	Data Screening

6. Common benchmarking

Common benchmarking was based on reference sites (sites in near-natural conditions), selected using common reference criteria.

Reference criteria for screening of sites in near-natural conditions in summary (see mdore details in Annex G):

- The first step taken for deriving maximum ecological potential water bodies was to do a pre-screening according to several different geographical **land use** variables. The other pressures considered are **overall population density in the catchment and total phosphorus.**
- Two sets of thresholds (Table G.1 in Annex G) are established in order to designate maximum ecological potential reservoirs.
  - The first is a **rejection threshold**, where if the reservoir fails to pass on a single pressure variable it won't be considered reference, and the second is a reference (MEP) threshold.
  - A maximum of two pressure variables can be over the **reference threshold**, but if three are over such limit, the reservoir fails to be included in the reference group.
- **Three secondary pressures** were considered: "Recreation activities", "Exploitation of fish population by fishery" and "Presence-absence of *Dreissena polymorpha* (zebra mussel)". The first two pressures were classified according to a discrete system with four classes: None, Low, Medium and Strong. None of these two pressures can be classified as strong in the MEP reservoirs. *Dreissena polymorpha* presence/absence is an exclusion criterion, since its effects on phytoplankton are well documented.

The same approach was followed for lakes and reservoirs.

Selection of Reservoir MEP (Maximum Ecological Potential) sites:

Following the IC Guidance, common reference criteria was applied to the data :

- In the first step, reservoirs which fulfilled these criteria were chosen ;
- Second, reservoirs with outlier biological values were eliminated (Annex G).

The reservoirs chosen as MEP according to these GIG´s criteria are listed in Annex G. All of them match up with the national ecological classifications.

In figure below the boxplots show the differences in chlorophyll-a and biovolume between reference (MEP) and non-reference reservoirs (non-MEP).

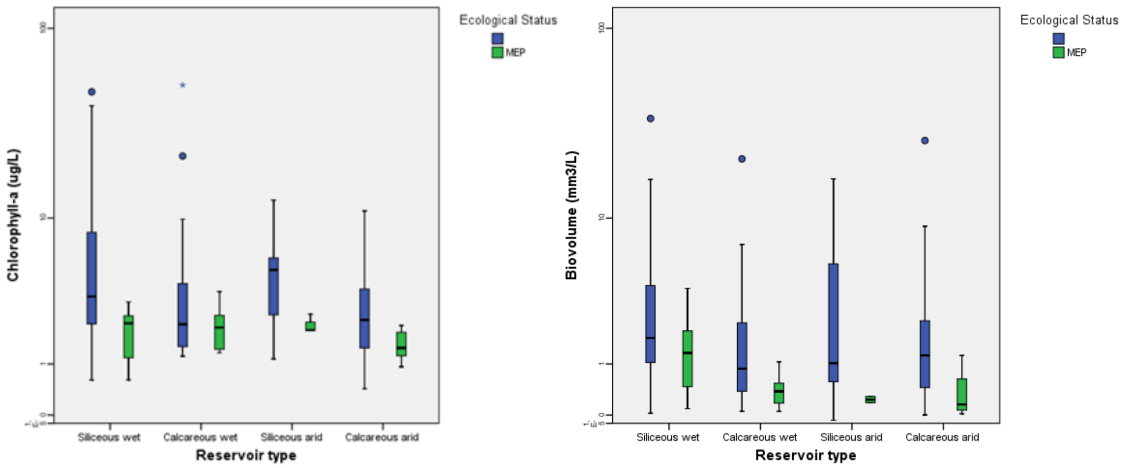


Figure 6.1Box-plot diagram presenting Chlorophyll-a and biovolume per reservoir type, separating MEP reservoirs colored in green and all reservoirs together colored in blue.

Screening of the biological data:

In reservoirs, four metrics (chlorophyll-a, biovolume, IGA and percentage of cyanobacteria) were used for the screening of the biological data:

- If two or more of samples of each reservoir had any of these 4 metrics above the G/M boundaries established in the first phase, the reservoir is not considered as MEP;
- The group that passed this first filter was subjected to another filter. Those reservoirs whose median value for any of these metrics (chlorophyll-a, biovolume, IGA or percentage of cyanobacteria) was above the third quartile of the set selected with the first filter were marked as doubtful (A1);

The rest of the reservoirs were marked as MEP:

- Those reservoirs proposed as MEP by the MS that do not fit the pressure criteria but have good biological values were classified as doubtful (A2), and the pressures were checked;
- After a last checking comparing the distribution of both chlorophyll-a and biovolume among the MEP sites taken for sure and those doubtful (Annex G) it

was decided that the three categories will be considered as MEP for calculation issues during the rest of the intercallibration process.

Reference sites: RESERVOIRS.

In total, 47 reservoirs were selected as MEP reservoirs: CY - 2; ES - 35; FR - 0; GR - 1; IT - 0; PT - 6; RO - 3.

Not enough MEP sites for each Member State in each common IC type are available, taking into account that the total number of reservoirs in some MS is very low (1 in Greece or 6 in France), but **there are enough MEP reservoirs among all the countries for intercalibration purposes:** calcareous arid type - 9 reservoirs, calcareous wet - 12, siliceous arid - 7, siliceous wet - 19.

Reference sites : LAKES.

Only Spain has reference lakes (in total 6) in the database. The countries with its assessment method finished (FR and IT) had no reference lakes in the database. It was not possible to follow the option pointed out in the IC Guidance when there are not enough reference sites (define alternative benchmarks) because the total number of lakes in the database from FR is 2 and from Italy is 4.

Benchmark standardisation

The 2nd step in the comparability analysis (Intercalibration Guidance. Annex V) is benchmark standardization, for correction of any sub-typological differences than can cause incomparability.

This step is not needed when the EQR values of a given national method for the benchmark sites does not differ between these subtypes (countries) of the two reservoirs types intercalibrated (siliceous wet reservoir and calcareous), according to the Students´s T test and the Generalized Linear Model (GLM) (Annex H).

7. Comparison of methods and boundaries

IC option and common metrics

It was decided use Option 3 (direct comparison of all assessment methods) for the intercalibration of the Mediterranean reservoir assessment methods. There was only one obstacle to apply Intercalibration Option 3:

- IT method`s sampling period is different than the rest of the Mediterranean countries (see Chapter 2 and Annex A). If the assessment method of one country can't be applied to the data of the other countries Option 2 should be used (Intercalibration Guidance);
- It was concluded that there were no important differences between the Italian method when considering samples of the whole year (sampling frequency in the Italian method) and only summer samples (sampling frequency in the rest of the Mediterranean countries methods) (Annex F). Therefore it was decided to apply the Italian method just to summer samples and use Option 3 for the intercalibration of the Mediterranean assessment methods.

As explained in Chapter 3, in a very advanced state of the intercalibration process, FR and RO decide not to participate in it for different reasons, therefore, following option 3 these methods were compared:

- Mediterranean Assessment system for Reservoirs Phytoplankton (MASRP) of Spain;
- the New Mediterranean Assessment System for Reservoirs Phytoplankton (NMASRP) of Cyprus and Portugal;
- Italian method (NITMET).

In reservoirs, comparisons were made using IC spreadsheets created by S. Birk et al (2012). These spreadsheets use the mean from all PCMs when calculating the average high-good and good-mod to obtain the "harmonisation guideline"against which the national boundaries are compared.

For lakes, only two methods, from IT and FR were initially intercalibrated, as the other countries with Mediterranean lakes (GR and ES) have not finalized their assessment methods. Since France left the IC process, the IC was not possible.

A pseudo-common metric (formed from the average of two countries against the national EQR of the remaining country) was used. All methods have significant correlations with common metrics (Table 7.1, Figure 7.1and Figure 7.2)

Table 7.1 Regression characteristics (National EQRs vs. pseudo-common metrics PCM)

Member State/Method	Siliceous Wet			Calcareous		
	r	p	slope	r	p	slope
Cyprus (NMASRP)				0.983	<0.01	0.88
Italy (NITMET)	0.970	<0.01	0.98	0.941	<0.01	1.02
Portugal (NMASRP)	0.978	<0.01	1.01			
Spain (MASRP)	0.969	<0.01	0.91	0.959	<0.01	0.96

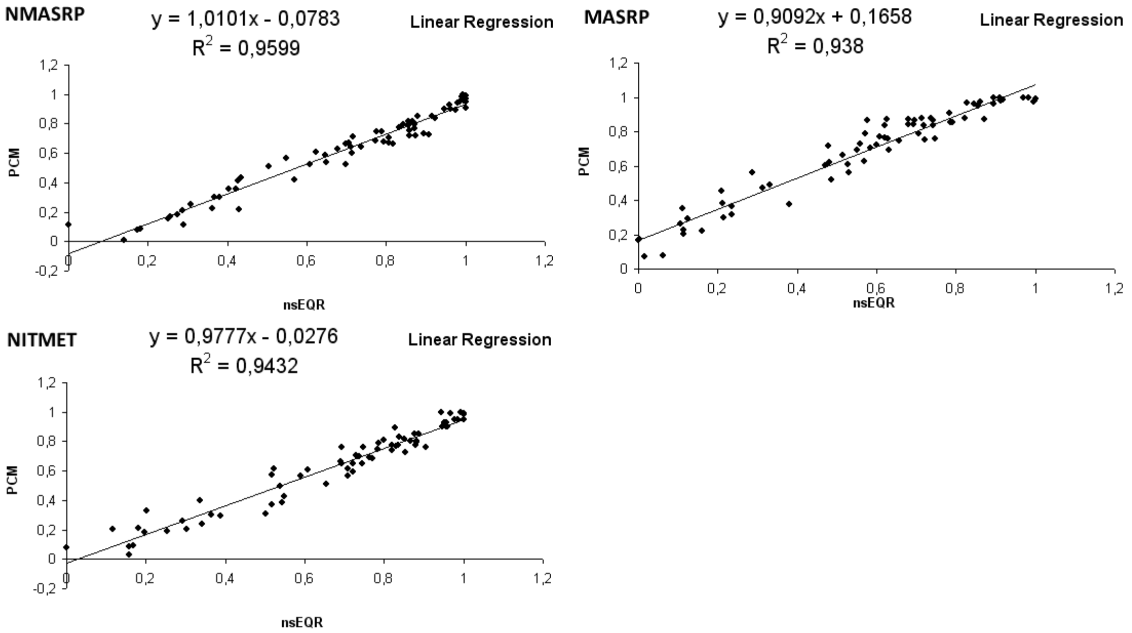


Figure 7.1. Linear least squares regression between the three methods and the pseudo-common metric (PCM) on siliceous wet reservoirs (NMASRP – Cyprus and Portugal, MASRP – Spain, NITMET – Italy reservoirs)

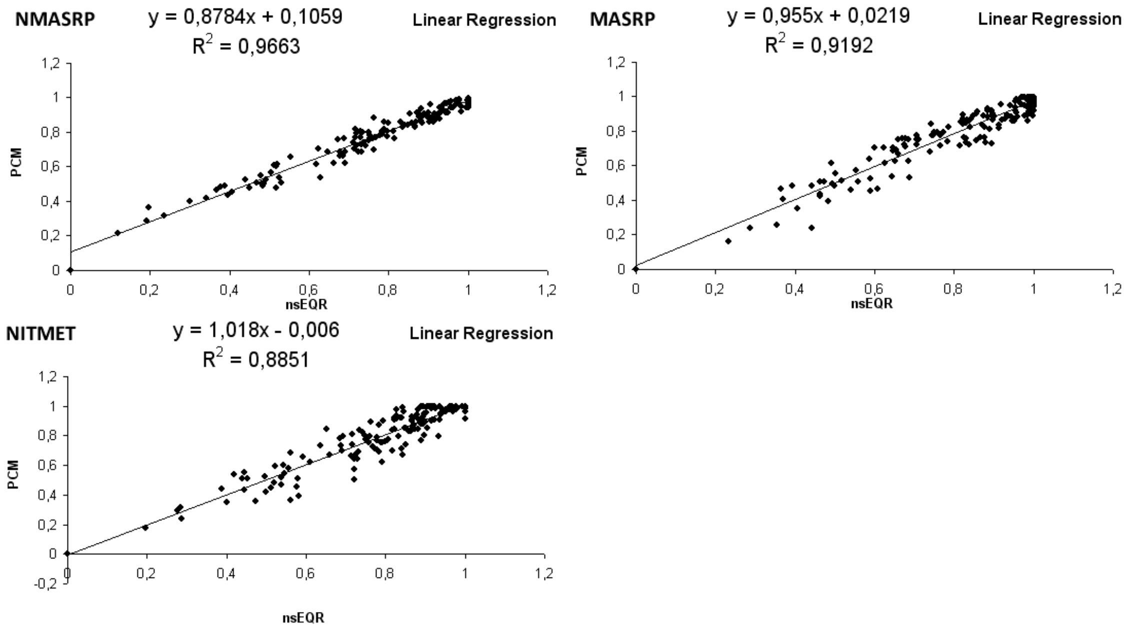


Figure 7.2. Linear least squares regression between the three methods and the pseudo-common metric (PCM) on calcareous reservoirs (NMASRP – Cyprus and Portugal, MASRP – Spain, NITMET – Italy reservoirs)

The following criteria were fulfilled in the relationships of each method with the pseudo-common metric both intercalibration types:

- The slopes were between 0.5 to 1.5;
- Correlations were significant, the Pearson correlation coefficient > 0.5;
- In the regression model, the minimum $R^2 \geq$ maximum R^2.

Next, IC comparability criteria were calculated and compared to the following criteria (Table 7.2.)

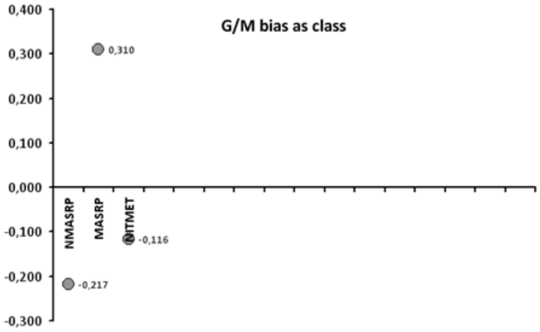
- The G/M bias should be lower than ±0.25;
- Furthermore, the absolute class difference has to be smaller than 1.

Table 7.2 Overview of the IC comparability criteria of Mediterranean phytoplankton method comparison

Method/Type	G/M boundary bias		Absolute Class Difference	
	Siliceous „wet“	Calcareous	Siliceous „wet“	Calcareous
Cyprus (NMASRP)	-0.22	0.02	0.18	0.09
Italy (NITMET)	-0.12	0.07	0.17	0.14
Portugal (NMASRP)	-0.22	0.02	0.18	0.09
Spain (MASRP)	0.31/0.246*	-0.09	0.24	0.11

For siliceous “wet” reservoirs the MASRP method (Spain) was slightly too strict in the G/M boundary, and had to adjust class boundary to reduce bias to 0.25 class equivalent units (Figure 7.3).

a) Before boundary adjustment



b) After boundary adjustment

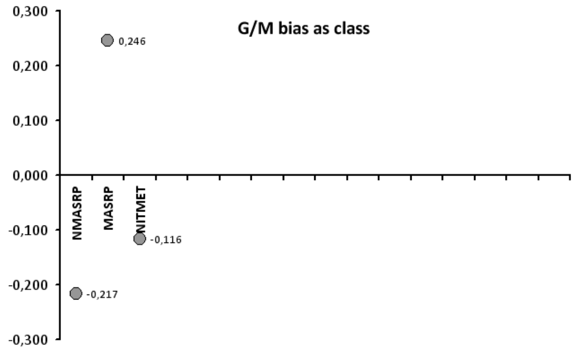


Figure 7.3G/M boundary bias between methods in siliceous wet reservoirs. The points mark the deviation in class equivalent units of the G/M boundary of each method to the global median: a) Before boundary adjustment b) After boundary adjustment.

For calcareous reservoirs all boundary biases are <0.25 class equivalent units, so no boundary adjustment is needed (Figure 7.4).

