THE GOULANDRIS NATURAL HISTORY MUSEUM
GREEK BIOTOPE/WETLAND CENTRE

MINISTRY OF ENVIRONMENT & ENERGY

REPORT

ON THE DEVELOPMENT OF THE NATIONAL METHOD FOR THE ASSESSMENT OF ECOLOGICAL STATUS OF NATURAL LAKES IN GREECE, USING THE BIOLOGICAL QUALITY ELEMENT "PHYTOPLANKTON"

1st revision

January 2017

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.
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1. INTRODUCTION

This report discusses the development of a national method for the assessment of ecological status of natural lakes in Greece, based on the Biological Quality Element (BQE) “phytoplankton”; it is a revised version of the 2016 report, following comments by JRC and Intercalibration Review Panel experts.

The main changes include an amendment to modNygaard index (exclusion of Centrales, see 2.1), the exclusion of a lake’s data from the reference dataset in order to avoid circularity in the assessment, and construction of pressure response curves with phytoplankton and TP data from lake years 2014 and 2015.

At the Mediterranean Lake Geographical Intercalibration Group, Member States defined two common water body types for reservoirs (L-M5/7 and L-M8). In spite of common efforts, within the Mediterranean GIG, there was no possibility to intercalibrate natural Mediterranean lakes because of the absence of common types with enough lakes[1]. As a result, no assessment methods for phytoplankton of natural lakes have been intercalibrated within the Med GIG.

The operation of the Greek water monitoring network started in 2012, following the publication of a Joint Ministerial Decision in 2011 and comprises 23 natural lakes. The development of the current assessment method, as described in this report, is based on the data from this national water monitoring network. Natural lakes in Greece are grouped into 3 types:

- warm monomictic, deep natural lakes with mean depth >9 m (type GR-DNL, 7 lakes);
- polymictic, shallow natural lakes with mean depth 3-9 m (type GR-SNL, 8 lakes);
- very shallow lakes < 3 m (type GR-VSNL, 8 lakes)

Up to now, 74 lake-years for all 3 types are available from the national dataset, taken from the 2012-2015 sampling campaign, i.e. 26 lake-years for GR-DNL, 31 lake-years for GR-SNL and 17 lake-years for GR-VSNL. Data from certain very shallow lakes (GR-VSNL) are scarce because of their drying up during summer months.

The national phytoplankton assessment method, developed and applied for GR-DNL and GR-SNL types, consists of metrics indicative of biomass and composition and is used to address eutrophication pressure in lakes. It differs from the NMASRP used for L-M 5/7 and L-M 8 reservoirs (de Hoyos et al. 2014), in the composition metric. In particular, it uses the Modified Nygaard Index as suggested by Estonia (Ott and Laugaste 1996, from Phillips et al. 2015) with further exclusion of Centrales, instead of the IGA index (Catalan 2003). It is also noted that a new phytoplankton community index has been recently published for lakes and reservoirs in Greece (Katsiapi et al. 2016). We checked it for compliance with regard to the detailed requirements of the WFD (boundary setting procedure, assessment results as EQRs); however, its scoring system is rather based on expert judgment and EQR’s are not derived.

In the following chapters, the development of the national assessment method and its application in 2 of the 3 types of natural lakes (GR-DNL and GR-SNL) are given. In order to develop the method, phytoplankton data from years 2012-2015 were used. The index will be tested for application in very shallow lakes (type GR-VSNL) when more data become available from the monitoring network.

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[1] A synopsis of the lake intercalibration efforts in the L-M GIG has been submitted to the ECOSTAT (9 Oct 2013).

