THE GOULANDRIS NATURAL HISTORY MUSEUM
GREEK BIOTOPE/WETLAND CENTRE

MINISTRY OF ENVIRONMENT & ENERGY

REPORT

ON THE APPLICATION OF PHYTOPLANKTON INDEX NMASRP FOR RESERVOIRS IN GREECE

June 2016

The present study has been prepared in the framework of the Greek National Water Monitoring Network, according to the Joint Ministerial Decision 140384/2011. The Network is supervised by the Directorate for the Protection and Management of Water Resources of the Special Secretariat for Waters of the Ministry of Environment and Energy. The data used in this report come from the Acts MIS. 371010, 371138, 371140, 371144, 371145 of the Operational Program "Environment and Sustainable Development" financed by the European Regional Development Fund.

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1. INTRODUCTION

During the two rounds of Med GIG Intercalibration Excersice (IC), phytoplankton assessment methods had been intercalibrated for lake types LM 5/7 (deep siliceous reservoirs) and LM 8 (deep calcareous reservoirs) (de Hoyos et al. 2014). In the first round of IC that Greece participated, boundaries for individual phytoplankton metrics (chlorophyll-a, total biovolume, percentage of cyanobacteria, IGA and MedPTI) were agreed (using option 1 of IC) and formally included in the 2008 EC Intercalibration Decision. During the second round of IC, national assessment methods based on the above metrics (NMASRP for Cyprus and Portugal, MASRP for Spain and NITMET for Italy) were intercalibrated for LM 5/7 and LM 8 types. Greece had not started the operation of the monitoring network at that time, and thus had not submitted its national assessment method for the second round of IC. The operation of the Greek monitoring network started in 2012, following the publication of a Joint Ministerial Decision in 2011. Fifty lake water bodies have been included in the monitoring network, out of which 15 are LM 5/7 and 5 are LM 8 reservoirs.

The national phytoplankton assessment method applied for Greek LM 5/7 and LM 8 reservoirs is the New Mediterranean Assessment System for Reservoirs Phytoplankton (NMASRP), that has been intercalibrated in the Med GIG, as applied by Cyprus and Portugal (i.e. the metric *percentage of cyanobacteria* is replaced by the metric *biovolume of cyanobacteria*, in order to account for algal blooms) (de Hoyos et al. 2014). Spain uses MASRP (i.e., with the metric *percentage of cyanobacteria*). NMASRP addresses eutrophication pressure in Mediterranean reservoirs.

This report does not discuss the development of new or revised ecological assessment method for phytoplankton according to finalized IC results, but the application of the existing NMASRP phytoplankton assessment method as intercalibrated in the Med GIG (including existing MEP values and EQRs), into the national dataset. NMASRP values are calculated as annual mean. This dataset contains data collected during the 2012-2015 sampling campaign, and in particular 53 NMASRP values for LM 5/7 and 18 NMASRP values for LM 8 reservoirs arising from 139 and 56 samplings, respectively.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

Greece applies the New Mediterranean Assessment System for Reservoirs Phytoplankton (NMASRP) in LM 5/7 and LM 8 reservoirs. The method is described in Annex A.2 of the JRC Technical Report (de Hoyos et al. 2014). The main features are: 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) Total Biovolume (mm^3/L)
Composition	IGA (Index Des Grups Algals) BV of cyanobacteria (mm^3/L)

Table 1. Overview of the metrics included in the NMASRP

MS	Taxonomic composition	Abundance	Frequency and intensity of algal blooms
GR	IGA index	Chl-a concentration Total biovolume	Biovolume of cyanobacteria

The description of NMASRP is given in A.2. of JRC Technical Report (de Hoyos et al. 2014), and briefly described below.

NMASRP is a mulitmetric index of 4 parameters where all of them have equal weights. These parameters are either related with biomass or composition and are the following:

1. <u>Chlorophyll-a (μg/l)</u>

An easily measurable parameter related to biomass which has a strong relationship with eutrophication pressure as expressed by Total Phosphorus.

2. <u>Total Biovolume (mm^3/l)</u>

This parameter is considered to be the most robust for freshwater phytoplankton as far as biomass is concerned.

3. <u>IGA (Index Des Grups Algals)</u>

The IGA index is calculated according to the following equation and is based on the percentage of major taxonomic groups found in the sample. This equation is suitable only for the samples where the represented taxonomic groups add up to70% or more of the sample.

$$CI = [1+0.1Cr+Cc+2(Dc+Chc) + 3Vc +4Cia] / [1+ 2(D+Cnc) + Chnc+Dnc]$$

4. <u>Biovolume of cyanobacteria (mm^3/L)</u>

This parameter is considered to be an indicator of algal blooms and it takes into account all species of cyanobacteria, excluding class Chroococcales with the exception though of *Woronichinia* and *Microcystis*, to avoid misinterpretation of eutrophic conditions.

The EQR values for each parameter are calculated according to the formulae given in Table 2 and then these values are being normalized (EQR → nEQR) following the equations in Table 3.

Table 2. Equations built for the calculations of EQR values for LM 5/7 and LM 8 reservoirs. MEP 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	LM 5/7	1.7	(1/x)/(1/1.7)
	LM 8	1.9	(1/x)/(1/1.9)
Total Biovolume	LM 5/7	1.2	(1/x)/(1/1.2)
	LM 8	0.9	(1/x)/(1/0.9)
IGA	LM 5/7	2	(400-x)/(400-2)
	LM 8	2.1	(400-x)/(400-2.1)
Cyano Biovolume	LM 5/7	0.02	(1/x)/(1/0.02)
	LM 8	0.005	(1/x)/(1/0.005)

Table 3. Normalization equations for EQR values for all different parameters and types. Each equation should be selected from left to right.