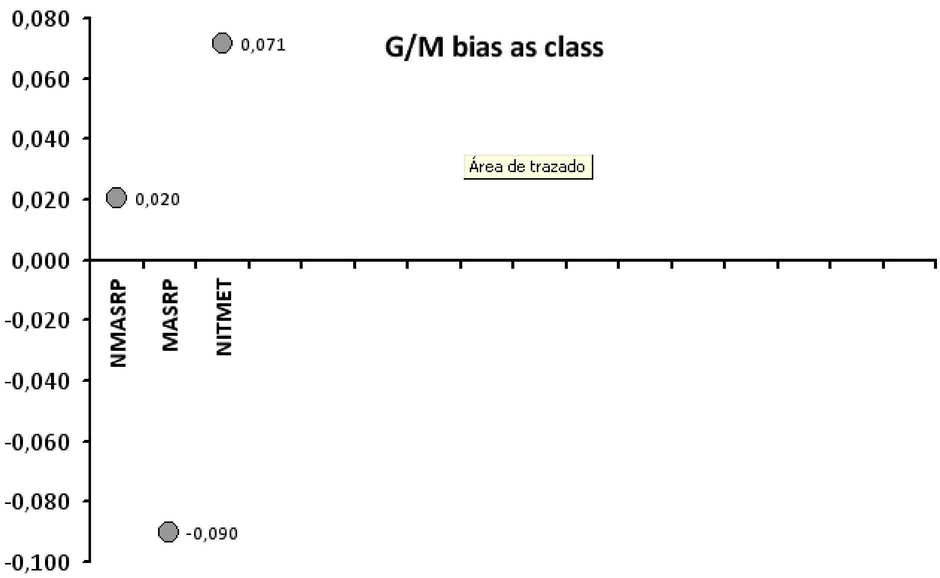


Figure 7.4. Calcareous reservoirs G/M boundary bias between methods. The points mark the deviation in class equivalent units of the G/M boundary of each method to the global median

IC results

After the comparison of the methods these are the resulting G/M boundaries for each method in each type:

Table 7.3 Overview of the IC results: EQRs of the Alpine GIG phytoplankton assessment methods

Member State	Classification	Ecological Quality Ratios	
	Method	G/M boundary before harmonization	G/M boundary after harmonization
		Siliceous "Wet" reservoirs	
ES	MASRP	0.60	0.58
PT	NMASRP	0.60	0.60
IT	NITMET	0.60	0.60
		Calcareous reservoirs	
ES	MASRP	0.60	0.60
CY	NMASRP	0.60	0.60
IT	NITMET	0.60	0.60

8. Description of biological communities

8.1. Description the biological communities at reference sites

Siliceous wet reservoirs

The MEP community type is composed, mainly of chrysophytes together with 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 are typical from MEP sites. Some species such as Crucigenia tetrapedia, Monoraphidium minutum from the Chlorococcales group and Ulnaria ulna from the diatoms group are also representative of good quality sites (Annex I).

Calcareous reservoirs

The MEP community type is composed, mainly of diatoms. The genres Cyclotella and Achnanthes, together with species as Ulnaria acus and Ulnaria ulna are typical from communities at MEP sites (Annex I).

8.1.1. Description of biological communities representing the "borderline" conditions between good and moderate ecological status

Siliceous wet reservoirs

The genera *Dinobryon*, *Pseudopedinella* and *Ochromonas*, from the chrysophytes, *Ankyra*, *Sphaerocystis* and *Coenochloris* from the Chlorococcales and *Asterionella*, *Nitzschia* and *Discostella* from the diatoms are typical from above G/M communities, and peak at MEP sites. Some species such as *Crucigenia tetrapedia*, *Monoraphidium minutum* from the Chlorococcales group and *Ulnaria ulna* from the diatoms group are also representative of good quality sites. They steadily decrease towards the G/M status, and almost disappear at that point. In parallel to this change in the community, cyanobacterial presence, which starts at low values, increase greatly in the community at approximately the same point where the other groups tend to disappear (the G/M boundary). The genus *Anabaena*, *Woronichinia* and *Aphanizomenon* are the main representatives of this change in the community (Annex I).

Calcareous reservoirs

There is a clear relation of certain phytoplanktonic groups and taxa with the change of classes from good to moderate in Calcareous reservoirs. An important part of the community is composed, when quality is still high, near the MEP community type, of diatoms. The genera *Cyclotella and Achnanthes*, together with species as *Ulnaria acus* and *Ulnaria ulna* are typical from high quality communities, and peak at MEP sites. They steadily decrease towards the G/M status, and below this limit, in parallel to the increase in cyanobacterial (*Anabaena*, *Microcystis*, *Aphanizomenon*) and chlorococcales (*Coelastrum*, *Scenedesmus* and *Pediastrum*) (Annex I).

Annexes

A. Lake phytoplankton method descriptions (included in the current IC results)

A.1 MASRP (Mediterranean Assessment System for Reservoir's Phytoplankton).

General information

This method is exclusively applied in Spain and it is composed of 4 parameters that are aggregated in a multimetric index where all of them have equal weights, and divided according to the parameters being related to biomass or composition. These parameters are the following:

Biomass	Chlorophyll-a (µg/L)
	Biovolume (mm^3/L)
Composition	IGA (Index Des Grups Algals)
	Percentage cyanobacteria (%)

Sampling

Sampling frequency

Water bodies to which the MASRP applies must be sampled twice a year between June and September (2 summer samples).

Sampling method and sample analysis

Samples have to be integrated samples from the euphotic zone, defined as 2.5* Secchi depth observed.

Biovolume and composition:

1. EN15204. 2006. Water quality – Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique).
2. CEN TC 230/WG 2/TG 3: 'Phytoplankton biovolume determination using inverted microscopy Utermöhl technique' (draft).

The Spanish protocol for chlorophyll-a, cell counting and biovolume calculation is under development.

Parameter description and calculation

IGA index

The IGA index is based on the coloniality of superior taxonomic groups and their trophic preferences, and it is applied according to the following equation, considering the percentage of each group in the sample. Only those samples where the represented groups add up to 70% or more of the sample will the index be suitable for application.

$$IGA = \frac{1 + 0{,}1 \times Cr + Cc + 2 \times (Dc + Chc) + 3 \times Vc + 4 \times Cia}{1 + 2 \times (D + Cnc) + Chnc + Dnc}$$

The groups are defined in the following table:

Table A.1 Correspondence of the IGA groups with the acronyms used in the equation above.

Dinoflagellates	D
Non colonial chrysophytes	Cnc
Non colonial chlorococcals	Chnc
Non colonial diatoms	Dnc
Cryptophytes	Cr
Colonial chrysophytes	Cc
Colonial diatoms	Dc
Colonial chlorococcals	Chc
Colonial volvocals	Vc
Cyanobacteria	Cia

Total biovolume

Total biovolume is considered to be a very good biomass indicator for freshwater phytoplankton. The robustness of the metric surpasses that of other biomass metrics such as dry weight, etc. It therefore comprises half of the weight of the biomass part of the multimetric method under description.

Chlorophyll-a

Chlorophyll-a has been broadly used for trophic studies and status of different freshwater ecosystems. It is easily measurable and it closely relates to pressures such as TP. Due to this, this broadly accepted parameter comprises 50% of the weight of the biomass part of the multimetric method under description.

Cyanobacteria percentage

This metric can account for the boom sensitive metric required by the phytoplankton method guidelines.

To avoid obtaining bad quality results for this metric in oligotrophic or mesotrophic water bodies, this metric is calculated excluding class *Chroococcales* with the sole exception of *Woronichinia* and *Microcystis*, since it is considered that within this class many species are typically found in nutrient poor habitats.

Application

Each parameter has a G/M Boundary defined. In the case of chlorophyll-a, a range was given. This is needed in order to calculate the nEQR values:

Table A.2 G/M values for the different parameters that compose the MASRP. In parenthesis, the exact value applied in Spain, where needed.

Parameter	Calcareous	Siliceous wet
Chlorophyll-a (µg/L)	4.2-6.0 (6)	6.7-9.5 (9.5)
Biovolume (mm^3/L)	2.1	1.9
IGA	7.7	10.6
Percentage cyanobact.	28.5	9.2

When calculating the correspondent EQR values for the different parameters, type specific formulae are applied as follows:

Table A.3 Equations built for the calculations of the EQR values for the different types of reservoirs. The MPE values for each parameter are used in order to rescale the parameters in a 0-1 scale.

	Reservoir IC type	MEP value	EQR calculation
Chlorophyll-a	Siliceous wet	2	(1/x)/(1/2)
	Calcareous	2,6	(1/x)/(1/2)
Biovolume	Siliceous wet	0,36	(1/x)/(1/0,36)
	Calcareous	0,76	(1/x)/(1/0,76)
IGA	Siliceous wet	0,1	(400-x)/(400-0,1)
	Calcareous	0,61	(400-x)/(400-0,61)
Cyano percentage	Siliceous wet	0	(100-x)/(100-0)
	Calcareous	0	(100-x)/(100-0)

Table A.4 Normalization equations for EQR values for all different parameters and types. Each equation should be selected from left to right.

	IC Res type	G/M	Normalizing equation
Chloro.	Siliceous wet	>9.5	nEQR = 2.857*EQR
		<9.5	nEQR = 0.5063*EQR + 0.4937
	Calcareous	>6	nEQR = 1.3953*EQR
		<6	nEQR = 0.7018*EQR + 0.2982
Biovol.	Siliceous wet	>1.9	nEQR = 3.1579*EQR
		<1.9	nEQR = 0.4938*EQR + 0.5062
	Calcareous	>2.1	nEQR = 1.6667*EQR
		<2.1	nEQR = 0.625*EQR + 0.375
IGA	Siliceous wet	>10.6	nEQR = 0.6162*EQR
		<10.6	nEQR = 15.234*EQR - 14.233
	Calcareous	>7.7	nEQR = 0.6108*EQR
		<7.7	nEQR = 22.533*EQR – 21.533
Cyan(%)	Siliceous wet	>9.2	nEQR = 0.6593*EQR
		<9.2	nEQR = 4.444*EQR - 3.444
	Calcareous	>28.5	nEQR = 0.8333*EQR
		<28.5	nEQR = 1.4268*EQR - 0.4268

At this point, all those samples where the values observed are below the MEP of the type, and are therefore assigned an EQR value above 1, should be truncated down to this value.

The last step before joining of the different metrics to give final results is the normalization of the EQR values (EQR→nEQR). To do this the following equations are applied.

Once the methodology for obtaining nEQR values has been established, the concordance of IC types must be matched up with the national types. With this, the method can be finally calculated.

The final part of the application of the method is, once you have all the different parameters transformed in nEQR values, the aggregation of them according to the formula below. This can be done as long as you have at least one composition and one biomass parameter.

$$MASRP = \frac{\left(\frac{nEQR(Chl) + nEQR(BV)}{2}\right) + \left(\frac{nEQR(IGA) + nEQR(\%Cya)}{2}\right)}{2}$$

The results obtained from this equation are already normalized, since all of its variables should have been normalized previously.

A.2 NMASRP (New Mediterranean Assessment System for Reservoir's Phytoplankton).

General information

This method is exclusively applied in Cyprus and Portugal and it is composed of 4 parameters that are aggregated in a multimetric index where all of them have equal weights, and divided according to the parameters being related to biomass or composition. These parameters are the following:

- Biomass
  - Chlorophyll-a (µg/L)
  - Biovolume (mm^3/L)
- Composition
  - IGA (Index Des Grups Algals)
  - BV of cyanobacteria (mm^3/L)

<u>Sampling</u>

Sampling in Portugal

<u>Sampling frequency</u>

Water bodies to which the NMASRP applies must be sampled thrice per growing season for metrics calculations, and an additional three times in autumn, winter and spring, although these last three samples will not be considered in the assessment.

<u>Sampling method and sample analysis</u>

Samples have to be integrated samples from the euphotic zone, defined as 2.5* Secchi depth observed.

Biovolume and composition:

1. Official protocol for chlorophyll-a analysis, based on NP 4327, 1996 – National Standard; EN ISO 10260, 1992 and Standard Methods 10200H), cell counting (based on EN 15204, 2006) and biovolume calculation (based on CEN TC 230/WG 2/TG 3) published.
2. Biovolume determination based on a Portuguese Standardized Biovolume Table and procedures described in CEN TC 230/WG 2/TG 3: 'Phytoplankton biovolume determination using inverted microscopy Utermöhl technique' (draft).

Sampling in Cyprus

Sampling frequency

Water bodies to which the NMASRP applies must be sampled twice a year between June and September (2 summer samples).

Sampling method and sample analysis

Samples have to be integrated samples from the euphotic zone, defined as 2.5* Secchi depth observed.

Biovolume and composition:

1. Chlorophyll-a: Method 10200 H. Chlorophyll. Standard Methods for the examination of water & wastewater. 21st Edition (2005). Edited by Eaton A. D., Clesceri L.S., Rice E.W., Greenberg A.E.
2. EN 15204. 2006. 'Water quality- guidance standard for the routine analysis of phytoplankton abundance and composition using inverted microscopy (Utermöhl technique)'.
3. CEN TC 230/WG 2/TG 3: 'Phytoplankton biovolume determination using inverted microscopy Utermöhl technique' (draft).

Parameter description and calculation

IGA

The IGA index is based on the coloniality of superior taxonomic groups and their trophic preferences, and it is applied according to the following equation, considering the percentage of each group in the sample. Only those samples where the represented groups add up to 70% or more of the sample will the index be suitable for application.

$$IGA = \frac{1 + 0{,}1 \times Cr + Cc + 2 \times (Dc + Chc) + 3 \times Vc + 4 \times Cia}{1 + 2 \times (D + Cnc) + Chnc + Dnc}$$

Total biovolume

Total biovolume is considered to be a very good biomass indicator for freshwater phytoplankton. The robustness of the metric surpasses that of other biomass metrics such as dry weight, etc. It therefore comprises half of the weight of the biomass part of the multimetric method under description.

Chlorophyll-a

Chlorophyll-a has been broadly used for trophic studies and status of different freshwater ecosystems. It is easily measurable and it closely relates to pressures such as TP. Due to this, this broadly accepted parameter comprises 50% of the weight of the biomass part of the multimetric method under description.

Cyanobacterial biovolume

This metric can account for the boom sensitive metric required by the phytoplankton method guidelines (WISER Deliverable D3.1-2: Report on phytoplankton bloom metrics).

The groups are defined in the following table:

Table A.5 Correspondence of the IGA groups with the acronyms used in the equation above.

Taxonomic group	Acronym
Dinoflagellates	D
Non colonial chrysophytes	Cnc
Non colonial chlorococcals	Chnc
Non colonial diatoms	Dnc
Cryptophytes	Cr
Colonial chrysophytes	Cc
Colonial diatoms	Dc
Colonial chlorococcals	Chc
Colonial volvocals	Vc
Cyanobacteria	Cia

Application

Each parameter has a G/M Boundary defined. In the case of chlorophyll-a, a range was given. This is needed in order to calculate the nEQR values:

Table A.6 G/M values for the different parameters that compose the NMASRP.

Parameter	Calcareous	Siliceous wet
Chlorophyll-a (µg/L)	5.3	7.9
Biovolume (mm^3/L)	2.5	2.8
IGA	6.5	37.6
Cyano BV (mm^3/L)	0.5	0.8

When calculating the correspondent EQR values for the different parameters, type specific formulae are applied as follows:

Table A.7 Equations built for the calculations of the EQR values for the different types of reservoirs. The MPE values for each parameter are used in order to rescale the parameters in a 0-1 scale.

	Reservoir IC type	MEP value	EQR calculation
Chlorophyll-a	Siliceous wet	1.7	(1/x)/(1/1.7)
	Calcareous	1.9	(1/x)/(1/1.9)
Biovolume	Siliceous wet	1.2	(1/x)/(1/1.2)
	Calcareous	0.9	(1/x)/(1/0.9)
IGA	Siliceous wet	2	(400-x)/(400-2)
	Calcareous	2.1	(400-x)/(400-2.1)
Cyano BV	Siliceous wet	0.02	(1/x)/(1/0.02)
	Calcareous	0.005	(1/x)/(1/0.005)

At this point, all those samples where the values observed are below the MEP of the type, and are therefore assigned an EQR value above 1, should be truncated down to this value.

The last step before the joining of the different metrics to give final results is the normalization of the EQR values (EQR→nEQR). To do this the following equations are applied.

Table A.8 Normalization equations for EQR values for all different parameters and types. Each equation should be selected from left to right.

	IC Res type	G/M	Normalizing equation
Chloro.	Siliceous wet	>7.9	nEQR = 2.7882*EQR
		<7.9	nEQR = 0.5097*EQR + 0.4903
	Calcareous	>5.3	nEQR = 1.6737*EQR
		<5.3	nEQR = 0.6235*EQR + 0.3765
Biovol.	Siliceous wet	>2.8	nEQR = 1.4*EQR
		<2.8	nEQR = 0.7*EQR + 0.3
	Calcareous	>2.5	nEQR = 1.6667*EQR
		<2.5	nEQR = 0.625*EQR + 0.375
IGA	Siliceous wet	>37.6	nEQR = 0.6589*EQR
		<37.6	nEQR = 4.4719*EQR – 3.4719
	Calcareous	>6.5	nEQR = 0.6067*EQR
		<6.5	nEQR = 36.173*EQR – 35.173
Cyan BV	Siliceous wet	>0.8	nEQR = 24*EQR
		<0.8	nEQR = 0.4103*EQR + 0.5897
	Calcareous	>0.5	nEQR = 60*EQR
		<0.5	nEQR = 0.404*EQR + 0.596

Once the methodology for obtaining of nEQR values has been established, the concordance of IC types must be matched up with the national types. With this, the method can be fully applied.

The final part of the application of the method is, once you have all the different parameters transformed in nEQR values, the aggregation of them according to the formula below. This can be done as long as you have at least one composition and one biomass parameter.

$$NMASRP = \frac{\left(\frac{nEQR(Chl) + nEQR(BV)}{2}\right) + \left(\frac{nEQR(IGA) + nEQR(CyaBV)}{2}\right)}{2}$$

The results obtained from this equation are already normalized, since all of its variables should have been normalized previously.

A.3 NITMET (New Italian Method).