This is the first effort to establish a national phytoplankton method for natural lakes. It may need additions, improvements and revisions in the future, e.g., trying also the IGA2 index (de Hoyos pers. comm. 2016), as well as intercalibration exercises among Member States in the Mediterranean GIG. We also consider that the adding up of data from 2016 and 2017 sampling campaigns will make the dataset more robust to statistical analyses, in order to test the application in GR-VSNL and potentially revise and/or refine reference conditions and boundaries in the other 2 types.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

Greece applies the Hellenic Phytoplankton Assessment System for Natural Lakes (HeLPhy) in types GR-DNL and GR-SNL. It is composed of 4 parameters, which 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	Modified Nygaard Index (NB) BV of cyanobacteria (mm^3/L)

2.1. METHODS AND REQUIRED BQE PARAMETERS

Table 1. Overview of the metrics included in HeLPhy

MS	Taxonomic composition	Abundance	Frequency and intensity of algal blooms
GR	Modified Nygaard Index	Chl α concentration Total biovolume	Biovolume of cyanobacteria

The HeLPhy assessment method consists of two biomass and two composition metrics as follows:

<u>Chlorophyll α</u>

Chlorophyll α has been broadly used for assessing trophic status in different freshwater ecosystems. It is easily measurable and closely related to eutrophication pressure. Due to this, this broadly accepted parameter comprises 50% of the weight of the biomass part of the multimetric method under description.

<u>Total biovolume</u>

Total biovolume is considered to be a very good biomass indicator for freshwater phytoplankton. Due to the robustness of the metric it comprises half of the weight of the biomass part of the multimetric method under description.

<u>modified Nygaard Phytoplankton Compound Quotient</u>

The modified Nygaard index is used to determine the taxonomic composition, using biomass of major groups. Ott & Laugaste (1996) have added two additional elements to the original formula: Cryptophyta and Chrysophyceae. The modified PCQ calculation (by Ott & Laugaste 1996) is further amended to exclude Centrales, since they are substantially represented both in high and good quality lakes, suggesting that they could not always validate eutrophic conditions in Greek lakes (Moustaka-Gouni and Nikolaidis 1992, Katsiapi et al. 2016). The final formula used is given below:

Equation 1

$$PCQ = \frac{Cyanophyta + Chlorococcales + Euglenophyceae + Cryptophyta + 1}{Desmidiales + Chrysophyta + 1}$$

This metric comprises 50% of the weight of the composition part of the multimetric method.

Cyanobacteria biovolume

This metric can account for the bloom sensitive metric required by the phytoplankton method guidelines (WISER Deliverable D3.1-2: Report on phytoplankton bloom metrics). It takes into account all species of cyanobacteria, excluding class Chroococcales with the exception though of *Woronichinia* and *Microcystis*, as in the NMASRP for reservoirs (de Hoyos et al. 2014) and as also foreseen in EEA specifications for national reporting. It accounts for 50% of the composition part of the multimetric method under description.

WFD compliance

Overall, the HeLPhy assessment method meets the criteria needed for WFD compliance. Parameters for taxonomic composition, abundance and algal blooms are assessed by the metrics described above. The final lake phytoplankton score is calculated by determining the arithmetic average of each parameter score. Abundance and composition metrics are combined with equal weights in a final Ecological Quality Ratio for each lake, with 5 classes of ecological assessment (High, Good, Moderate, Poor, and Bad).

2.2. SAMPLING AND DATA PROCESSING

Overview

Table 2. Overview of sampling and data processing for HeLPhy assessment method.

Item	Description
Frequency per year	2-4 samples during the growing season (May to October).
Sampling methods	Integrated sampling from euphotic zone (2.5 x Secchi Depth), in open waters, from the deepest part of the lake.
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	Genus level, species level when possible

2.3. NATIONAL REFERENCE CONDITIONS

The setting of national reference conditions is based on existing near-natural reference sites according to the procedure recommended by the REFCOND Guidance document No. 10, on River and lakes typology, reference conditions and classification systems (European Commission 2003). The method chosen for establishing reference conditions is based on pressure criteria which are used as a screening tool, and then, on estimating spatially based reference conditions, using data from monitoring sites.