	Reservoir IC type	MEP value	Normalizing equation
Chlorophyll-a	LM 5/7	>7.9	nEQR = 2.7882*EQR
		<7.9	nEQR = 0.5097*EQR + 0.4903
	LM 8	>5.3	nEQR = 1.6737*EQR
		<5.3	nEQR = 0.6235*EQR + 0.3765
Total Biovolume	LM 5/7	>2.8	nEQR = 1.4*EQR
		<2.8	nEQR = 0.7*EQR + 0.3
	LM 8	>2.5	nEQR = 1.6667*EQR
		<2.5	nEQR = 0.625*EQR + 0.375
IGA	LM 5/7	>37.6	nEQR = 0.6589*EQR
		<37.6	nEQR = 4.4719*EQR – 3.4719
	LM 8	>6.5	nEQR = 0.6067*EQR
		<6.5	nEQR = 36.173*EQR – 35.173
Cyano Biovolume	LM 5/7	>0.8	nEQR = 24*EQR
		<0.8	nEQR = 0.4103*EQR + 0.5897
	LM 8	>0.5	nEQR = 60*EQR
		<0.5	nEQR = 0.404*EQR + 0.596

The last step of the method is the application of the equation given below which requires at least one composition and one biomass parameter.

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

The combination rule used is the arithmetic average of normalized EQRs. The NMASRP is WFD compliant, since parameters indicative of phytoplankton taxonomic composition, abundance, and frequency and intensity of algal blooms according to Annex V (1.2.2.) of WFD are included.

2.2. SAMPLING AND DATA PROCESSING

Greece uses similar sampling strategies and data processing techniques with all Mediterranean countries, as follows:

Table 4. Phytoplankton sampling and data processing description.

Item	Description
Frequency per year	2-4 samples during the growing season (May to October).
Sampling methods	Integrated sampling from euphotic zone (2.5xSecchi Depth), in open waters, from the deepest part of the lake, at a distance >100 m from the dam.
Data processing	Chl α is determined using 90% acetone and applying the trichromatic equation (Jeffrey and Humphrey 1975) (APHA 2012); phytoplankton composition and biovolume, using inverted microscopy (Utermöhl technique) (ISO EN 15204: 2006)
Level of identification	Species level when possible, genus level

2.3. NATIONAL REFERENCE CONDITIONS

The criteria for deriving the maximum ecological potential (MEP) conditions from existing reservoirs were set in the Med GIG, as described by de Hoyos et al. (2014) and Pahissa et al. (2015) and were applied in the national dataset. These were CORINE landcover parameters (i.e. Artificial Land Use %, Intensive Agriculture % and Natural and Semi-Natural Areas %), Population Density (no of inhabitants/km^2) and Total Phosphorus (mean annual values). Two threshold values were selected for each variable: a “Rejection limit” and a “Reference limit” as follows:

Table 5. Rejection and reference limits for the five selected pressures.

	ALU (%)	IA (%)	NASNA (%)	PD (no/km^2)	TP (μg/l)
Rejection limits	<4	<20	>70	<30	<30
Reference limits	<1	<10	>80	<10	<12

Source: Pahissa et al. 2015

Nine out of 20 reservoirs of the national dataset (8 LM 5/7 and 1 LM 8) were selected as potential MEP sites through pressure screening, since they passed the reference threshold values. It is noted that reservoir no 8 had been also considered as a MEP when included in the IC dataset (1 of 29 in the Med GIG). After this first step, in order to validate or disqualify reservoirs as MEP sites, the phytoplankton community was taken into consideration, and especially the contribution of cyanobacteria to total biovolume (fig. 1). During the second screening, one reservoir was removed from the list of MEP sites (fig. 2.). The list of all sites, with their characteristics, including the final list of MEP sites, is given in table 6.

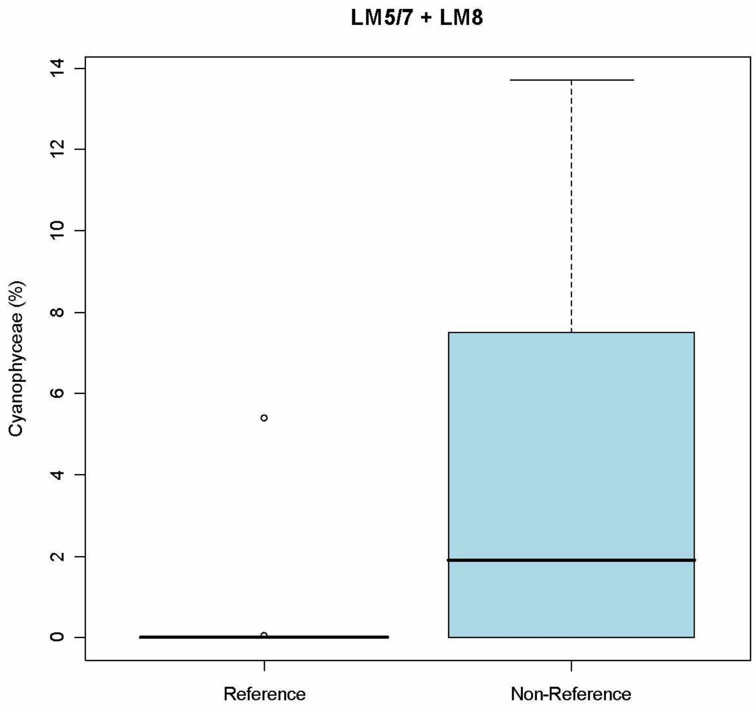


Figure 1. Contribution of cyanobacteria biovolume to total biovolume in potential MEP and non MEP sites (before removal of reservoir no 9).