General information

This method is exclusively applied in Italy and it is composed of 4 parameters that are aggregated in a multimetric index where all of them have equal weights, and divided according to the parameters being related to biomass or composition. These parameters are the following:

Biomass	Chlorophyll-a (µg/L) Biovolume (mm^3/L)
Composition	MedPTI (Mediterranean Phytoplankton Trophic Index) BV of cyanobacteria (mm^3/L)

Sampling

<u>Sampling frequency</u>

According to the published official protocol, to apply the NITMET, 6 samplings per year, 4 of them between April and October, one sampling at the end of the autumn and one between January and March must be taken.

<u>Sampling method and sample analysis</u>

Samples have to be integrated samples from the euphotic zone, defined as 2.5* Secchi depth observed.

Biovolume and composition:

1. Phytoplankton counting and biovolume determination: EN 15204. 2006. 'Water quality- guidance standard for the routine analysis of phytoplankton abundance and composition using inverted microscopy (Utermöhl technique).
2. Chlorophyll-a: A national reference protocol does not exist. A common reference paper is Lorenzen, C.J. 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnol. Oceanogr., 12: 343-346. Others use: Method 10200 H. Chlorophyll. Standard Methods for the examination of water & wastewater. 21st Edition (2005). Edited by Eaton A. D., Clesceri L.S., Rice E.W., Greenberg A.E.

Parameter description and calculation

<u>MedPTI</u>

The MedPTI is applied as described in "Marchetto A., Padedda B. M., Mariani, M. A., Lugliè, A. and Sechi, N. *A numerical index for evaluating phytoplankton response to changes in nutrient levels in deep Mediterranean reservoirs*, J. Limnol., 68(1); 106-121, 2009."

<u>Total biovolume</u>

Total biovolume is considered to be a very good biomass indicator for freshwater phytoplankton. The robustness of the metric surpasses that of other biomass metrics such as dry weight, etc. It therefore comprises half of the weight of the biomass part of the multimetric method under description.

Chlorophyll-a

Chlorophyll-a has been broadly used for trophic studies and status of different freshwater ecosystems. It is easily measurable and it closely relates to pressures such as TP. Due to this, this broadly accepted parameter comprises 50% of the weight of the biomass part of the multimetric method under description.

Cyanobacterial biovolume

This metric can account for the boom sensitive metric required by the phytoplankton method guidelines (WISER Deliverable D3.1-2: Report on phytoplankton bloom metrics).

Application

Each parameter has a G/M Boundary defined. In the case of chlorophyll-a, a range was given. This is needed in order to calculate the nEQR values:

Table A.9 G/M values for the different parameters that compose the NITMET.

Parameter	Calcareous	Siliceous wet
Chlorophyll-a (µg/L)	5.3	7.9
Biovolume (mm^3/L)	2.5	2.8
MedPTI	2.13	2.13
Cyano BV (mm^3/L)	0.5	0.8

When calculating the correspondent EQR values for the different parameters, type specific formulae are applied as follows:

Table A.10 Equations built for the calculations of the EQR values for the different types of reservoirs. The MPE values for each parameter are used in order to rescale the parameters in a 0-1 scale.

	Reservoir IC type	MEP value	EQR calculation
Chlorophyll-a	Siliceous wet	1.7	(1/x)/(1/1.7)
	Calcareous	1.9	(1/x)/(1/1.9)
Biovolume	Siliceous wet	1.2	(1/x)/(1/1.2)
	Calcareous	0.9	(1/x)/(1/0.9)
MedPTI	Siliceous wet	3.1	x/3.1
	Calcareous	3.1	x/3.1
Cyano BV	Siliceous wet	0.02	(1/x)/(1/0.02)
	Calcareous	0.005	(1/x)/(1/0.005)

The last step before the joining of the different metrics to give final results is the normalization of the EQR values (EQR→nEQR). To do this the following equations are applied.

Table A.11 Normalization equations for EQR values for all different parameters and types. Each equation should be selected from left to right.

	IC Res type	G/M	Normalizing equation
Chloro.	Siliceous wet	>7.9	nEQR = 2.7882*EQR
		<7.9	nEQR = 0.5097*EQR + 0.4903

	IC Res type	G/M	Normalizing equation
	Calcareous	>5.3	nEQR = 1.6737*EQR
		<5.3	nEQR = 0.6235*EQR + 0.3765
Biovol.	Siliceous wet	>2.8	nEQR = 1.4*EQR
		<2.8	nEQR = 0.7*EQR + 0.3
	Calcareous	>2.5	nEQR = 1.6667*EQR
		<2.5	nEQR = 0.625*EQR + 0.375
MedPTI	Siliceous wet	NA	nEQR = 0.6-0.2 (EQR - 0.69)/(0.59-0.69)
	Calcareous		
Cyan BV	Siliceous wet	>0.8	nEQR = 24*EQR
		<0.8	nEQR = 0.4103*EQR + 0.5897
	Calcareous	>0.5	nEQR = 60*EQR
		<0.5	nEQR = 0.404*EQR + 0.596

Once the methodology for obtaining nEQR values has been established, the concordance of IC types must be matched up with the national types. With this, the method can be fully applied.

At this point, all those samples where the values observed are below the MEP of the type, and are therefore assigned an nEQR value above 1, should be truncated down to this value.

The final part of the application of the method is, once you have all the different parameters transformed in nEQR values, the aggregation of them according to the formula below. This can be done as long as you have at least one composition and one biomass parameter.

$$NITMET = \frac{\left(\frac{nEQR(Chl) + nEQR(BV)}{2}\right) + \left(\frac{nEQR(MedPTI) + nEQR(CyaBV)}{2}\right)}{2}$$

The results obtained from this equation are already normalized, since all of its variables should have been normalized previously.

B. Lake phytoplankton method descriptions (not included in the current IC results)

B.1 Description of the Romanian assessment method for reservoirs

General background

The evaluation system for Romanian Reservoirs is based on Phytoplankton and follows the one for Natural lakes. The EQRs are established on statistical bases (taking into account the median values of phytoplankton metrics).

For reservoirs, no MEP sites were identified but some less impacted (best available) sites were selected and taken into consideration.

Classification scheme

One multimetric index includes 5 metrics combined into a multimetric index in different proportions, according to their representativity and response to pressures:

0.1*TAX+0.2*CYANO+0.3*BIO+0.15*CHL+0.25*ID = MULTIMETRIC INDEX

The Phytoplankton 5 metrics are:

- Total taxa number (= TAX);
- % Cyanobacteria from the total no of individuals/l (=CYANO) (as a bloom metric)
- Total Biomass/biovolume (mg/l or mm3/l)(=BIO)
- Chlorophyll-a (micrograms/l) (=CHL)
- Shannon –Wiener Diversity index (= ID)

Evaluation system was set for 14 types of Romanian reservoirs.

The 5 indices selected reflect the main pressures on algal communities: nutrient load, organic pollution, water level variations, general degradation.

Some correlations between different phytoplankton metrics and pressure parameters are illustrated bellow (especially nutrient load and organic pollution) for reservoirs which have significant data in our data base (Table B.1, Figure B.1 and Figure B.2).

Table B.1 Regression characteristics (R^2 and significance) between phytoplankton metrics and pressure indicators

Pressure indicator	Phytoplankton metrics	R2	Significance
N-NH4	Number of taxa	0.53	<0.0001
N-NH4	Diversity index	0.51	<0.0001
TP	Chlorophyll-a	0.13	0.001
TP	% Cyanobacteria	0.41	<0.0001

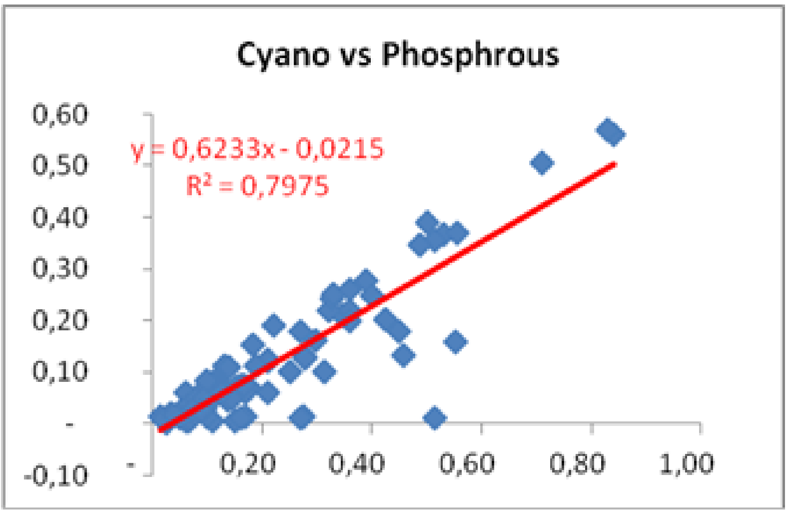


Figure B.1 Correlation between cyanobacteria abundance and total phosphorus for ROLA08 type.

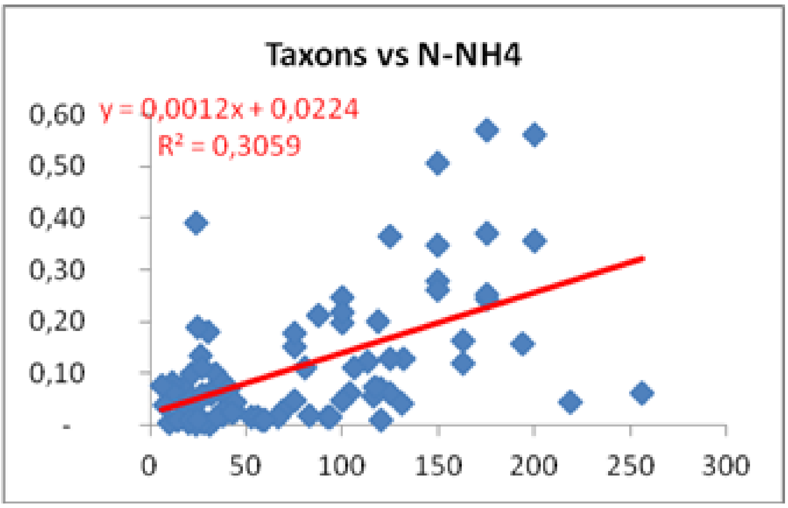


Figure B.2 Correlation between total taxa number and ammonia for ROLa12 type.

Boundary setting

Values were set for 3 classes of ecological potential: maximum, good and moderate for typological categories (some reservoir types which seemed to have similar responses to pressures have been grouped), based on available data set from at least 3 years, ecological arguments and also expert judgment.

The 25th percentile (P25) represents the reference status, P50 Maximum ecological potential and P75 Good Ecological potential (for % Cyanobacteria index, chl-a and biomass). For Diversity index and total nº of taxa: Reference-P75, MEP – P50, GEP-P25.

The values of multimetric index are the following:

- maximum ecological potential (min. 0.64)
- good ecological potential (min. 0.41)
- moderate ecological potential (max.0.41)

On maximum ecological potential, the algal communties show high diversity and different sensitive taxa are present (eg. *Fragilaria arcus var. arcus, Asterionella formosa, Cerantium hirundinella, Peridinium sp.*), no algal blooms, Cyanobacteria or other tolerant taxa are absent or low.

On good ecological potential, the algal communities show moderate diversity and some sensitive taxa are present (eg. *Dinobryon sertularia, Fragilaria ulna var. acus*), Cyanobacteria or algal blooms can be present (with Bacillariophyta species).

On moderate ecological potential, the algal communties show low diversity and tolerant taxa are present (ex. *Fragilaria crotonensis, Anabaena solitaria, Aphanocapsa sp.*). Cyanobacteria are also present (including spring and autumn), frequently algal blooms (often with Cyanobacterial taxa).

Romanian position regarding WFD compatibility of the method for evaluation of reservoirs

Romania has developed its methods for the evaluation of ecological status and ecological potential on the basis of existing data at national level and on the experience of national experts. The selection of metrics was done taking into account WFD requirements, Romanian national lake typology, and specificity of lakes, main pressures and existing data. Until now, the methods used allowed the evaluation of water bodies. From our point of view, none of the metrics is perfect; each one has its advantages and disadvantages and could be considered more or less subjective.

Taking into account the existing financial resources, in 2012/2013 the evaluation methods will be re-analysed in order to identify the most sensitive metrics to the pressures that could affect the lakes.

It seems unfair that in this particular moment, at the end of Intercalibration Exercise, to say that Romanian method is not compatible with WFD requirements. Obviously, each one of our arguments will induce a counter-argument…

Our request is the following: to take into consideration and to clearly specify that the Romanian method is <u>not comparable with other methods in the GIG and not that it is not compatible to the WFD requirements</u>. The incompatibility resulted from the metrics used and also from the limits proposed."

B.2 Description of assessment method used in azores´ natural volcanic lakes

Typology

In Azores, 24 volcanic lakes have been identified as water bodies within the scope of the WFD. These include all volcanic lakes larger than 0.01 km^2 surface area. Initially three volcanic lake types were defined using the following descriptors: Latitude, Longitude, Altitude, Lake size, Geology and Mean depth. Basically these three lake types were differentiated according to lake size and mean depth (Table B.2).

Table B.2 Azores Volcanic Natural Lakes types initially defined under the WFD and descriptors

Type	Altitude (m)	Latitude (º)	Longitude (º)	Size (km^2)	Geology	Mean depth (m)
Mid-altitude, Very small, Siliceous, Deep Volcanic Lakes	Mid-altitude (200-900)	36º45'-39º43'	24º32'-31º17'	Very small (0,5–5)	Siliceous	Deep (3–15)
Mid-altitude, Micro, Siliceous, Deep Volcanic Lakes	Mid-altitude (200-900)	36º45'-39º43'	24º32'-31º17'	Micro and Very small (0,01–0,5)	Siliceous	Deep (3–15)
Mid-altitude, Micro, Siliceous, Shallow Volcanic Lakes	Mid-altitude (200-900)	36º45'-39º43'	24º32'-31º17'	Micro (0,01–0,5)	Siliceous	Shallow (<3)

More recently this abiotic typology was validated with biological data resulting in only two types, since the Micro and Very Small Deep Volcanic Lakes were merged (Gonçalves, 2008; Gonçalves *et al.* 2008a; Gonçalves *et al.* 2008b). These two types and descriptors are described in Table B.3.

Table B.3 Azores Volcanic Natural Lakes final typology and descriptors

Type	Altitude (m)	Latitude (º)	Longitude (º)	Size (km^2)	Geology	Mean depth (m)
Mid-altitude, Micro and Very small, Siliceous, Deep Volcanic Lakes	Mid-altitude (200-900)	36º45'-39º43'	24º32'-31º17'	Micro and Very small (0,01–5)	Siliceous	Deep (3–15)
Mid-altitude, Micro, Siliceous, Shallow Volcanic Lakes	Mid-altitude (200-900)	36º45'-39º43'	24º32'-31º17'	Micro (0,01–0,5)	Siliceous	Shallow (<3)

Eleven (11) of the 24 Azores volcanic lakes belong to the Mid-altitude, Micro and Very small, Siliceous, Deep Volcanic Lakes type, whereas the remaining 13 volcanic lakes belong to the Mid-altitude, Micro, Siliceous, Shallow Volcanic Lakes type.

Sampling and analytical methods

Due to the climate conditions in Azores, the phytoplankton growing period is not well established (*i.e.* across seasons), therefore 4 samples per year, one per season, are considered to be representative of the phytoplankton annual cycle. Samples are collected using a Water Sampler (e.g. Van Dorn, Nasen, Niskin).

In Mid-altitude, Micro, Siliceous, Shallow Volcanic Lakes type sampling is done at sub-superficial level only. For this type this sampling procedure will continue to be followed in the future.

For Mid-altitude, Micro and Very small, Siliceous, Deep Volcanic Lakes type sampling procedures include three depth levels integrated samples during the mixing period and sub-superficial samples during the stratification period. The sampling procedures described for this type have been followed since 1998 through 2010. From summer of 2011 on, the sampling procedures will include integrated samples of the euphotic layer (2.5*Secchi depth).

Phytoplankton identification down to species level is required whenever possible. Official protocol for chlorophyll-a analysis, based on NP 4327, 1996 – National Standard; EN ISO 10260, 1992 and Standard Methods 10200H), cell counting (based on EN 15204, 2006) and biovolume determination (based on CEN TC 230/WG 2/TG 3) published. The Standardized Biovolume Table is under development.

Quality Assessment Method

For the assessment of Azores Natural Volcanic Lakes status the method Phytoplankton - Index of Biotic Integrity (P-IBI), a multimetric index, was developed by Gonçalves (2008) and adopted. Metrics were selected according to correlation with total phosphorous (p≤0,01 and Pearson r > 0,5). The P-IBI also shows a good correlation with total phosphorous (p≤0,01 and Pearson r > 0,5). Thus this index includes metrics based on phytoplankton composition, abundance and biomass, detecting pressures mainly related with eutrophication (see Gonçalves, 2008 for more detailed information).

For the application of this method only one sampling site per water body is needed (due to the small size of Azores lakes) with at least 4 samples per year. The list of all biological metrics and scores is presented in Table B.5. The final index score is obtained through the sum of all metric scores.

The ecological status is expressed in 5 quality classes and the P-IBI is expressed in EQR by dividing the observed P-IBI value with the reference value, thus waterbodies are assessed against type-specific near-natural reference conditions.

Reference sites were identified by pressure analysis. Several pressures were quantified (*e.g.* land use, pollution sources, recreation) and the lakes with lowest scores were selected for reference sites. After biological validation based on present phytoplankton community structure or paleolimnological analysis, some of the previously selected reference sites were rejected (Gonçalves, 2008; Gonçalves *et al.* 2008a; Gonçalves *et al.* 2008b).

Ecological status quality classes were set in line with the WFD normative definitions. The 95th percentile of the distribution in reference sites was used for High/Good boundary. The other boundaries resulted from splitting the remaining gradient into 4 equal width classes. Since metric scores are different for each Volcanic Lake type the reference and boundaries values are the same for both lake types.

Table B.4 shows the reference and boundaries values for P-IBI.

The Phytoplankton – Index of Biotic Integrity developed and adopted to assess ecological status of Azores Volcanic Lakes includes the following parameters specified in the WFD normative definitions for phytoplankton: biomass, abundance and taxonomic composition. Yet it does not cover frequency and intensity of algal blooms. Also, the P-IBI shows a relation with the most relevant pressure effect in this region (eutrophication), displaying a significant inverse correlation with total phosphorous. Therefore it is considered to be adequate to assess ecological status in Azores Volcanic Lakes, meeting most of the requirements of the normative definitions of the WFD.

Table B.4 Reference and quality classes boundary values for Phytoplankton – Index of Biotic Integrity (P-IBI)

Quality Index	Reference value	High/Good (EQR)	Good/Moderate (EQR)	Moderate/Poor (EQR)	Poor/Bad (EQR)
P-IBI	4,7	0,94	0,74	0,53	0,31

Table B.5 Phytoplankton – Index of Biotic Integrity (P-IBI) metrics and scores for each lake type

Lake types	Metrics	Metric scores (anual mean)			Units
		5	3	1	
Mid-altitude, Micro and Very small, Siliceous, Deep Volcanic Lakes	Cyanobacteria biomass	<0,01	0,01-0,1	>0,1	mg/L
	% of cyanobacteria	<1	1-5	>5	%
	Cryptophyceae biomass	<0,1	0,1-0,2	>0,2	mg/L
	% of dinoflagelates	>10	1-10	<1	%
	Total phytoplankton biomass	<1	1-5	>5	mg/L
	Chlorophyll *a* (sub-superficial samples only)	<3	3-10	>10	µg/L
Mid-altitude, Micro, Siliceous, Shallow Volcanic Lakes	Cyanobacteria biomass	<0,01	0,01-0,1	>0,1	mg/L
	% of cyanobacteria	<0,5	0,5-5	>5	%
	Cryptophyceae biomass	<0,1	0,1-0,2	>0,2	mg/L
	% of dinoflagelates	>10	1-10	<1	%
	Total phytoplankton biomass	<2,5	2,5-10	>10	mg/L
	Chlorophyll *a* (sub-superficial samples only)	<4	4-12	>12	µg/L

C. WFD compliance check: Bloom metrics

C.1 GIG position regarding inclusion of bloom metrics in the phytoplankton assessment methods

Since all relevant parameters for the biological element phytoplankton must be included in the Methods employed in each country (See IC guidance document), and the inclusion of a bloom sensitive metric hasn't been specifically addressed in the methods of the Mediterranean countries, this document clarifies the position and solutions reached.

In the official Mediterranean methods (MASRP, ROMET, and IPLAC), a distinct bloom sensitive metric is absent, but the following considerations have to be made:

As has been pointed out within other GIGS "there is no clear agreement regarding the definition of a bloom, (…) this is a significant short-coming of the directive". An emerging definition of a bloom is "an elevated biomass of cyanobacteria" (Position paper on bloom metric from Germany, presented 2011-06-24 for ECOSTAT meeting).

Some Mediterranean methods actually include metrics on Cyanobacteria occurrence such as Cyanobacteria percentage of total phytoplankton biovolume (MASRP) or Cyanobacteria percentage of total cell number (ROMET), covering the summer - time where the main bloom episodes could be found in Mediterranean reservoirs.

The WISER project recommended adopting a cyanobacteria metric based on abundance (biovolume) rather than percentage of abundance (Lake Phytoplankton Bloom Metrics-Discussion Paper. L. Carvalho. 9th March 2011).

There is a good and significant correlation between the parameter percentage of biovolume of cyanobacteria and the parameter proposed in the WISER project as bloom metric: mean of Cyanobacteria biovolume of the summer period (Figure C.1, Table C.1). Also there is a significant correlation between the latter and the percentage of cyanobacteria cell abundance (Figure C.2, Table C.1).

Table C.1 Pearson correlation coefficient and significance of the relations represented in Figure C.1 and Figure C.2.

Significance of regressions in Figure C.1 and Figure C.2.		Calcareous		Siliceous wet	
		Cyanobacteria biovolume percentage	Cyanobacteria cell percentage	Cyanobacteria biovolume percentage	Cyanobacteria cell percentage
Total Cyanobacteria biovolume	Pearson	0.658	0.218	0.527	0.376
	Sig.	<0.001	0.003	<0.001	0.004

The metrics on cyanobacteria occurrence included in the current (official) Mediterranean methods (MASRP and ROMET) are satisfactorily related to bloom specific parameters.

Other GIGs have defended other parameters such as chlorophyll-a, that are in fact, partially sensitive to blooms too, and which is also applied in all the Mediterranean methods intercalibrated.

Furthermore, within the L-M GIG second phase reservoir intercalibration, two new methods have been developed, including Cyanobacteria BV as a baseline parameter

(NITMET and NMASRP). These methods have been established as official in Cyprus, Portugal and Italy.

The MASRP and ROMET are, therefore, to different extents, sensitive to cyanobacterial abundance, since them have incorporated specific metrics to follow the abundance and importance of this group in the planktonic community. Therefore the methods should be sensitive to blooms, especially those concerning cyanobacteria.

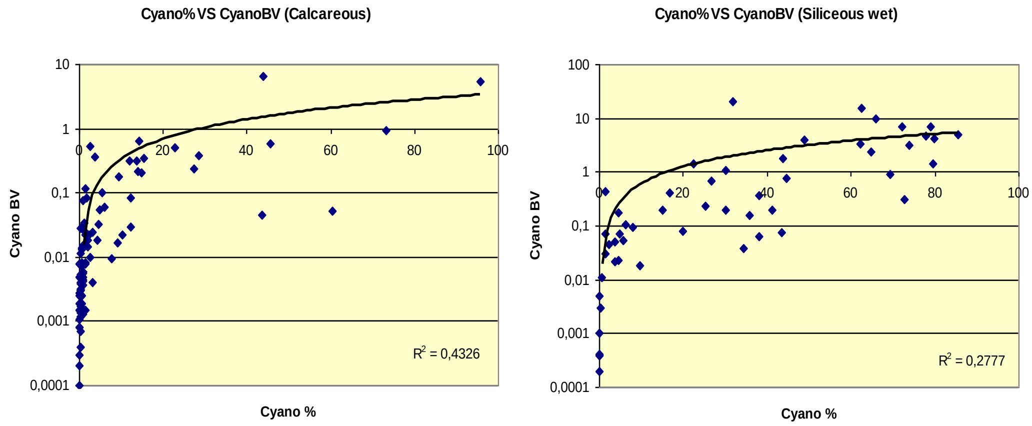


Figure C.1 Scatter plot and best linear fit for Cyanobacterial biovolume percentage metric (Applied in MASRP) VS Total Cyanobacterial BV (Recommended Bloom Sensitive metric), for Calcareous and Siliceous wet types.

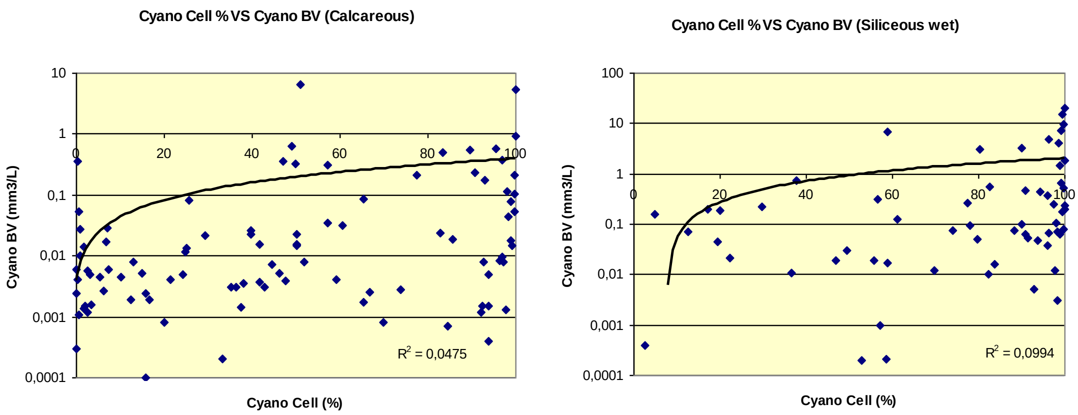


Figure C.2 Scatter plot and best linear fit for Cyanobacterial cell abundance percentage metric (Applied in ROMET) VS Total Cyanobacterial BV (Recommended Bloom Sensitive metric), for Calcareous and Siliceous wet types.

D. WFD compliance check: Boundary setting procedure

D.1 New Mediterranean Assessment System for Reservoirs Phytoplankton (NMASRP)

Metrics

Four metrics have been used in this method:

- Chlorophyll a;
- Total biovolume of phytoplankton;
- Biovolume of cyanobacteria. All species of cyanobacteria are included except those classified as chroococcals, but not excluding the genres Woronichinia and Microcystis;
- IGA (Catalan et al, 2003). In this analysis, the algae groups percentage used in the IGA was calculated referred to the total biovolume of the phytoplankton and only was calculated when the sum of biovolume of IGA groups was higher than the 70 % of total biovolume.

G/M boundaries

In order to establish the G/M boundaries, two parallel methodologies have been employed, each of them giving weight to different aspects of the data:

1. The first one use data statistical distribution in different TP groups;
2. The second approach is based on a equidistant division using both ends (upper and lower) of the data.

The use of these two methods in parallel increases the robustness of the results.

In the first methodology the response of each parameter is studied throughout the trophic gradient. First of all, in order to simplify and reduce the amount of analysis to handle, all pressures where checked in order to find if one of them could act as a surrogate of all the rest (Figure D.1 and Figure D.2). Total Phosphorus was chosen for such purpose since it is known to be a driving factor for phytoplankton populations in reservoirs. In the calcareous type, reservoirs from Jucar and Guadalquivir river basin districts were not taken into account**.**

It can be concluded:

- High levels of intensive agriculture and high population density are generally associated to high TP measurements in the reservoirs;
- Total Phosphorus can be used as a representative pressure, in direct relation to the other pressures considered in the analysis.

The next step in good/moderate boundary establishment consists on generating total phosphorus groups in an ecologically relevant manner. The groups created are: 0-5 µg/L, 5-10 µg/L, 10-20 µg/L, 20-50 µg/L, 50-100 µg/L and 100-MAX µg/L. The four parameters which build up the New Mediterranean Assessment System of Reservoirs Phytoplankton (NMASRP) where plotted in box-plots according to total phosphorus groups (Figure D.3 and Figure D.4).

The G/M boundary is thought to follow the 75 percentile of the parameter in the 20-50 µg TP/L group. It has been confirmed that there is a major shift in the parameters on the step between 20-50µg/L and 50-100µg/L. Limits for the metrics in the siliceous wet and calcareous types where set in this manner (Table D.1).

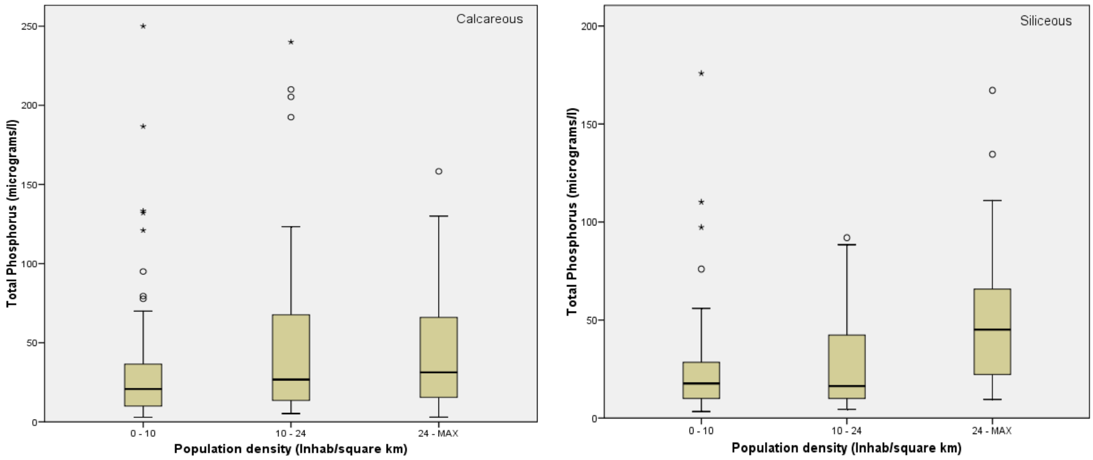


Figure D.1 Box-plots showing Total Phosphorus data (annual mean) plotted according to Population density groups in Calcareous (left) and Siliceous wet reservoirs (right).

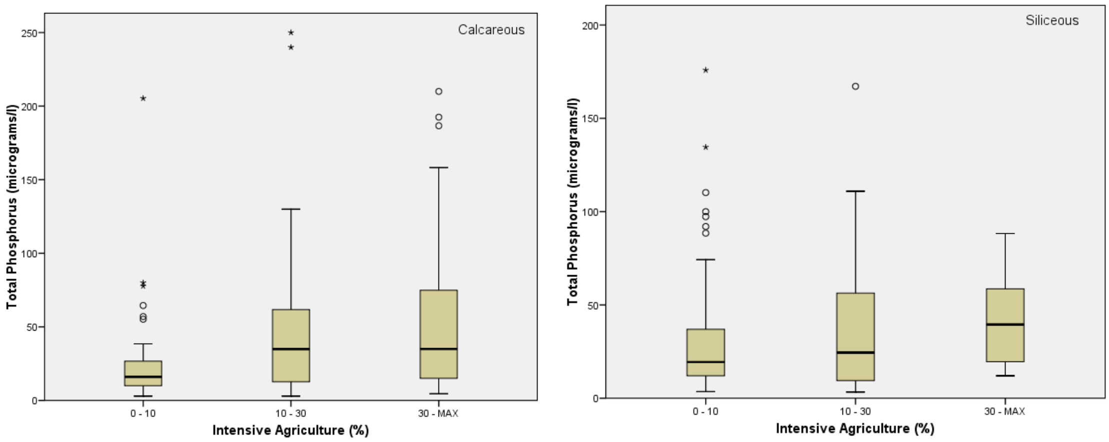


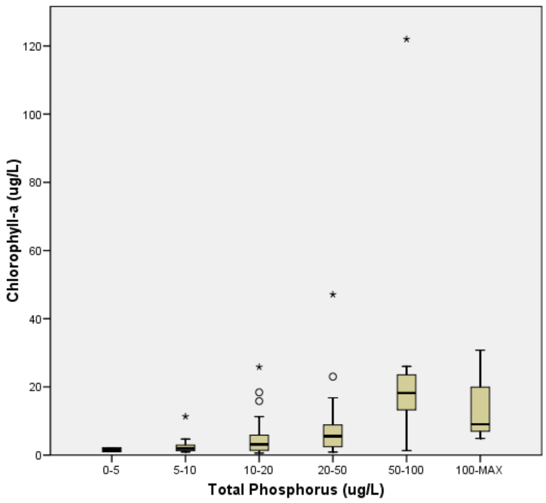
Figure D.2 Box-plots showing Total Phosphorus data (annual mean) plotted according to Intensive agriculture (%) groups in Calcareous and Siliceous wet reservoirs.