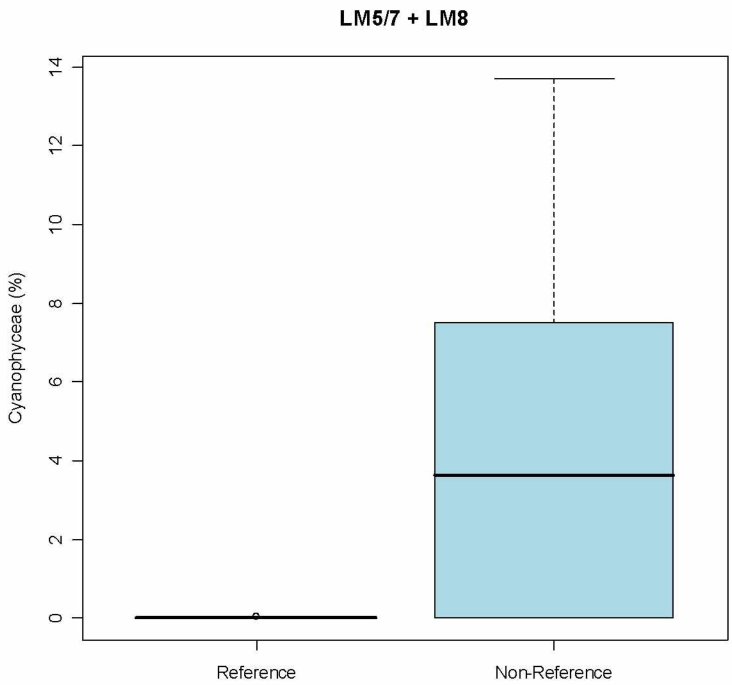


Figure 2. Contribution of cyanobacteria biovolume to total biovolume in potential MEP and non MEP sites (after removal of reservoir no 9).

Table 6. List of all LM 5/7 and LM 8 reservoirs, including those finally fitting the criteria for maximum ecological potential.

No	Lake name	TYPE	**Artificial land use** (% on EXISTING clc coverage)	**Intensive agriculture** (% on EXISTING clc coverage	**Natural and semi-natural areas** (% on EXISTING clc coverage)	**Population density** (no of inhabitants /km² of catchment)	**TP** (μg/l, mean annual)[1]	**Potential MEP**	**Final MEP**
1	Techniti Limni Pigon Aoou	LM 5/7	0,00	1,84	98,16	0,00	13,94	**Y**	**Y**
2	Techniti Limni Mornou	L-M5/7	0,88	2,34	90,08	9,67	9,55	**Y**	**Y**
3	Techniti Limni Kastrakiou	LM 5/7	0,31	1,23	90,03	9,88	12,46	**Y**	**Y**
4	Techniti Limni Polyfytou	LM 5/7	1,36	26,17	60,87	30,61	21,94	N	N
5	Techniti Limni Evinou	LM 5/7	0,56	0,49	93,19	6,06	10,26	**Y**	**Y**
6	Techniti Limni Sfikias	LM 5/7	1,35	25,60	61,41	30,05	16,80	N	N
7	Techniti Limni Asomaton	LM 5/7	1,39	25,34	61,62	29,74	20,07	N	N
8	Techniti Limni Tavropou	LM 5/7	0,00	6,42	85,25	14,57	10,96	**Y**	**Y**
9	Techniti Limni Gratinis	LM 5/7	0,16	0,00	95,68	1,80	17,41	**Y**	N
10	Techniti Limni Feneou	LM 5/7	0,00	0,00	95,47	0,00	10,85	**Y**	**Y**
11	Techniti Limni Pournariou	LM 5/7	0,54	0,93	83,24	15,47	8,63	**Y**	**Y**
12	Techniti Limni Platanovrysis	LM 5/7	1,39	5,06	82,28	30,07	21,25	N	N
13	Techniti Limni Faneromenis	LM 5/7	0,72	31,45	66,52	35,68	18,67	N	N
14	Techniti Limni Bramianon	LM 5/7	0,00	44,55	46,56	10,98	10,86	N	N
15	Techniti Limni Thisavrou	LM 5/7	1,50	5,54	80,63	32,91	21,36	N	N
16	Techniti Limni Marathona	LM 8	11,92	26,07	43,12	193,80	8,98[2]	N	N
17	Techniti Limni Ladona	LM 8	0,30	9,18	74,31	10,22	17,99	N	N
18	Techniti Limni Kremaston	LM 8	0,29	0,72	91,40	9,82	10,74	**Y**	**Y**
19	Techniti Limni Smokovou	LM 8	1,72	23,51	66,02	11,46	17,06	N	N
20	Techniti Limni Pineiou	LM 8	0,13	26,18	43,04	20,08	13,26	N	N

[1] TP for 2015 only. Seasonal samples were averaged for mean annual, except for reservoir no 5, where only 2 values were available.

[2] Reservoir no 16 is part of a drinking water supply network for Athens, and receives water from reservoirs no 2 and 5 and a natural lake.

The distribution of total phosphorus, chlorophyll α and total biovolume in LM 5/7 and LM 8 Greek reservoirs are shown in Figure 3. The differences between MEP and non-MEP sites are observed.