Table D.1 Good/Moderate boundaries established with the box-plot method described above, divided in calcareous and siliceous wet types, including the potential new substitute metric for cyanobacteria percent: cyanobacteria biovolume (BV)

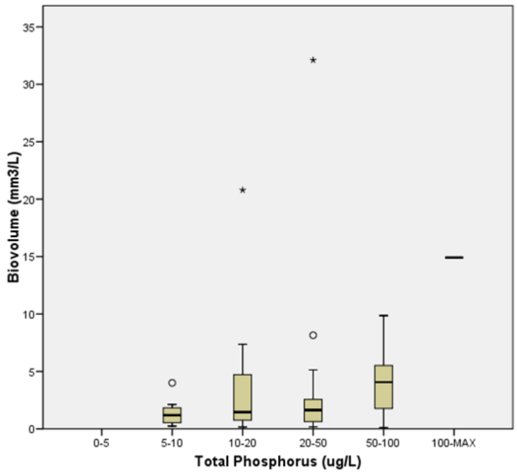
Type/ parameter	Chlorophyll-a (µg/l)	Total biovolume (mg/l)	IGA index	Cyanobacteria Biovolume (mg/l)
Calcareous	4.0	2.0	2.1	0.01
Siliceous wet	8.7	2.6	15.1	0.4

In the second methodology, a parallel approach has been applied with a double purpose. This approach has been used within the WISER project to establish metric thresholds and calculate EQRs in metrics like Cyanobacteria BV (WISER Deliverable D3.1-2: Report on phytoplankton bloom metrics), and it will be referred to from now on as the “Anchor method”. It consists of setting an anchor value for each parameter by calculating the median value for all reference sites in a certain type. After this a second anchor point has to be chosen, to establish the maximum value (or most degraded case). 85% of cases in siliceous reservoirs and 95% of cases in calcareous reservoirs were used, excluding one outlyer in chlorophyll-a data and another one in biovolume data. The differential consideration of percentage of the data was based on variability of parameters within the types. The most degraded value was selected and the following formula applied:

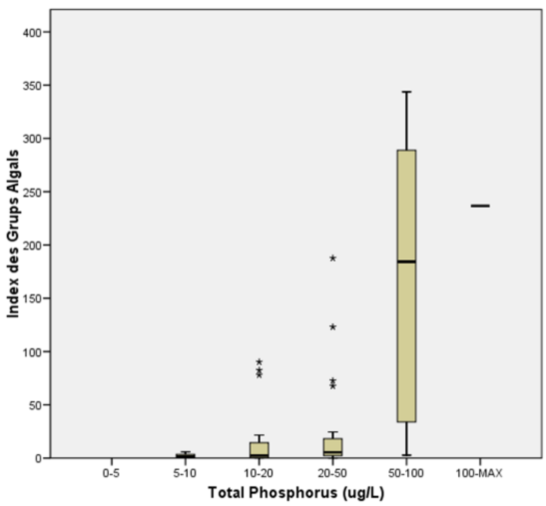
a) chlorophyll-a



b) Total biovolume



c) IGA index



d) Biovolume of Cyanobacteria

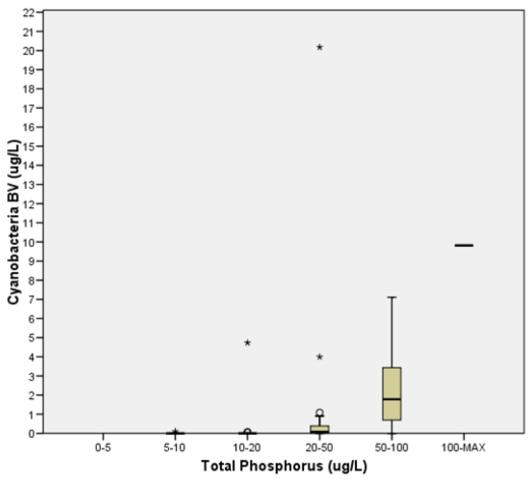
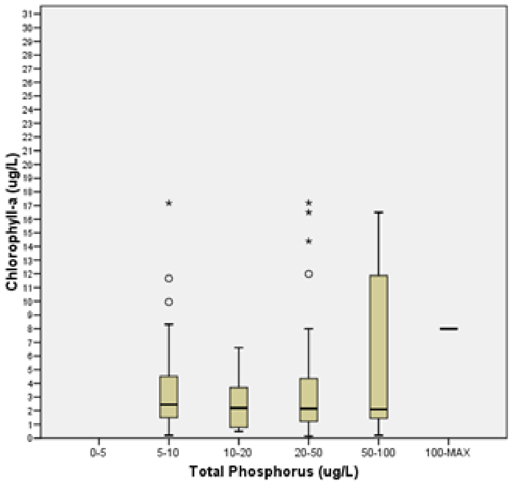
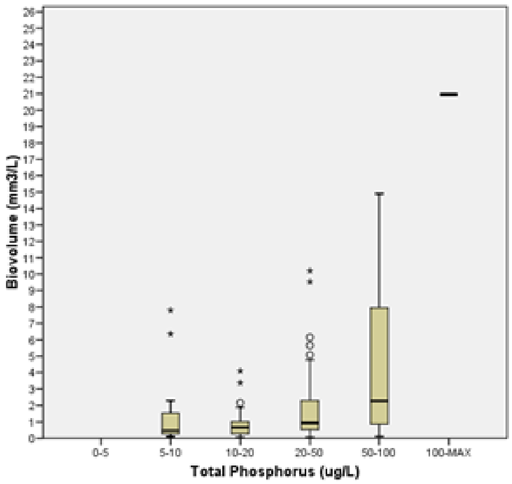


Figure D.3 Box-plots showing a) Chlorophyll-a summer mean, b) Biovolume summer mean, c) IGA summer mean, d) Cyanobacteria biovolume summer mean divided in TP groups (annual mean) for siliceous wet reservoirs. The third quartile of the 20-50 TP group was chosen as the G/M boundary

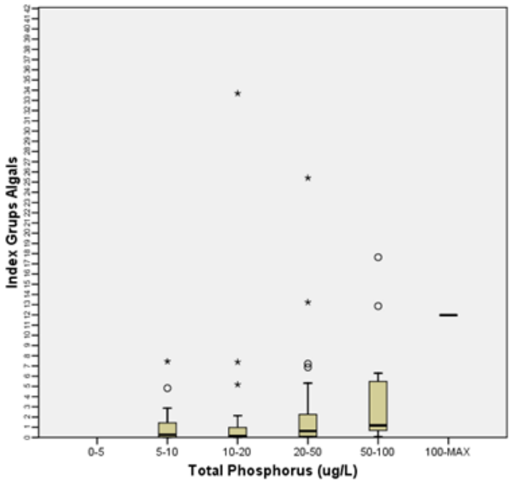
a) chlorophyll-a



b) Total biovolume



c) IGA index



d) Biovolume of Cyanobacteria

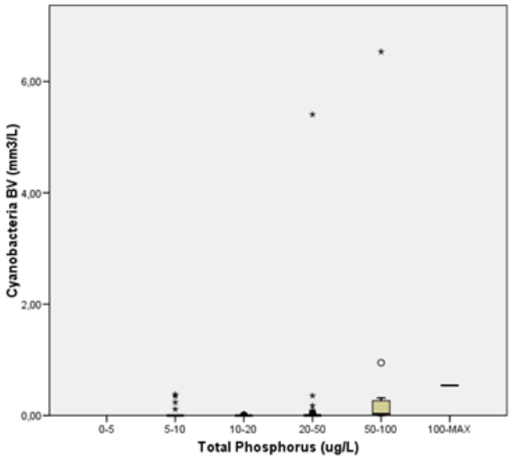


Figure D.4 Box-plots showing a) Chlorophyll-a summer mean, b) Biovolume summer mean, c) IGA summer mean, d) Cyanobacteria biovolume summer mean divided in TP groups (annual mean) for calcareous reservoirs (without Jucar and Guadalquivir river basis districts). The third quartile of the 20-50 TP groups (annual mean) was chosen as the G/M boundary

$$G/M = 0.6*(E1 - E0) + E0$$

E1: MEP median

E0: Maximum (worst quality) value of 85% or 95% of cases

The double purpose of the employment of an alternative approach is both to check on the feasibility of the limits, and second, to smooth the final results by calculating a mean between both boundaries derived in different ways. The anchor method has produced, therefore G/M boundaries for all parameters (Table D.2).

Table D.2 Good/Moderate boundaries established with the anchor method described above, divided in calcareous and siliceous wet types, including the potential new substitute metric for cyanobacteria percent: cyanobacteria biovolume

	Chlorophyll-a (µg/l)	Total biovolume (mg/l)	IGA index	Cyanobacteria Biovolume (mg/l)
Calcareous	6.6	3.08	10.9	0.1
Siliceous wet	7.2	3.1	60.2	1.3

.

A mean was calculated between the G/M boundaries established by both methods, therefore enabling to calculate normalization equations and finalize a new method (Table D.3).

Table D.3 Good/Moderate boundaries established by calculating the mean between both the anchor method and the box-plot method described above, divided in calcareous and siliceous wet types, including the potential new substitute metric for cyanobacteria percent: cyanobacteria biovolume

	Chlorophyll-a (µg/l)	Total biovolume (mg/l)	IGA index	Cyanobacteria Biovolume (mg/l)
Calcareous	5.3	2.5	6.5	0.1
Siliceous wet	7.9	2.8	37.6	0.8

The value of cyanobacterial biovolume good/moderate boundary for calcareous reservoirs is very low, and not coincident with the preliminary results about this parameter in other European lakes within the WISER project (Phillips, G. et al.). After consulting several phytoplankton experts, and considering the WHO guidance levels, and taking into account the overall low cyanobacterial biovolume of calcareous reservoirs in the data base (Figure D.5), it was decided that even though the above described boundary setting methods support placing the boundary at 0.1 mm^3/L, it is most sensible to move the boundary to 0.5 mm^3/L (Table D.4). This bias in cyanobacterial biovolume supports using 95% of the data as the top anchor for the second methodology in calcareous reservoirs instead of the 85% as in the siliceous reservoirs, since the value for cyanobacteria parameters is still very low.

Table D.4 Good/Moderate boundaries established by calculating the mean between both the anchor method and the box-plot method described above, divided in calcareous and siliceous wet types, including the potential new substitute metric for cyanobacteria percent: cyanobacteria biovolume. The boundary for this metric in Calcareous reservoirs is chosen differently to the other four parameters.

	Chlorophyll-a (µg/l)	Total biovolume (mg/l)	IGA index	Cyanobacteria Biovolume (mg/l)
Calcareous	5.3	2.5	6.5	0.5
Siliceous wet	7.9	2.8	37.6	0.8

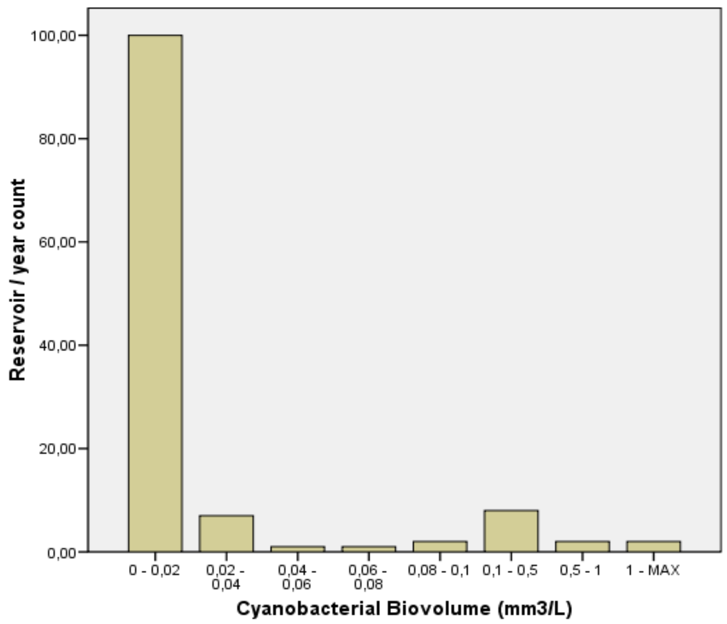


Figure D.5 Number of Calcareous reservoir years which fall within different cyanobacterial biovolume classes. Most cases fall in the first (and lowest) category (0-0.02 mm^3/L),

MEP values

The process of selection of MEP reservoirs and final MEP reservoir list is addressed in Annex G. The MEP for each metric was the mean of the values (reservoir-years) of this metric in all the MEP sites (Table D.5)

With these MEP values and G/M values, EQRs and normalizing equations for the NMASRP were calculated.

Table D.5 MEP in calcareous and siliceous wet types, including the potential new substitute metric for cyanobacteria percent: cyanobacteria biovolume.

	Chlorophyll-a (µg/l)	Total biovolume (mg/l)	IGA index	Cyanobacteria Biovolume (mg/l)
Calcareous	1.9	0.9	2.1	0.005
Siliceous wet	1.7	1.2	2	0.02

EQR Calculations

The way of calculating EQRs is parallel to the way in which it was done with the MASRP method as established in the first phase. The only major change in the methodology to establish these equations implies the change in reference values, (Table D.6).

Table D.6 Equations used to transform parameters into EQR scale. The variable "x" represents the parameter value you wish to transform. "Maximum Ecological Potential" MEP acts as the baseline to rescale parameters into a 0-1 scale.

Type	Parameter	Parameter → EQR equation	MEP (mean)

Sil. wet	Chl-a	(1/x)/(1/MEP)	1.7
	BV	(1/x)/(1/MEP)	1.2
	IGA	(400-x)/(400-MEP)	2
	Cya. BV	(1/x)/(1/MEP)	0.02
Calc.	Chl-a	(1/x)/(1/MEP)	1.9
	BV	(1/x)/(1/MEP)	0.9
	IGA	(400-x)/(400-MEP)	2.1
	Cya. BV	(1/x)/(1/MEP)	0.005

In the cases when the mean of Cyanobacteria of the summer was zero, the EQR considered was 1, nevertheless this issue should be further tested.

E. WFD compliance check: Pressure-impact relationships for the national methods

Lake phytoplankton methods used in the Mediterranean region are designed to assess eutrophication - which is the most relevant impact in this region.

Total phosphorus (TP) is considered one of the most significant pressure proxies to trigger eutrophication. The range of TP concentrations in a reservoir can be consequence of several pressures in the catchment. In principle, phosphorous loading to reservoirs is closely related to population density and land use in the catchment.

Relation of the ROMET (RO), MASRP (ES), ITMET (IT lakes) and IPLAC (FR) with pressures in reservoirs

Even though the typological analysis has concluded that there are four reservoir types that can be considered in the database (siliceous arid, siliceous wet, calcareous arid and calcareous wet), in the current analysis and in the comparison of the methods all calcareous reservoirs are considered as a single group. This merging can be done because the boundaries of the metrics in “wet” and “arid” reservoirs are very similar.

Both siliceous arid and siliceous wet types are showing a significant inversely proportional correlation between total phosphorus and the EQRs of every method applied, except in the case of ROMET in the siliceous wet reservoirs, where the correlation is low but significant (Table E.1).

Table E.1 Pearson’s correlation between the four methods (EQRs) and Total Phosphorus performed at a reservoir/year level and per type. The correlation coefficient, significance and sample size (N) are indicated.

Type	Parameter	ROMET	MASRP	ITMET	IPLAC
Siliceous wet	Correlation	-0.29	-0.57	-0.69	-0.65
	Significance	0.003	<<0.001	<<0.001	<<0.001
Siliceous arid	Correlation	-0.27	-0.46	-0.48	-0.46
	Significance	n.s.	0.003	0.002	0.011
Calcareous	Correlation	0.13	-0.03	0.17	0.18
	Significance	0.109	0.703	0.029	0.023

The ecological quality in calcareous reservoirs apparently is uncorrelated to total phosphorus, except for the Italian and French methods, although even in this case the significance of the correlations is low. Other proxies for nutrient pressure (population density and proportion of natural and semi-natural land use) were, therefore, analyzed (Table E.2). The correlation with these other pressures is significant in most cases but not very high. This is consistent with the fact that these pressures are not directly related to the amount of nutrients in a reservoir, and the relations are acknowledged as generally fuzzy. All in all, if correlations can be observed among the methods and potential sources of nutrient pressure in the catchment, there must be something misleading in the total phosphorus data, as phosphorus is a key driving factor for phytoplankton growth and ecosystem eutrophication. This may be related to low analytical quality in some cases, but also to the presence of biologically non-available phosphorus in the TP pool, which may be particularly relevant in some turbid hard waters. Investigation of this use is beyond the present exercise, but should be taken into account in future assessments.

Table E.2 Pearson's correlation between the four methods and total phosphorus (TP), population density (PD) and natural and semi-natural land use (NASN%).

Type			PD	NASN%	TP
Calcareous	ROMET	Correlation	-0.16	0.24	0.13
		Significance	0.044	0.004	0.109
	MASRP	Correlation	-0.28	0.31	-0.03
		Significance	<<0.001	<<0.001	n.s.
	ITMET	Correlation	-0.23	0.28	0.17
		Significance	0.002	<<0.001	0.029
	IPLAC	Correlation	-0.14	0.27	0.18
		Significance	n.s.	<<0.001	0.023

Since the general level of relatedness to total phosphorus appears to be lower than expected on calcareous reservoirs, further analysis was performed for spotting those river basin districts or subsets of the data that could be originating the lack of correlation between the methods and total phosphorus. Two river basin districts from Spain were identified as a large source of noise in the relationship. These were Jucar RBD and Guadalquivir RBD. They were spotted through analysis of the correlation of total phosphorus to both chlorophyll-a and biovolume and the relation of these two last parameters between themselves. The correlations with TP increases significantly when excluding the above mentioned data subsets from the analysis (Table E.3).

Table E.3 Bivariate correlation between the four methods and total phosphorus (TP). Jucar RBD and Guadalquivir RBD are excluded from the analysis. Orange implies low, but significant correlation. Green shows high correlation and significance.

Type	Method	Parameter	TP
Calcareous	ROMET	Correlation	-0.41
		Significance	<<0.001
	MASRP	Correlation	-0.46
		Significance	<<0.001
	ITMET	Correlation	-0.45
		Significance	<<0.001
	IPLAC	Correlation	-0.19
		Significance	n.s.

In summary, it can be concluded that these methods are significantly sensitive to eutrophication pressures and, therefore, suitable for the assessment of the ecological status of these water bodies. This result confirms the compliance with the Water Framework Directive requirements.

Relation of the NMASRP and NITMET with pressures in reservoirs

In next tables there are relationship between the NMASRP and NITMET and the pressures. They are similar than in the case of MASRP and ITMET.

Table E.4 Pearson's correlation between the NMASRP , the NITMET and total phosphorus (TP), artificial land use (ALU), natural and semi-natural land use (NASN%), population density (PD) and intensive agriculture.

Type: silicious wet		IA	PD	NASN	ALU	TP
NMASRP	Pearson	-0.13	-0.43	0.23	-0.57	-0.66
	Significance.	n.s.	<<0.01	n.s.	<<0.01	<<0.01
NITMET	Pearson	-0.13	-0.42	0.24	-0.58	-0.7
	Significance.	n.s.	<<0.01	0.042	<<0.01	<<0.01

Table E.5 Pearson's correlation between the New Mediterranean Assessment System for Reservoir Phytoplancton (NMASRP), the new Italian Assessment method and total phosphorus (TP), artificial land use (ALU), natural and semi-natural land use (NASN%), population density (PD) and Intensive agriculture.

Type: calcareous		IA	PD	NASN	ALU	TP
NMASRP	Pearson	-0.1	-0.3	0.2	-0.15	0.000
	Significance.	n.s.	<<0.01	<<0.01	0.05	n.s.
NITMET	Pearson	-0.12	-0.37	0.21	-0.13	-0.03
	Significance.	n.s.	<<0.01	<<0.01	n.s.	n.s.

Table E.6 Pearson's correlation between the New Mediterranean Assessment System for Reservoir Phytoplancton (NMASRP), the new Italian Assessment method and total phosphorus (TP), artificial land use (ALU), natural and semi-natural land use (NASN%), population density (PD) and intensive agriculture.

CAL without JU & GUA.		IA	PD	NASN	ALU	TP
NMASRP	Pearson	-0.06	-0.45	0.23	-0.17	-0.39
	Significance.	n.s.	<<0,01	0,019	n.s.	<<0,01
NITMET	Pearson	-0,1	-0.48	0.25	-0.15	-0.42
	Significance.	n.s.	<<0,01	0,01	n.s.	<<0,01

Relation of the IPLAC and ITMET with pressures in lakes

In the case of lakes, only one type is being intercalibrated, corresponding to calcareous lakes. Similarly as in Calcareous reservoirs, there is no significant relation between total phosphorus and the two methods intercalibrated. Similarly again, we found a significant relation between both national methods and a secondary pressure, in this case artificial land use (%) (Table E.6).

The lack of relatedness between the methods and Total Phosphorus can be due, again in a similar way as it has been described in the case of Calcareous reservoirs, to mistakes in the measurement or posterior handling of the data or to a low availability of the measured phosphorus for the phytoplankton. This can be confirmed when we check that the relation between Chlorophyll-a and biovolume with total phosphorus is not significant, while there should be a strong significance of the positive correlation between them. This would support the quality of the method results above that of total phosphorus data (Table E.8).

Table E.7 Pearson's correlation table showing a summary of all methods against different pressure parameters. Pearson coefficient, significance and sample size

		Total Phoshorus (µg/l)	Artificial Land Use %
IPLAC	Correlation	0.24	-0.36
	Significance	n.s.	0.014
	N	38	45
ITMET	Correlation	0.11	-0.46
	Significance	n.s.	<<0.001
	N	40	54

Table E.8 Pearson's correlation table showing the relation between Chlorophyll-a and Biovolume with total phosphorus and intensive agriculture.

Metrics		Total Phoshorus (µg/l)	Artificial Land Use %	Chlorophyll-a (µg/l)
Chlorophyll-a (µg/l)	Correlation	0,11	0.28	
	Significance	n.s.	0.009	
	N	73	87	
Total biovolume (mg/l)	Correlation	-0,07	0.74	0.48
	Significance	n.s.	<<0.001	<<0.001
	N	40	54	53

F. IC feasibility check: Italian assessment method

The Italian method has posed a problem for the comparisons between methods, since it demands samples to be taken not only during summer, but in winter too. The number of reservoir years in which the full method could be calculated decreased greatly (from 303 to 68 reservoir years), making the comparisons much less effective.

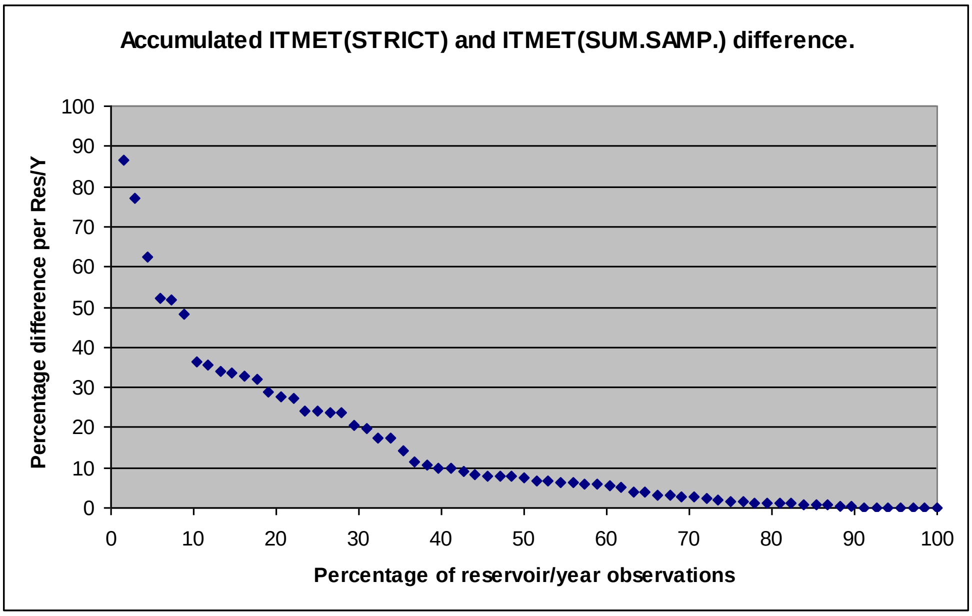


Figure F.1 Plot presenting the difference between the final results of the Italian method (ITMET) per reservoir/year expressed as percentages, ordered from those observations with greater divergence to the most similar observations. ITMET (STRICT) is the method calculated with both winter and summer samples and ITMET (SUM.SAMP.) refers to the method calculated with only summer samples.

An analysis of the real difference between both methods, when calculated with only summer samples and with both winter and summer samples, was made (Figure F.1). The results show how 80 % of the reservoir years which could be analysed by the two methods, show less than 30 % difference between them. The amount of comparable samples is, therefore, significantly high. Furthermore, when using summer samples the ITMET could be calculated on more than twice the number of reservoir years.

It was decided, according to the above mentioned criteria, that the Italian method would be applied considering samples as the MASRP does.

G. Deriving reference conditions (maximum ecological potential)

This analysis was carried out taking into account pressure criteria and biological criteria, following the Annex III of the Intercalibration Guidance (2010)

Pressure criteria

The first step taken for deriving maximum ecological potential water bodies was to do a pre-screening according to several different geographical land use variables. These variables were established through the CORINE LANDCOVER ANALYSIS. The different pressures considered were the following:

- Artificial land use percentage: Composed of the sum of all the categories of CLC class 1. (Urban areas continuous and discontinuous, industrial and commercial zones, communication infrastructures and networks, mines, etc.);
- Intensive agriculture percentage: Composed of the sum of the CLC categories corresponding to a high potential impact from agricultural activities: arable land (including irrigated land), permanent crops (with associated annual crops), vineyards, orchards, olive groves, complex cultivation patterns, - CLC codes : 2.1, 2.2, 2.4.1, 2.4.2;
- Natural and semi-Natural land use: Composed of forest and natural areas, wetlands, water bodies - codes CLC codes: 3.1.1, 3.1.2, 3.1.3, 3.2, 3.3, 4 and 5.

The other pressures considered are overall population density in the catchment and total phosphorus. Many of these pressures are believed to be correlated (even surrogates of each other), but nonetheless employing all of them will surely give a finer designation of low impacted sites.

Two sets of thresholds (

Table G.1) are established in order to designate maximum ecological potential reservoirs as has been done in the Fish Cross GIG. The first is a rejection threshold, where if the reservoir fails to pass on a single pressure variable it won't be considered reference, and the second is a reference threshold. A limit of two pressure variables can be over the reference threshold, but if three are over such limit, the reservoir fails to be included in the reference group. The thresholds are placed as shown below, and were based on:

- Proposed pressure screening criteria for selecting potential reference condition sites or values. (REFCOND Guidance)
- Criteria and thresholds used by the L-M GIG countries in the first round of IC.
- Criteria and thresholds used for the Med lakes and reservoirs in other biological elements (e.g. fish).
- Criteria and thresholds used in other GIG.

Table G.1 Rejection and reference limits for the five different selected parameters: ALU(%) → Artificial Land Use percentage; IA(%) → Intensive Agriculture percentage; NASN(%) → Natural and Semi-natural Land Use percentage; PD → Population density; TP → Total Phosphorus.

	ALU (%)	IA (%)	NASN (%)	PD (hab/km^2)	TP (µgP/l)
Rejection limits	< 4	< 20	> 70	< 30	< 30
Reference limits	< 1	< 10	> 80	< 10	< 12

Total Phosphorus rejection limit is set at a sensible boundary, by considering the mean total phosphorus value of the reservoirs which pass the reference "test" and applying the mean (12,48 µg P/L) plus three times the standard deviation (4,507 µg P/L) of TP within that subset from the data base. These results in a rejection limit of approximately 30 µg P/L.

Finally we realized how some particular uses that reservoirs may have can actually affect ecological response to a high degree, therefore three secondary pressures were considered. The three pressures considered are "Recreation activities", "Exploitation of fish population by fishery" and "Presence-absence of *Dreissena polymorpha* (Zebra mussel)". The first two pressures were classified according to a discrete system with four classes: None, Low, Medium and Strong. None of these two pressures can be classified as strong in the MEP reservoirs. *Dreissena polymorpha* presence/absence is an exclusion criterion, since its effects on phytoplankton are well documented.

Biological criteria

From the whole set of MEP reservoirs produced after filtering pressure criteria, a thorough revision of the metrics within the selected sites was performed in order to spot those reservoirs where, even though the pressures acting seem to be low, there are some other factors altering the maximum ecological potential community. These may be other pressures not included in the analysis, such as contamination sources other than agricultural or urban ones.

Following this line of reasoning, two more filtering criteria were considered. They intend to spot those sites that have low pressures but whose low quality metrics would compromise the quality of the reference data subset.

The first filter consists of a sample by sample check of the metrics of all reservoirs that had passed the pressure check. Biological metrics revised where chlorophyll-a, biovolume, IGA and % Cyanobacteria. If two or more of these samples are above the G/M boundaries established in the first phase the reservoir is not considered as reference.

The second filter tries to exclude from the MEP group those reservoirs that have metric values excessively high from an overall perspective. Some reservoirs are on a high profile metrics level and would hinder the quality of MEP sites, but their levels are still lower than those established as G/M on the first IC phase. This is why on this step Those reservoirs whose median value for any of these metrics (chlorophyll-a, biovolume, IGA or percentage of cyanobacteria) was above the third quartile of the set selected with the first filter where marked as doubtful (A1). The rest of the reservoirs were marked as MEP (Figure G.1 and Figure G.2). This ensures that the analysis would not get “contaminated” by bad status metrics in the MEP data set, but at the same time, that if for whatever the reason these reservoirs recovered the quality associated to the low pressure level they enjoy, they can be recovered. Those reservoirs whose median value for any of both metrics (chlorophyll-a or biovolume) is below the third quartile of the MEP data set where marked as MEP

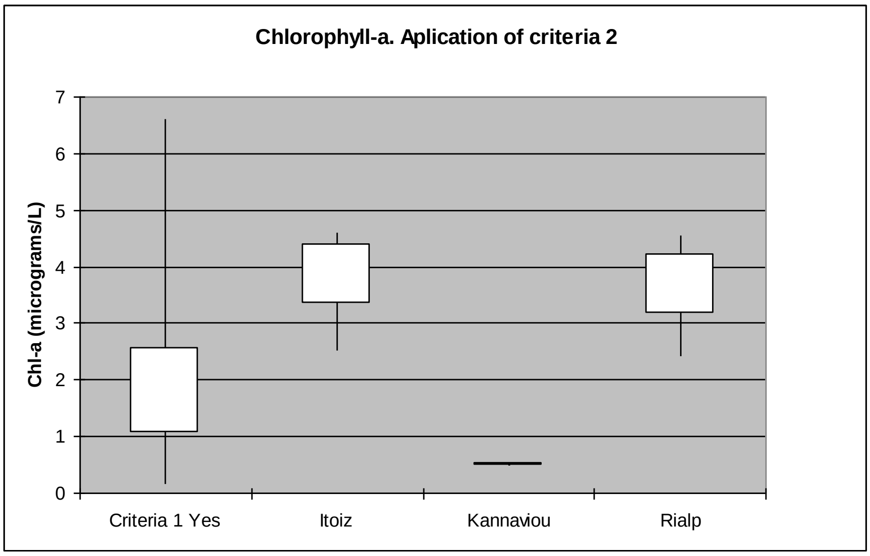


Figure G.1 Three reservoirs´ chlorophyll-a box-plotted together with the data set of reservoirs that passed the first set of criteria, showing how the second criteria is analyzed: Rialp does not fulfill the second set of criteria in chlorophyll-a. Kannaviou fulfils the second set of criteria in chlorophyll. Itoiz does not fulfill the second set of criteria in chlorophyll-a. All reservoirs were classified as A1 (see Figure G.2).

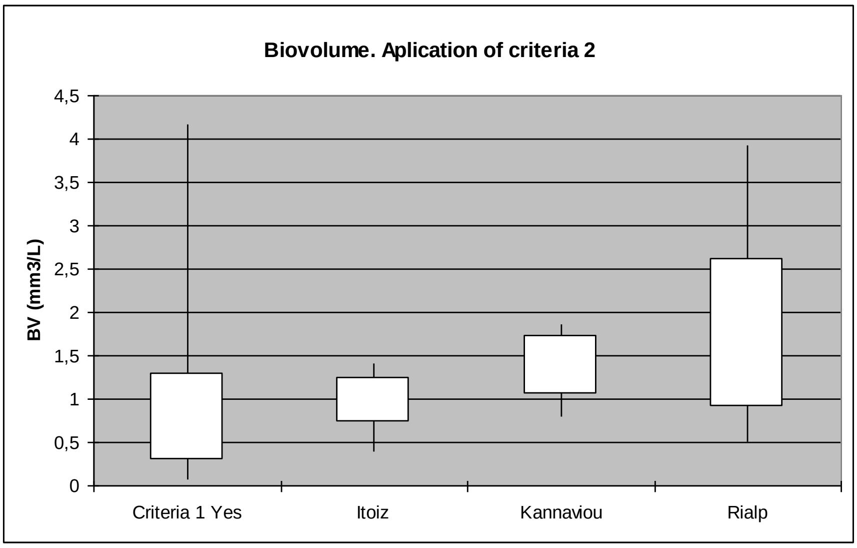


Figure G.2 Three reservoirs´ biovolume box-plotted together with the data set of reservoirs that passed the first set of criteria, showing how the second criteria are analyzed: Rialp does not fulfill the second set of criteria 2 in biovolume. Kannaviou does not fulfill the second set of criteria in biovolume.

Itoiz fulfils the second set of criteria in biovolume. All reservoirs classified as A1 (see Figure G.1).

The third filter concerns those reservoirs proposed as MEP by the MS that do not fit the pressure criteria but have good biological values. These were classified as doubtful (**A2**), and the pressures were checked. The total number of reservoirs within the above mentioned groups is: 8 A1 reservoirs, 10 A2 reservoirs and 29 MEP reservoirs in the database (Table G.2).

Table G.2 Total number of reservoirs per country, including the amount of reservoirs considered as A1 and A2 according to the abovementioned criteria, and those reservoirs strictly considered as Maximum Ecological Potential.

MS	A1	A2	MEP	Total
Spain	7	6	22	122
Cyprus	1	0	1	7
Greece	0	0	1	1
France	0	0	0	6
Romania	0	0	3	10
Portugal	0	4	2	18
Italy	0	0	0	15
TOTAL	8	10	29	179

Once an agreed classification has been reached, many different cross checkings with different data can be performed. The two main variables in the biological part of the database, since they are incorporated in one way or another to every method from the Mediterranean GIG, are chlorophyll-a and biovolume. The most basic check has been to view the distribution of these two variables among all reservoirs and only the MEP reservoirs (Figure G.3).