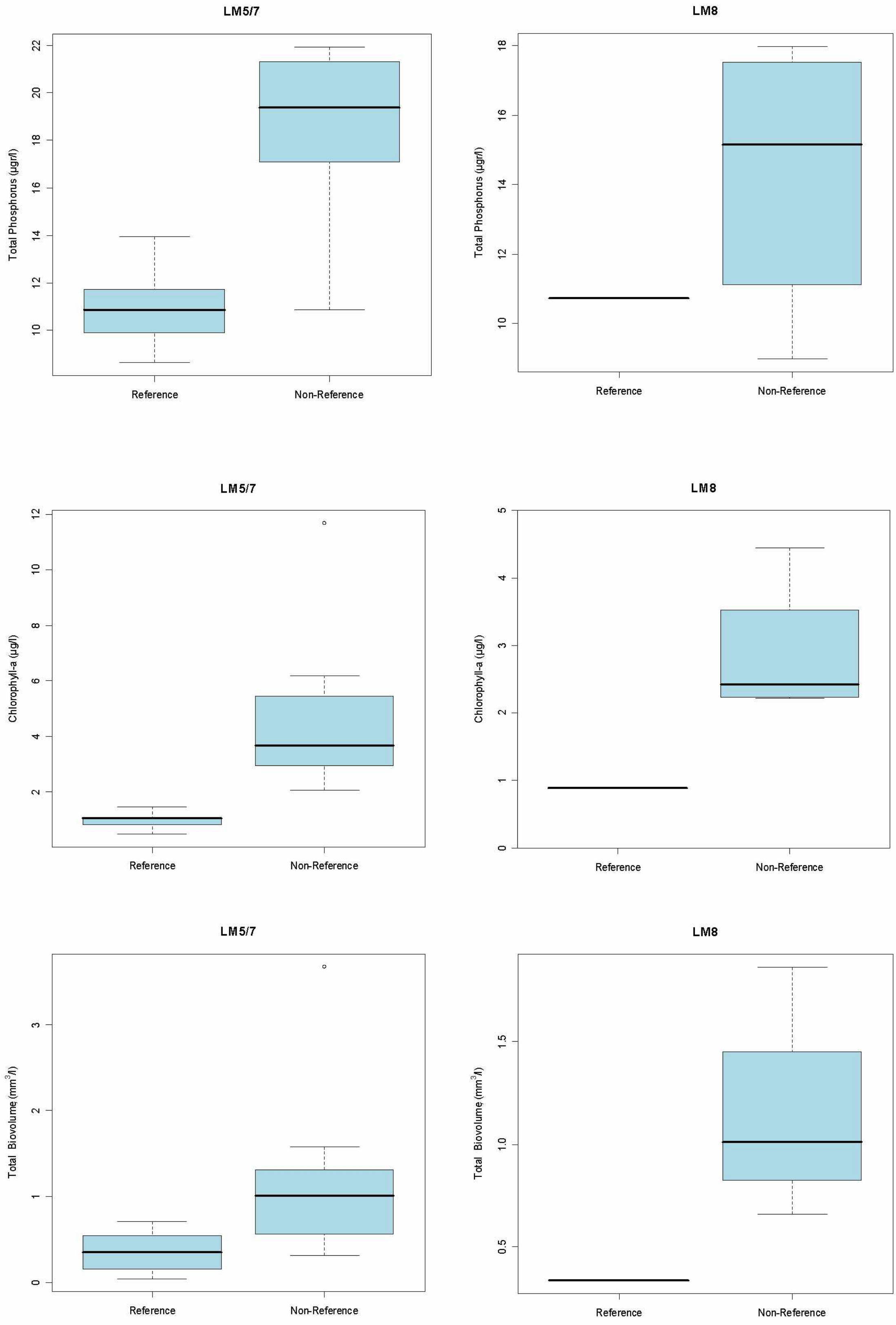


Figure 3. Distribution of total phosphorus, chlorophyll a and total biovolume in MEP and non- MEP LM 5/7 and LM 8 Greek sites. TP values available only for 2015.

2.4. NATIONAL BOUNDARY SETTING

Greece uses the same boundaries as Portugal and Cyprus for all parameters. The detailed description of methodology used to derive ecological class boundaries is given in the JRC Intercalibration Technical Report (de Hoyos et al. 2014), and in particular, in Annex D1 of this report.

Below we provide the statistical distribution of certain phytoplankton parameters in relation to TP, in order to demonstrate the response of each parameter throughout the trophic gradient in the national dataset. In particular, box-plots of Chl a and IGA values against TP are given for LM 5/7, LM 8 and LM 5/7+LM 8 reservoirs. With regard to total biovolume and cyanobacteria biovolume box-plots against TP, data from LM 8 reservoirs were not treated separately as they were limited and did not provide any meaningful explanation. In general, biomass and composition values are relatively low, cyanobacteria biovolume values are quite low, whereas maximum annual mean TP values do not exceed 22 μg/l, in any of the sites (see table 6).

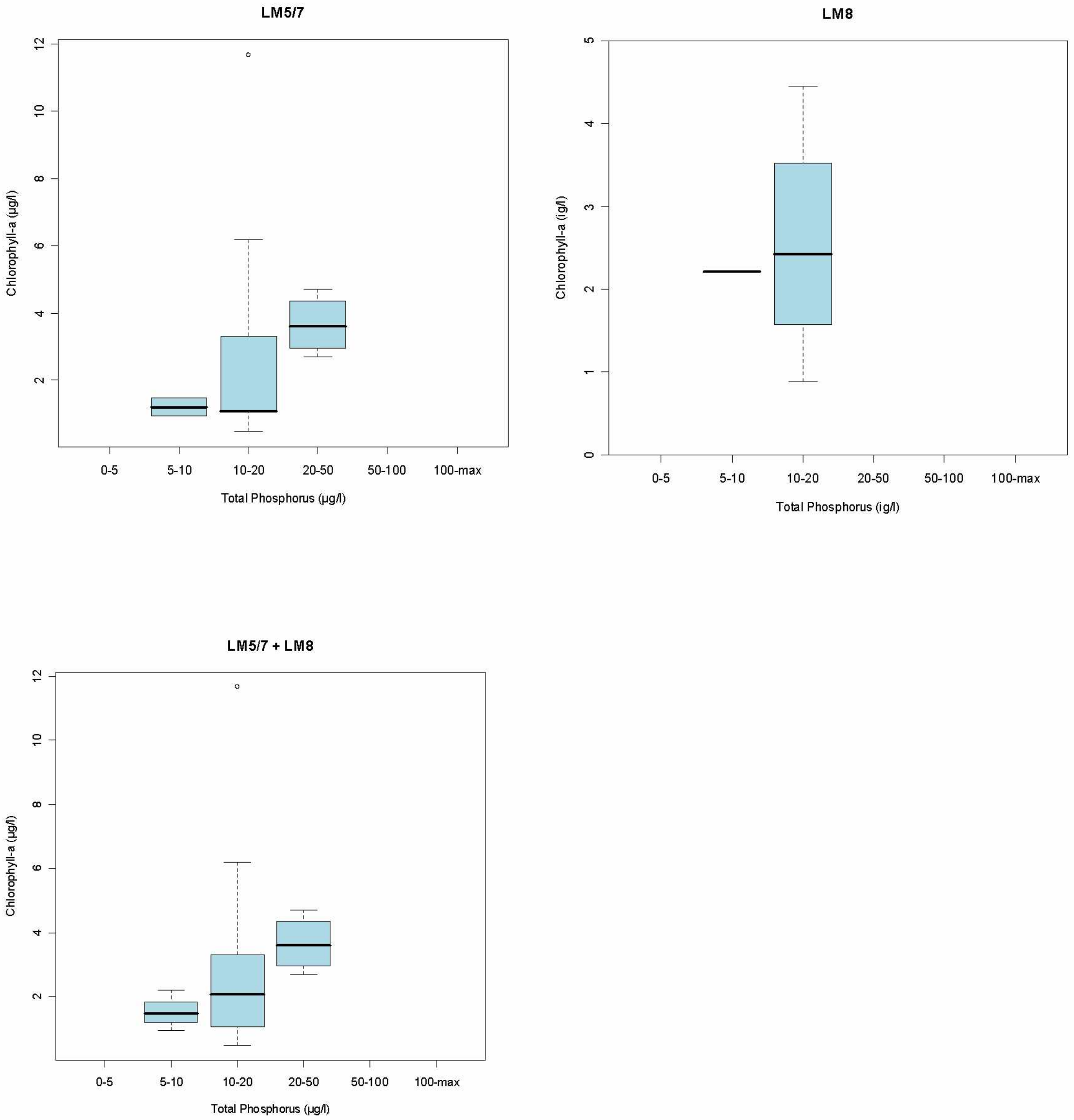
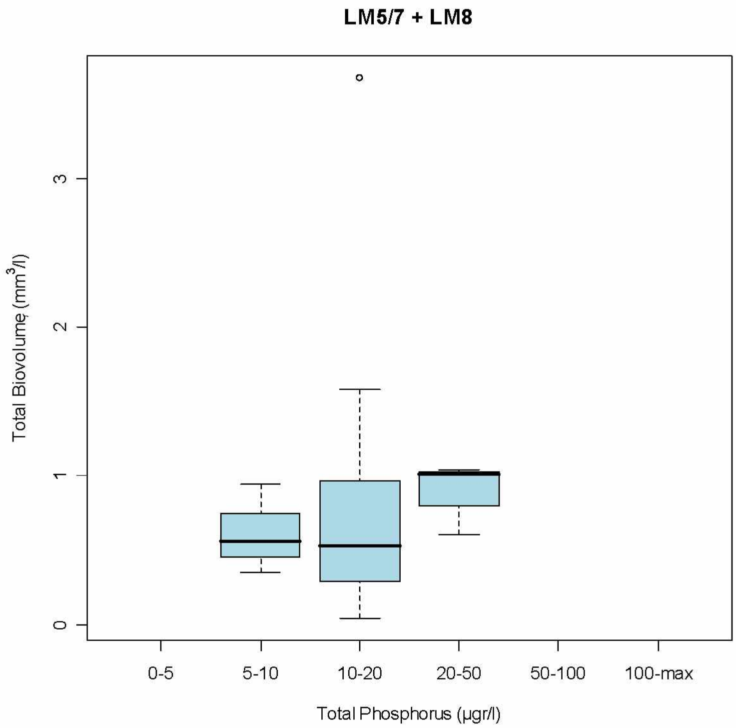


Figure 4. Chlorophyll α values (mean of growing season) plotted against Total Phosphorus groups of values (annual mean) in LM 5/7, LM8 and in all sites together.