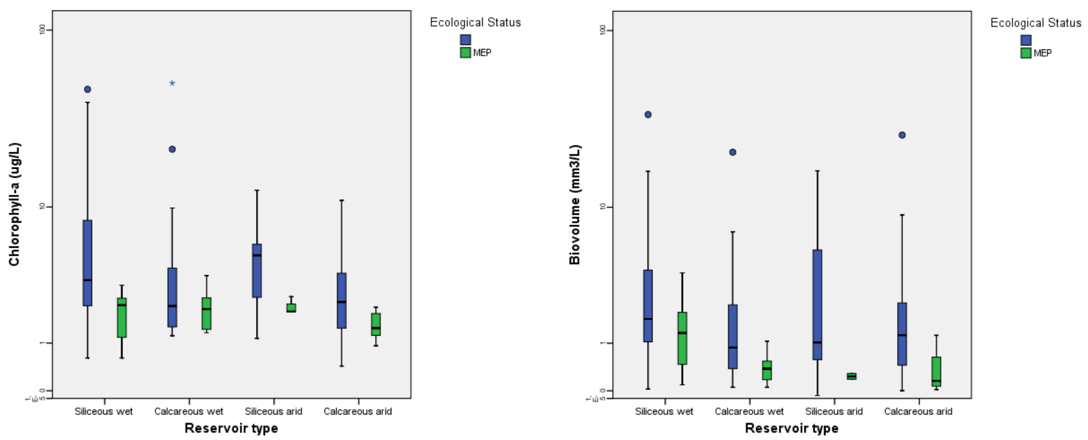


Figure G.3 Box-plot diagram presenting Chlorophyll-a and biovolume per reservoir type, separating MEP reservoirs colored in green and all reservoirs together colored in blue.

One of the most interesting conclusions, considering the homogeneity of the different pressures among the four types, is that Calcareous reservoirs seem to be less sensitive to pressures, with equal pressure increments resulting in differentiated higher responses in Siliceous reservoirs than in Calcareous ones (Figure G.3).

MEP sites

The selection of MEP sites was cross checked again with pressures to graphically visualize the distribution of these sites according to the five most relevant pressures. The main conclusion is that, in all cases, the median and the 3rd quartile (except in the case of TP) are below the reference threshold (the opposite in natural and semi-natural area). Those pressures which are least present in our MEP dataset are Artificial Land Use and Intensive Agriculture. Anyhow, the low pressures throughout all the MEP dataset confirm a sound selection of the sites.

Table G.3 Contents of columns from left to right per reservoir type: Number of reservoirs strictly considered MEP followed by percentage of reservoirs in this category; Number of reservoirs considered A1 followed by percentage of reservoirs in this category; Number of reservoirs in A2 followed by percentage of reservoirs in this category; Percentage of MEP+A1+A2 in relation to total number of reservoirs per type; Total number of reservoirs per type.

	MEP	A1	A2	MEP+A1+A2 (%)	TOTAL
Siliceous Wet	15 (23.07%)	2 (3%)	2 (3%)	29.07%	65
Siliceous Arid	3 (10.71%)	0 (0%)	4 (14.29)	25%	28
Calcareous Wet	7 (20%)	4 (11.43%)	1 (2.86%)	34.29%	35
Calcareous Arid	4 (7.84%)	2 (3.92%)	3 (5.88%)	17.64%	51

Since there are several reservoir types in the database where there is not a sufficient number of perfectly fitting MEP sites (Such as Siliceous arid and Calcareous arid types) (Table G3), and after thorough consensus among all the countries represented in the data base (Table G.4), the three categories will be considered as MEP for calculation issues during the rest of the intercallibration process. A last checking was done to compare the distribution of both chlorophyll-a and biovolume among 3 groups: MEP, MEP+A1 and MEP+A1+A2 (Figure G.4). If a big difference was spotted, joining the three categories may have been too risky for the results. The medians for chlorophyll-a are 1.5, 1.6 and 1.54 as arranged in the graph. The medians of biovolume are 0.4, 0.6 and 0.6 as arranged on the graph. All medians seem to fall under a reasonable limit for MEP reservoirs.

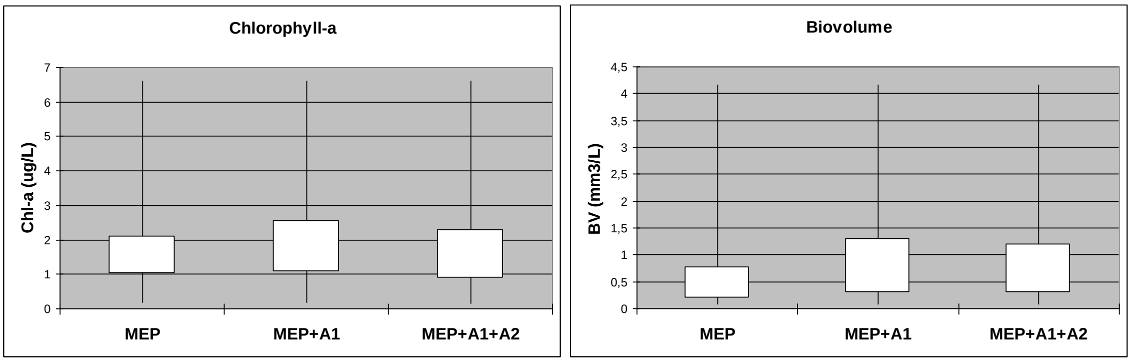


Figure G.4 Box-plots of chlorophyll-a and biovolume in the reservoirs selected as MEP, MEP + A1 and MEP + A1 + A2).

In table G.4 the 47 reservoirs considered as MEP for the intercallibration process are listed, and the type to which they belonged to assigned.

Table G.4. Reservoirs considered as MEP for the intercallibration process. CA: Calcacreous arid. CW Calcareous wet. SA: Siliceous arid. SW: Siliceous wet.

Country	Reservoir name	Type	National Eco. Status	Ecological Status
Cyprus	Lefkara	CA	MEP	MEP
Cyprus	Kannaviou	CA	MEP	A1
Spain	Tous	CA	MEP	A2
Spain	Grado 1	CA	MEP	MEP
Spain	El Portillo	CA	MEP	MEP
Spain	San Clemente	CA	MEP	A2
Spain	Camarasa	CA	MEP	A1
Spain	Arquillo de San Blas	CA	MEP	A2
Spain	Tranco de Beas	CA	MEP	MEP
Spain	Vadiello	CW	MEP	MEP
Spain	Mediano	CW	MEP	MEP
Spain	Beleña	CW	MEP	A1
Spain	Montearagón	CW	MEP	A1
Spain	Mansilla	CW	MEP	MEP
Spain	Riaño	CW	MEP	MEP
Spain	Porma	CW	MEP	MEP
Spain	Barrios de Luna	CW	MEP	MEP
Spain	La Requejada	CW	MEP	MEP
Spain	Itoiz	CW	MEP	A1
Spain	Ordunte	CW	MEP	A2
Spain	Tanes	CW	MEP	A1
Portugal	Beliche	SA	MEP	A2
Portugal	Santa Clara	SA	MEP	A2
Portugal	Odeleite	SA	MEP	A2
Spain	Rumblar	SA	MEP	MEP
Spain	Yeguas	SA	MEP	MEP
Spain	Andévalo	SA	MEP	A2

Country	Reservoir name	Type	National Eco. Status	Ecological Status
Spain	Chanza	SA	MEP	MEP
Greece	Tehniti Limni Tavropou	SW	MEP	MEP
Portugal	Santa Luzia	SW	MEP	MEP
Portugal	Castelo do Bode	SW	MEP	A2
Portugal	Vilarinho das Furnas	SW	MEP	MEP
Romania	Valea de Pesti	SW	MEP	MEP
Romania	Poiana Uzului	SW	MEP	MEP
Romania	Vidraru	SW	MEP	MEP
Spain	Camporredondo	SW	MEP	A1
Spain	Uzquiza	SW	MEP	MEP
Spain	San Sebastian	SW	MEP	MEP
Spain	Bao	SW	MEP	MEP
Spain	Salime	SW	MEP	A1
Spain	Doiras	SW	MEP	MEP
Spain	La Cohilla	SW	MEP	MEP
Spain	El Atazar	SW	MEP	MEP
Spain	Las Portas	SW	MEP	A2
Spain	Cenza	SW	MEP	MEP
Spain	Añarbe	SW	MEP	MEP
Spain	Matalavilla	SW	MEP	MEP

Reference Lakes

The same pressure criteria applied to the reservoirs was applied for lakes. In the lakes which passed the pressures criteria biomass and composition metrics (chlorophyll, biovolume and MedPTI) were analyzed. If, in one of these metrics, the median of all data from the lake was above the third quartile of the reference data set, this lake was excluded (the opposite for MedPTI).

Six lakes, all of them from Spain were classified as Reference (Table G.5).

Table G.5 Lakes considered as Reference for the intercallibration process. (*) Reference just for phytoplankton

Country	Lake name	Type	National Eco. Status	Ecological Status
Spain	Laguna del Marquesado	Calcareous	Reference	Reference
Spain	Laguna del Tejo	Calcareous	Reference	Reference
Spain	Laguna del Arquillo	Calcareous	Reference	Reference
Spain	Laguna Grande del Tobar	Calcareous	Reference(*)	Reference
Spain	Laguna de Taravilla	Calcareous	Reference	Reference
Spain	Estanque grande de Estanya	Calcareous	Reference	Reference

H. Benchmark standardization

Benchmarking in the context of the intercalibration intends to remove differences among national assessment methods that emerge from systematic discrepancies such as methodological, typological or biogeographical differences rather than the differentiated response to anthropogenic pressures. The consideration of the data without benchmarking can pose a problem for the comparability exercise, and is therefore a compulsory process to study these effects originating from discrepancies among countries. Several different approaches have been applied in order to perform the benchmark normalization.

Reservoirs

The process of standardization would be meaningless if "the EQR values of a given national method for benchmark sites do not differ between these subtypes" (IC Guidance).

In the two types being intercalibrated there are no MEP reservoirs from all countries (Annex G).

Calcareous reservoirs.

In calcareous type the MEP reservoirs are just from Cyprus (2) and Spain (19). Student´s T test analysis shows high p-values for the test chosen (Table H.1) therefore we have not got enough evidence to say that the EQR means of the MEP reservoirs in each of the three methods in the case of Spain and Cyprus is different.

Table H.1 Table showing p-values of the Student´s T test comparisons performed on the three methods for MEP reservoirs among countries within the Calcareous type.

P-values of the pairwise comparisons performed between the MEP reservoirs method results among countries within types.			**Calcareous**		
			Spain		
			MASRP	**NMASRP**	**NITMET**
Calcareous	**Cyprus**	**MASRP**	0,68		
		NMASRP		0,62	
		NITMET			0,89

Another, more visual way of checking for baseline differences among MEP reservoirs is to simply plot them in box-plots grouped per method, and separated per countries. Similar levels of response to pressure are expected to show in the section referring to a single method if the thesis that no benchmark standardization is needed can be supported (Figure H.1). In the case of Calcareous reservoirs it is clear that there is similar response at low levels of pressure in the case of Cyprus and Spain.

To know if there are biogeographical differences between the other Mediterranean countries belonging to this type, a Generalized Linear Model (GLM) was applied using all reservoirs in the DB from each county, not only the MEP sites.

Pressure variables should form the covariates in the model (In this case TP), while the country of origin of the data is specified as a random factor. The dependant variable is defined as the EQR value for the respective country method. Using these premises, three models per method were built. The first one (GLM1) considering country of origin as random factor, the second one (GLM2) not considering the country of origin as a random factor, and the third one (GLM3) considering as a random factor several groups: those countries that appeared as significant in the first GLM, and one group with all the other countries (not significant in the first GLM). If all country effects appear as not significant in GLM1, there is not enough evidence to say that there are differences in the response of the methods to the pressures in the different countries; therefore no benchmark standardization is needed.

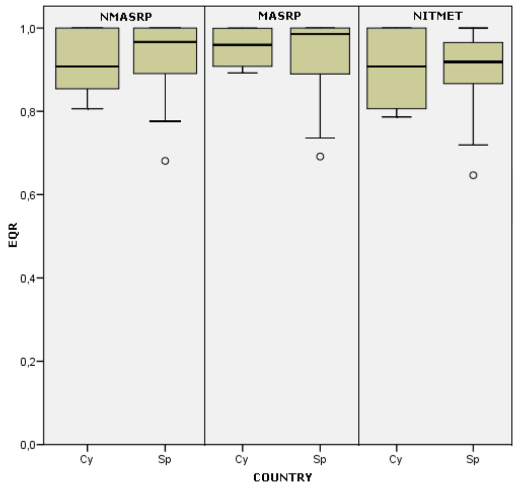


Figure H.1 Box-plots showing the distribution of MEP reservoir results from Spain and Cyprus to MASRP, NMASRP and NITMET.

The intersection of the GLM2 minus the intersection of the GLM1 gives the offset values for those countries which are not significant in the GLM1. In order to assess the offset of the significant countries, the GLM3 is used. This offset is calculated from the individual intersection of the significant country minus the offset applied to the non-significant countries.

After these offsets have been calculated, if existent, they should be applied to each EQR method result depending on the method and country of application as well as to the boundaries of the method. All GLM1s for the three different methods show a lack of significance in the country effect when applied (Table H.3 and Table H.4).This, in turn, results in an offset value of zero (i.e. There is no desirable transformation of those methods in any country which would render them more comparable).

Table H.2 GLM1 for the NMASRP, considering TP as covariate and country of origin as cofactor in Calcareous reservoir type.

Parameter	B	Std error	95% Wald confidence interval		Statistical hypothesis testing		
			Lower	Upper	Wald´s Chi sq.	df	Sig.
(Intersection)	-,028	,0268	-,081	,024	1,101	1	,294
[Country=Cy]	-,058	,0428	-,142	,026	1,840	1	,175
[Country=It]	-,113	,1046	-,318	,092	1,165	1	,280
[Country=Sp]	0[a]	.	.	.	.	.	.
TP	-,004	,0010	-,006	-,002	19,467	1	,000
(Scale)	,019[b]	,0027	,015	,025			

Dependent var: NMASRP

Table H.3 GLM1 for the MASRP, considering TP as covariate and country of origin as cofactor in Calcareous reservoir type.

Parameter	B	Std error	95% Walds confidence interval		Statistical hypothesis testing		
			Lower	Upper	Walds Chi sq	df	Sig.
(Intersection)	-,034	,0257	-,084	,016	1,767	1	,184
[Country=Cy]	-,029	,0402	-,108	,049	,536	1	,464
[Country=It]	-,121	,1015	-,320	,078	1,417	1	,234
[Country=Sp]	0[a]	.	.	.	.	.	.
TP	-,004	,0009	-,006	-,002	17,011	1	,000
(Scale)	,018[b]	,0025	,014	,024			

Dependent var: MASRP

Table H.4 GLM1 for the NITMET, considering TP as covariate and country of origin as cofactor in Calcareous reservoir type.

Parameter	B	Std error	95% Walds confidence interval		Statistical hypothesis testing		
			Lower	Upper	Walds Chi sq	df	Sig.
(Intersection)	-,034	,0255	-,084	,016	1,747	1	,186
[Country=Cy]	-,048	,0405	-,127	,032	1,394	1	,238
[Country=It]	-,092	,0978	-,284	,099	,891	1	,345
[Country=Sp]	0[a]	.	.	.	.	.	.
TP	-,005	,0009	-,007	-,003	23,925	1	,000
(Scale)	,017[b]	,0024	,013	,022			

Dependent var: NITMET

Siliceous wet reservoirs

In siliceous wet type the MEP reservoirs are from Portugal (3) and Spain (12), but Portugal has not inputted biovolume data for this reservoir type.

We selected a metric that both countries have in the dataset (chlorophyll-a) in order to check for subtypological differences (Table H.5).

Table H.5 Table showing p-values of the Student´s T test comparisons performed on the chlorophyll a for MEP reservoirs among countries within the Siliceous wet type.

P-values of the pair wise comparisons performed between the MEP reservoirs´ chlorophyll means among countries.	**Spain**
Portugal	0.128

Siliceous wet reservoirs show a high p-value for the test chosen (

Table H.5) therefore we have not got enough evidence to say that, at a confidence level of 95%, the chlorophyll-a means of the MEP reservoirs from both countries are different.

As in the case of calcareous reservoirs, Italy has no MEP reservoirs, therefore, it is not included in this analysis. To know if there are biogeographical differences with the other Mediterranean countries belonging to this type, a GLM was applied using all reservoirs in the DB from each country, not only the MEP. According to these GLMs there is no significant effect of the country of origin on the data; therefore not applying any benchmark standardization is supported, again, by this analysis (Table H.6, Table H.7 and Table H.8).

Table H.6 GLM for the NMASRP, considering TP as covariate and country of origin as cofactor in Siliceous wet type.

Parameter	B	Std error	95% Wald confidence interval		Statistical hypothesis testing		
			Lower	Upper	Wald Chi sq	df	Sig.
(Intersection)	-,038	,0512	-,138	,063	,537	1	,464
[country= Italy]	-,118	,1049	-,324	,087	1,270	1	,260
[country= Spain]	0[a]	.	.	.	.	.	.
TP	-,009	,0021	-,013	-,005	18,775	1	,000
(Scale)	,035[b]	,0061	,025	,050			

Dependent var: NMASRP

Table H.7 GLM for the MASRP, considering TP as covariate and country of origin as cofactor in Siliceous wet type.

Parameter	B	Std error	95% Wald confidence interval		Statistical hypothesis testing		
			Lower	Upper	Wald Chi sq	df	Sig.
(Intersection)	-,094	,0466	-,185	-,003	4,079	1	,043
[country=Italy]	-,121	,0944	-,306	,064	1,632	1	,201
[country=Spain]	0[a]	.	.	.	.	.	.
TP	-,010	,0019	-,013	-,006	25,667	1	,000
(Scale)	,025[b]	,0043	,018	,035			

Dependent var: MASRP

Table H.8 GLM for the NITMET, considering TP as covariate and country of origin as cofactor in Siliceous wet type.