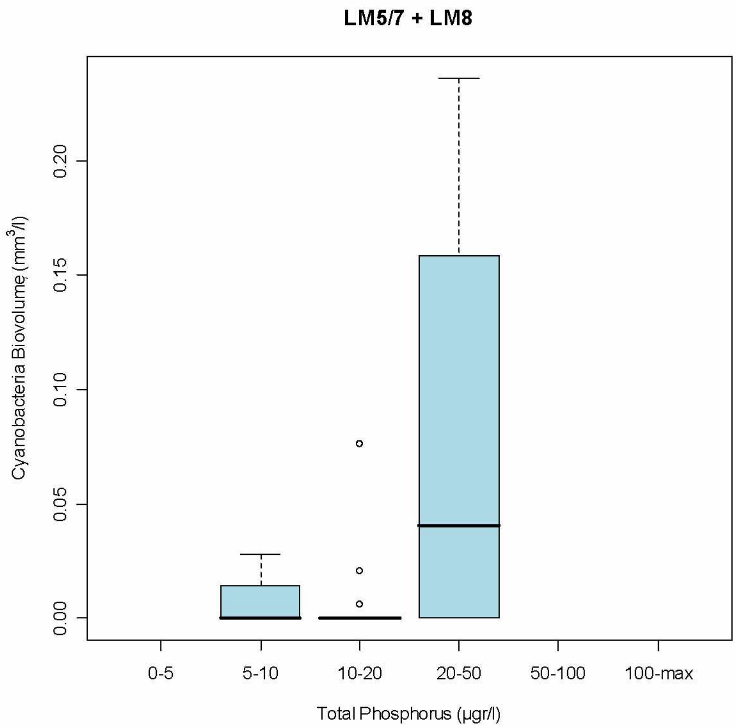


Figure 5. Total biovolume and cyanobacteria biovolume values of LM 5/7 + LM 8 reservoirs (mean of growing season), plotted against Total Phosphorus groups of values (annual mean).

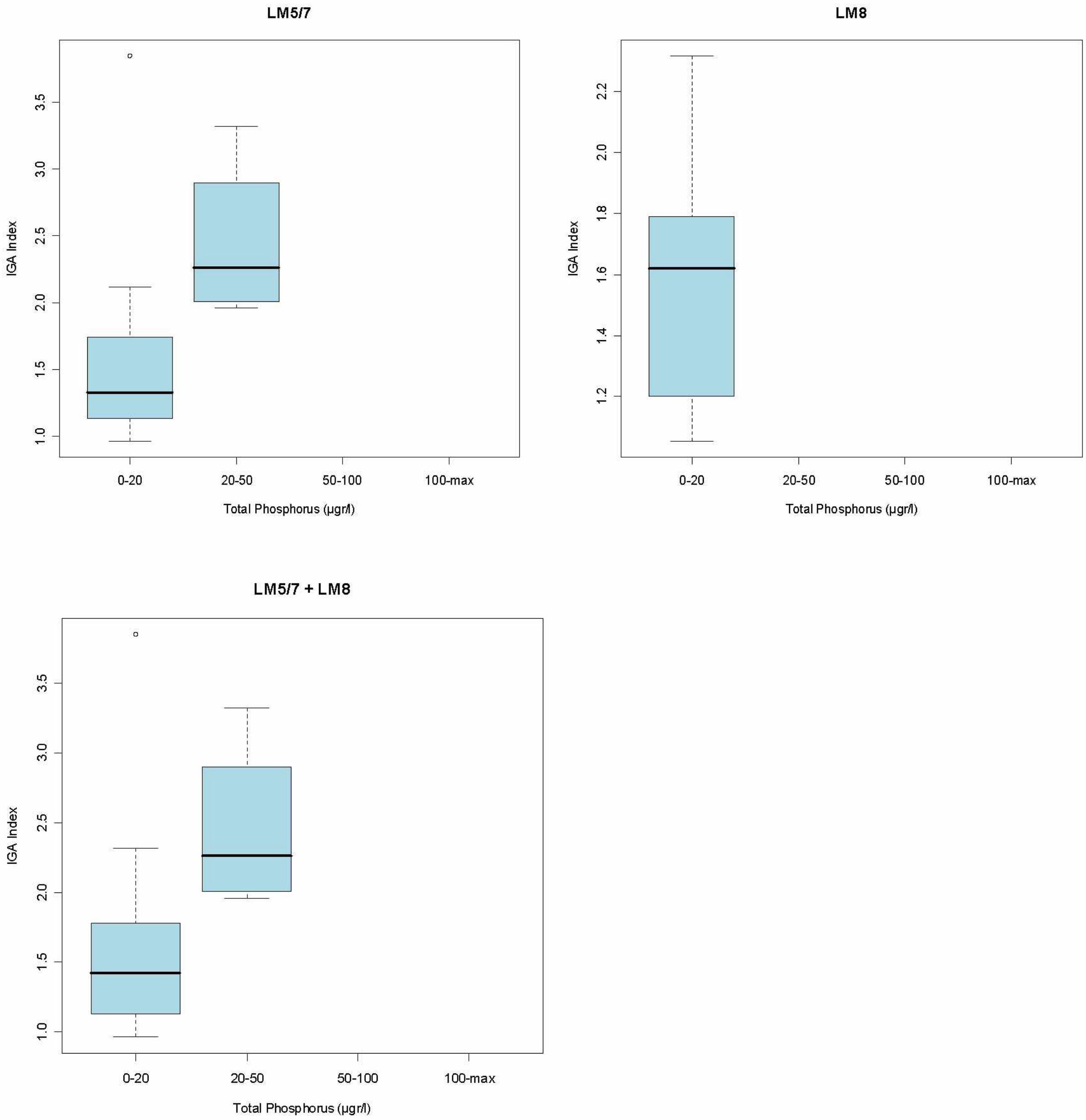
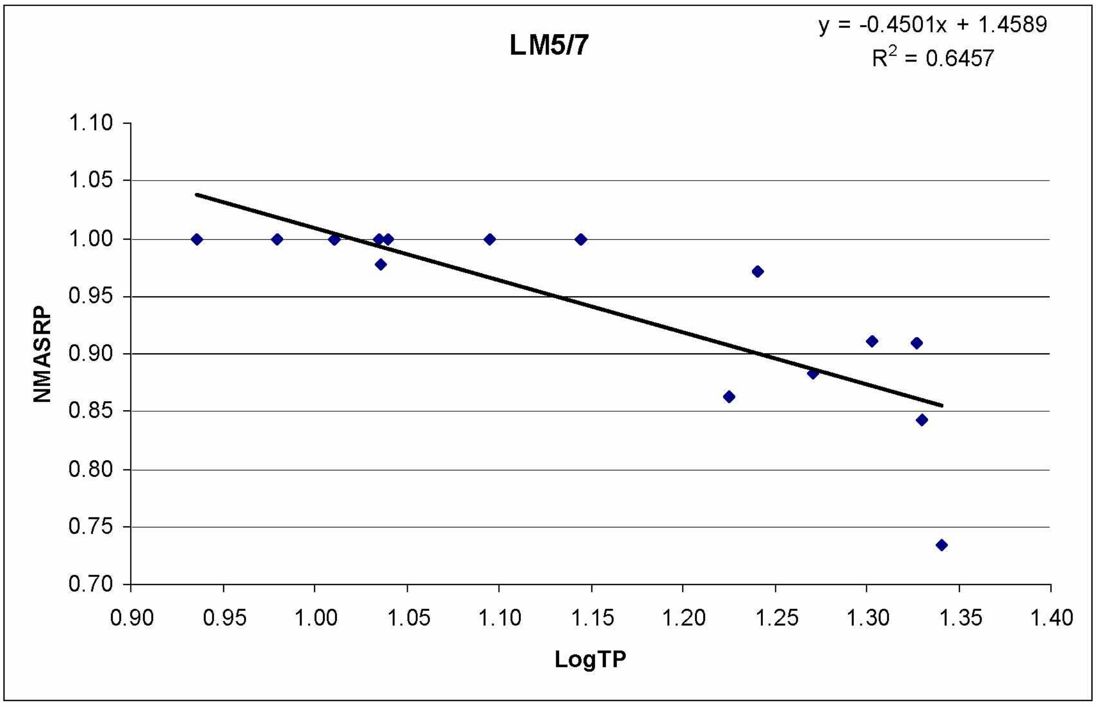


Figure 6. Distribution of IGA index (mean of growing season) in relation to Total Phosphorus (annual mean), in LM 5/7, LM8 and in all sites together.

NMASRP addresses eutrophication pressure in Mediterranean reservoirs. We use Total Phosphorus and Land uses as proxies to eutrophication and the pressure – response curves follow. The relationship of NMASRP with TP was significant in LM 5/7 sites and where all sites are grouped together. LM 8 does not exhibit significant correlation with TP (R^2 = 0.1784, p=0.47865), however, only a small dataset was available (only 5 reservoirs).



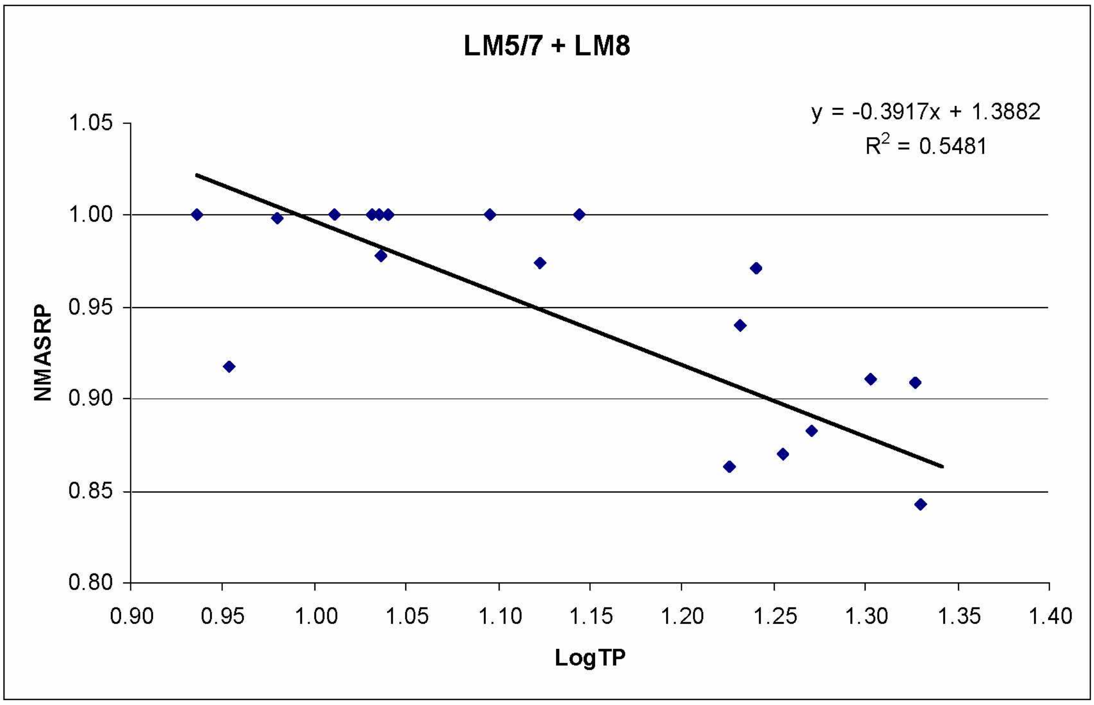


Figure 7. Relation between mean annual NMASRP and TP in LM 5/7 and LM 5/7 and LM8 reservoirs.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 7. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	We apply the method, as intercalibrated, i.e. with MEP and the boundary for Good/Moderate ecological potential. Due to data limitations, since all LM 5/7 and LM 8 reservoirs are above the Good Ecological Potential, no data are available in the national dataset for the whole trophic range. In the future, if applicable, 4 classes will be considered (High and Good classes merged into Good and above).
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	Yes, arithmetic average is the combination rule.
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	Yes, assessment applies for LM 5/7 and LM 8 common intercalibration types.
The water body is assessed against **type-specific near-natural reference conditions**	Yes
Assessment results are expressed as **EQRs**	Yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	Yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods (“apples and pears”) has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an “IC feasibility check” to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.