Parameter	B	Std error	95% Wald confidence interval		Statistical hypothesis testing		
			Lower	Upper	Wald Chi sq	df	Sig.
(Intersection)	-,036	,0467	-,127	,056	,584	1	,445
[country=Italy]	-,108	,0957	-,296	,079	1,282	1	,257
[country=Spain]	0[a]	.	.	.	.	.	.
TP	-,009	,0019	-,013	-,006	24,506	1	,000
(Scale)	,029[b]	,0051	,021	,041			

Dependent var: NITMET

I. Biological communities at reference sites and G/M sites

In order to assess the phytoplankton species that better describe the transition G/M, we evaluated for each species the biovolume weighted average of the MASRP values (BWAM) in the reservoirs where a species was appearing.

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

Where BV_{ij} is the species (i) biovolume in reservoir year (j) and $MASRP_j$ is 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 poor conditions. Species around G/M transition show BWAM around 0.6. Variance of BWAM estimation and number of observations of the species in the database were used to screen the species with too high BWAM estimation; high variance indicate tolerance of a broad range of conditions independent of BWAM values, low number of observations warns about spurious results.

Different species were selected with these criteria and afterwards grouped in classes. These classes where plotted, transformed to percentage biovolume of the total mean biovolume per reservoir year, against both chlorophyll-a and NMASRP classification. The variation of the indicator groups was studied throughout the span of these two parameters.

Siliceous wet reservoirs

There is a clear relation of certain phytoplanktonic groups and taxa with the change of classes from good to moderate. The community is composed, when quality is still high, near the MEP community type, mainly of Chrysophytes together with good quality indicator diatoms and Chlorococcals. The genera *Dinobryon*, *Pseudopedinella* and *Ochromonas*, from the Chrysophytes, *Ankyra*, *Sphaerocystis* and *Coenochloris* from the Chlorococcales and *Asterionella*, *Nitzschia* and *Discostella* from the diatoms are typical from above G/M communities, and peak at MEP sites. Some species such as *Crucigenia tetrapedia*, *Monoraphidium minutum* from the Chlorococcales group and *Ulnaria ulna* from the diatoms group are also representative of good quality sites. Not only they are typical, but the steadily decrease towards the G/M status, and almost disappear at that point. In parallel to this change in the community, cyanobacterial presence, which starts at low values, increase greatly in the community at approximately the same point where the other groups tend to disappear. The genera *Anabaena*, *Woronichinia* and *Aphanizomenon* are the main representatives of this change in the community (Figure I.1 and Figure I.2).

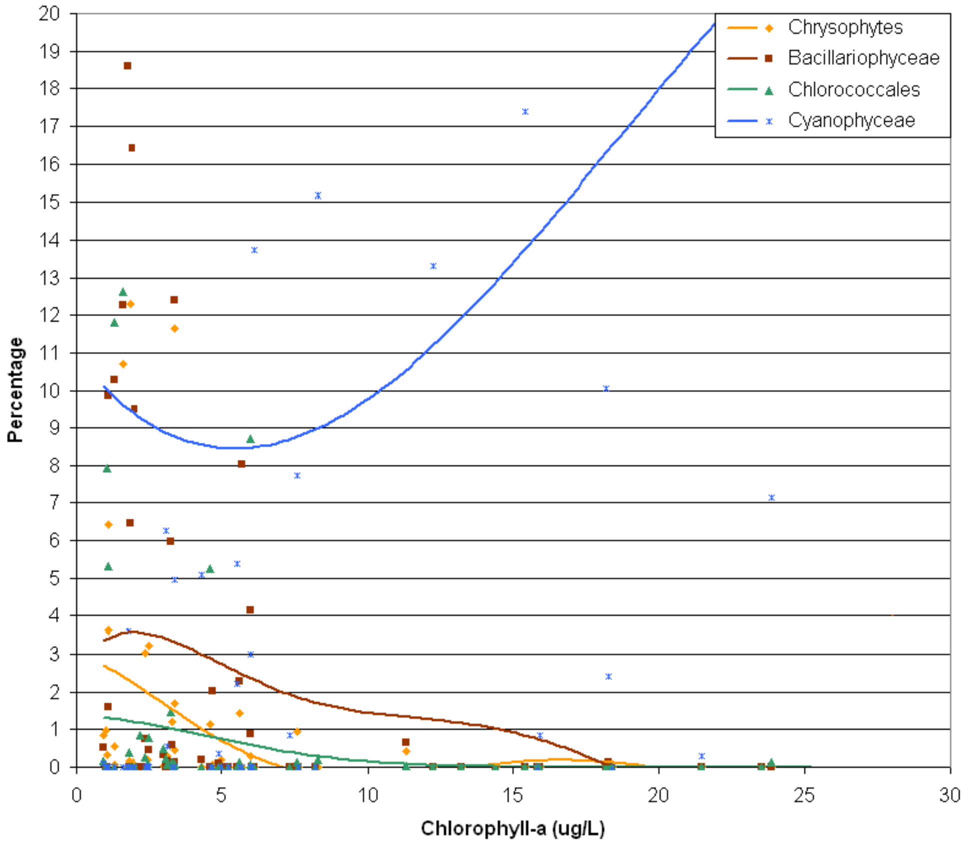


Figure I.1 Scatter-plot between chlorophyll-a and the mean percentage contribution of different groups to the mean total biovolume per reservoir year. Chrysophytes (Orange) include the genera Dinobryon, Pseudopedinella, Ochromonas and Chrysochromulina. Bacillariophyceae (Brown) include genres Asterionella, Nitzschia, Discostella and the species Ulnaria ulna. Chlorococcals include genera Ankyra, Sphaerocystis and Coenochloris, and the species Crucigenia tetrapedia and Monoraphidium minutum. Cyanophyceae includes the genera Aphanizomenon, Woronichinia and Anabaena. The lines represent polynomial adjustments.

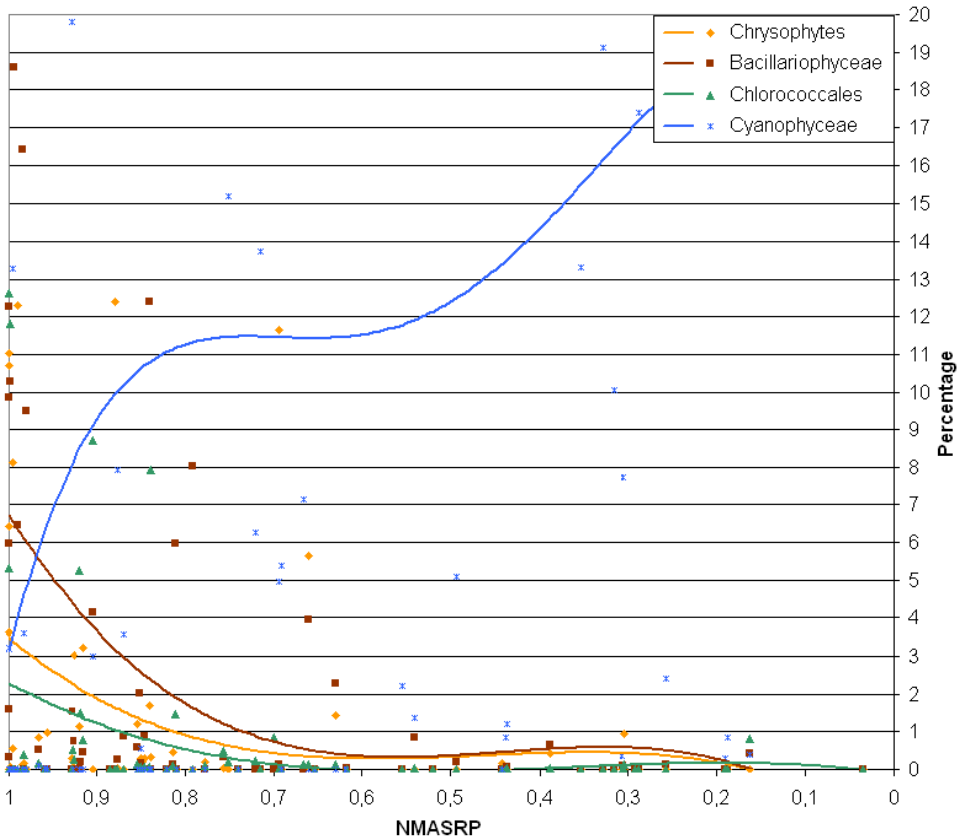


Figure I.2 Scatter-plot between NMASRP and the mean percentage contribution of different groups to the mean total biovolume per reservoir year. Chrysophytes (Orange) include the genera Dinobryon, Pseudopedinella, Ochromonas and Chrysochromulina. Bacillariophyceae (Brown) include genr Asterionella, Nitzschia, Discostella and the species Ulnaria ulna. Chlorococcals include genres Ankyra, Sphaerocystis and Coenochloris, and the species Crucigenia tetrapedia and Monoraphidium minutum. Cyanophyceae includes the genera Aphanizomenon, Woronichinia and Anabaena. The lines represent polynomial adjustments.

Calcareous reservoirs

There is a clear relation of certain phytoplanktonic groups and taxons with the change of classes from good to moderate in Calcareous reservoirs. The community is composed, when quality is still high, near the MEP community type, mainly of diatoms. The genera Cyclotella and Achnanthes, together with species as Ulnaria acus and Ulnaria ulna are typical from high quality communities, and peak at MEP sites. Not only they are typical, but the steadily decrease towards the G/M status, and below this limit, in parallel to the increase in cyanobacterial and Chlorococcales presence, which implies the main change in the community. Chlorococcales genera considered in this analysis are Coelastrum, Scenedesmus and Pediastrum. These three groups together with the main cyanobacterial genera such as Anabaena, Microcystis, Aphanizomenon and others start to dominate the community at the G/M threshold (Figure I.3 and Figure I.4).

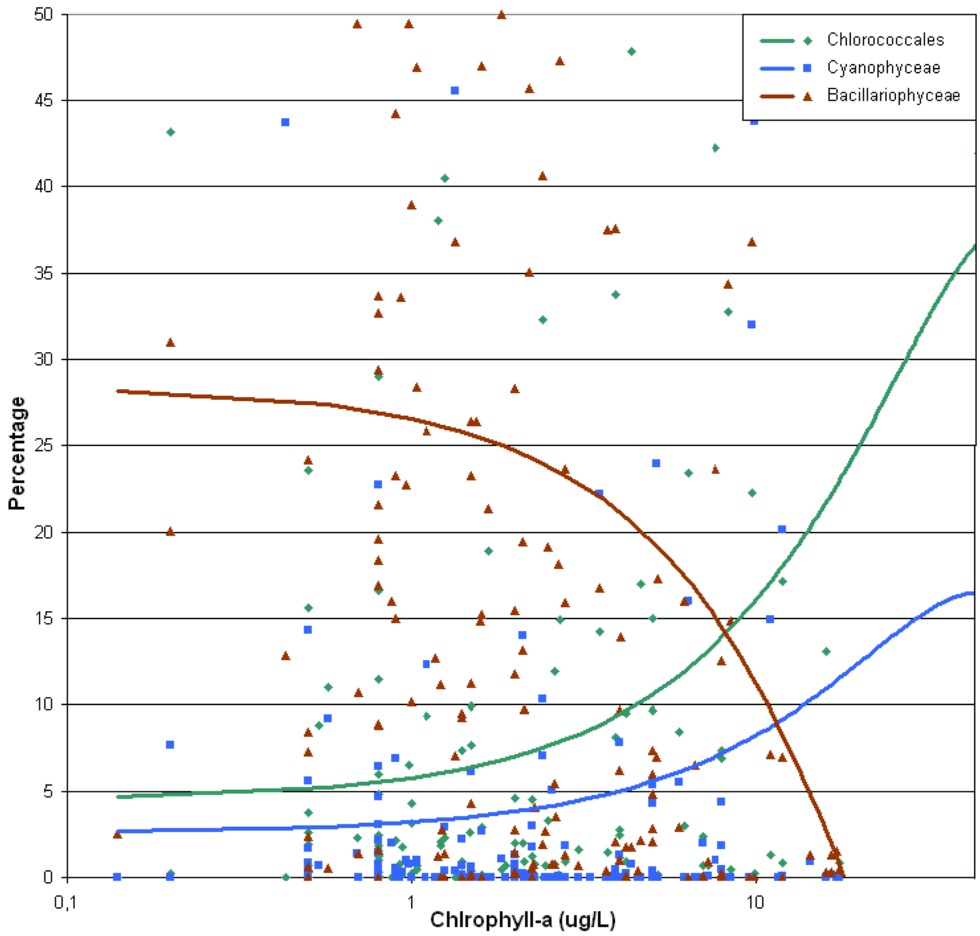


Figure I.3 Scatter-plot between chlorophyll-a and the mean percentage contribution of different groups to the mean total biovolume per reservoir year. Bacillariophyceae (Brown) include genera Cyclotella and Achnanthes, and the species Ulnaria ulna and Ulnaria acus. Chlorococcales include genera Coelastrum, Scenedesmus and Pediastrum. The lines represent polynomial adjustments.

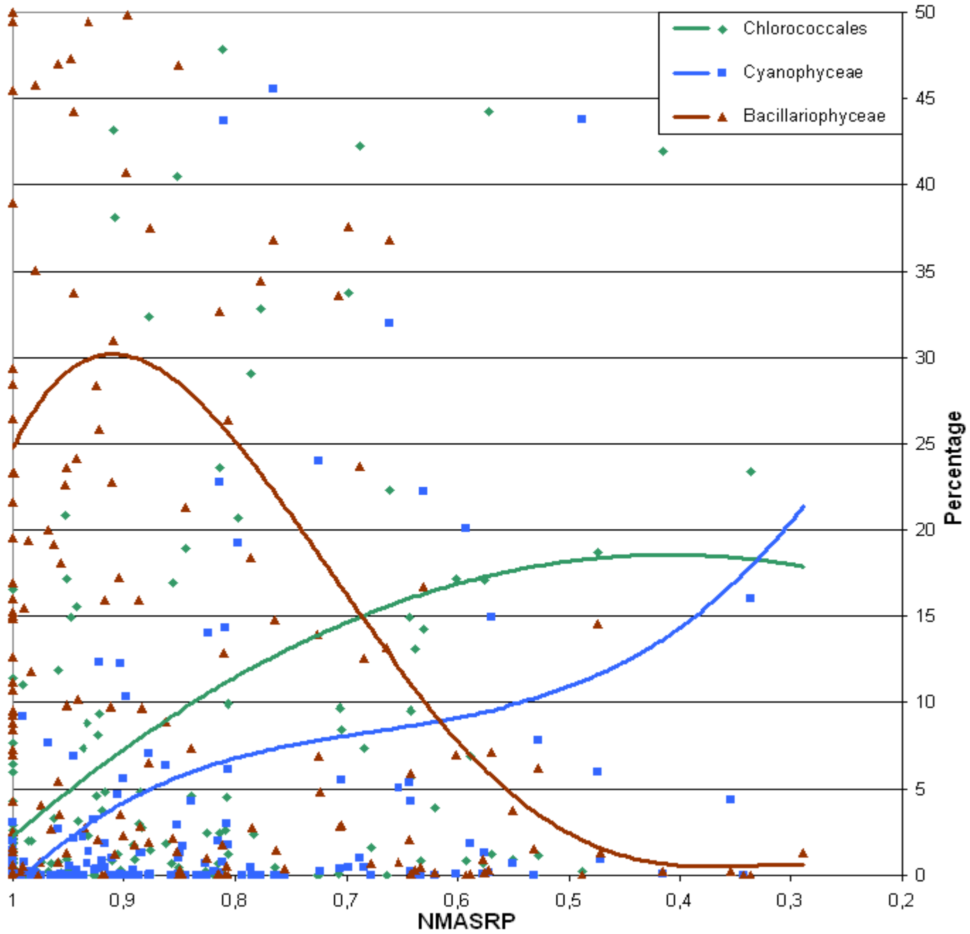


Figure I.4 Scatter-plot between NMASRP and the mean percentage contribution of different groups to the mean total biovolume per reservoir year. Bacillariophyceae (Brown) include genera Cyclotella and Achnanthes, and the species Ulnaria ulna and Ulnaria acus. Chlorococcales include genera Coelastrum, Scenedesmus and Pediastrum. The lines represent polynomial adjustments

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Abstract

One of the key actions identified by the Water Framework Directive (WFD; 2000/60/EC) is to develop ecological assessment tools and carry out a European intercalibration (IC) exercise. The aim of the Intercalibration is to ensure that the values assigned by each Member State to the good ecological class boundaries are consistent with the Directive's generic description of these boundaries and comparable to the boundaries proposed by other MS.
In total, 83 lake assessment methods were submitted for the 2nd phase of the WFD intercalibration (2008-2012) and 62 intercalibrated and included in the EC Decision on Intercalibration (EC 2013). The intercalibration was carried out in the 13 Lake Geographical Intercalibration Groups according to the ecoregion and biological quality element. In this report we describe how the intercalibration exercise has been carried out in the Mediterranean Lake Phytoplankton group.

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