Yes, the above method addresses the LM 5/7 and LM 8 common intercalibration types, according to 2013 Intercalibration Decision.

Common IC type	**Type characteristics**	**MS sharing IC common type**
LM 5/7	Reservoirs, deep, large, siliceous	Greece, France, Italy, Portugal, Romania, Spain
LM 8	Reservoirs, deep, large, calcareous	Greece, Cyprus, France, Italy, Romania, Spain

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

The NMASRP addresses eutrophication pressure. In table 8 we give the Pearson coefficient and p-values of the regression equations between NMASRP and land use and TP for Greece, Portugal and Cyprus that apply the index. The numbers of PT and CY are taken from de Hoyos et al. (2014). NMASRP values in LM 8 are not significantly related to Land Use and TP, however, a small dataset was available; we envisage that with the collection of more data, this will change. When both LM 8 and LM 5/7 are included in the equation, a significant relationship with TP is formed.

Table 8. Relations between NMASRP and Land Use, and TP.

Mean NMASRP 2015 ~ Land Use and TP

GR	LM 5/7		IA	PD	NASN	ALU	TP
		Pearson	-0,48761	-0,800264	0,57109	-0,75984	-0,80356
	NMASRP	**Significance**	0,06522	**0,00034**	0,02617	**0,000157**	**0,000307**

PT-CY	LM 5/7		IA	PD	NASN	ALU	TP
		Pearson	-0,13	-0,43	0,23	-0,57	-0,66
	NMASRP	**Significance**	n.s.	**<<0.01**	n.s.	**<<0.01**	**<<0.01**

GR	LM 8		IA	PD	NASN	ALU	TP
		Pearson	-0,13822	-0,237224	0,16156	-0,26408	-0,42239
	NMASRP	**Significance**	0,66772	0,700814	0,79519	0,66772	0,47865

PT-CY	LM 8		IA	PD	NASN	ALU	TP
		Pearson	-0,1	-0,3	0,2	-0,15	0
	NMASRP	**Significance**	n.s.	**<<0.01**	**<<0.01**	0,05	n.s.

GR	All lakes (LM 5/7 +LM 8)		IA	PD	NASN	ALU	TP
		Pearson	-0,43525	-0,27135	0,43472	-0,21604	-0,74031
	NMASRP	**Significance**	0,05510	0,24717	0,05544	0,36029	**0,00019**

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods

It is one common method, so the assessment concept is uniform.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Provide conclusions on the IC feasibility.

This report provides data and information on the application, in Greece, of NMASRP, a common method intercalibrated in the MED GIG, using option 1, with common MEP and G/M boundary. This method is identically applied in Portugal, Cyprus and Greece. Thus, there is a full compliance with the intercalibration results, in EQR, as presented in the Commission Decision 2013/480/EU.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

It is one common method with regard to sampling, data processing and analysis, boundary setting procedure, MEP values and Good/Moderate boundaries, applied by Cyprus, Portugal and Greece. It is has been intercalibrated within the MED GIG, for which Option 1 was chosen. Thus there is full compliance with the completed IC. This report describes the application of this method, as the national assessment method by Greece, since no monitoring data and national assessment method had been available by the time that round 2 of IC was carried out.

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

The description of phytoplankton communities at high and good status are described below.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

LM 5/7 reservoirs

The MEP phytoplankton community is relatively poor and is composed mainly of chrysophytes together with good quality indicator diatoms. The genus Dinobryon from chrysophytes and the genera Cyclotella, Synedra, Asterionella from the diatoms are typical from MEP sites. Certain chlorophytes such as genera Scenedesmus and Sphaerocystis and dinoflagellates, such as Ceratium and Peridinium are also present.

LM 8 reservoirs

The MEP phytoplankton community is relatively poor, composed mainly of diatoms and mostly genera Cyclotella and Synedra and Genus Dinobryon from Chysophytes. Dinoflagellates such as Ceratium and Peridinium are also present.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

LM 5/7 reservoirs

Chrysophytes generally decrease. With regard to diatoms, in addition to Cyclotella and Synedra, genera Aulacoseira and Nitzchia are becoming more abundant, whereas the presence of Fragilaria, and Tabellaria are noted. Chlorophytes are appearing in the phytoplankton community, represented by genera Scenedesmus, Planktonema, Tetraedron, Sphaerocystis and Oocystis. Cyanobacteria, although holding overall a small percentage of total biovolume, begin to appear, represented mostly by genera Planktothrix, Aphanizomenon, and Microcystis.

LM 8 reservoirs

Chrysophytes generally decrease. With regard to diatoms, Cyclotella are the main part of the community, whereas chlorophytes, such as Sphaerocystis appear. Cyanobacteria, although holding overall a small percentage of total biovolume, begin to appear, represented mostly by Microcystis.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

There aren't any sites in moderate status.

7. REFERENCES

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