The screening criteria elaborate the degree of acceptable change in an anthropogenic pressure that would provide the limits of high status for a lake. These criteria chosen for selecting potential reference condition sites, are among the ones proposed by REFCOND (European Commission 2003) and the pressure indicators used commonly in literature (Poikane et al. 2015):

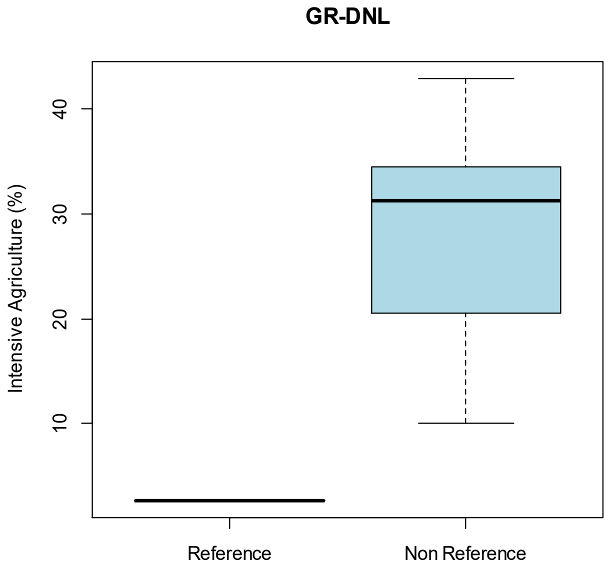
- Total phosphorus concentration (TP), calculated as annual mean for each lake;
- Artificial land use (ALU), composed of the sum of percentages of all the categories of Corine Landcover Analysis, CLC class 1 (Urban areas continuous and discontinuous, industrial and commercial zones, communication infrastructures and networks, mines, etc.);
- Intensive agriculture (IA), composed of the sum of percentages of the CLC categories corresponding to a high potential impact from agricultural activities (arable and irrigated land, permanent and annual crops, vineyards, orchards, olive groves, complex cultivation patterns, CLC codes 2.1, 2.2, 2.41, 2.4.2);
- Natural and semi-natural land use (NASN), composed of the sum of percentages of forest and natural areas, wetlands, water bodies, CLC codes 3.1.1, 3.1.2, 3.1.3, 3.2, 3.3, 4 and 5;
- Population density (PD), calculated as inhabitants per square kilometer in the catchment area of each lake.

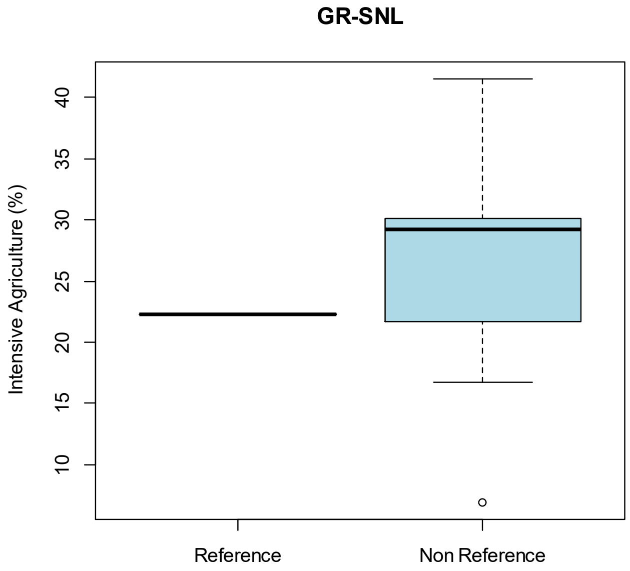
Many of these pressure criteria may be correlated strongly to each other, but applying all of them simultaneously is expected to give a better filtering of low impacted and potential reference sites. For each one of these pressure criteria, a threshold value has been determined, for accepting or rejecting a site as potential reference one. If a lake fails to pass even one of these pressure criteria, then it is not considered as reference. These threshold values (Table 3), were derived from bibliographical data (values adopted in the Med GIG or values proposed in publications) and were supported by expert judgment. They were also used in the development of the national assessment method for aquatic macrophytes (Zervas et al. 2016).

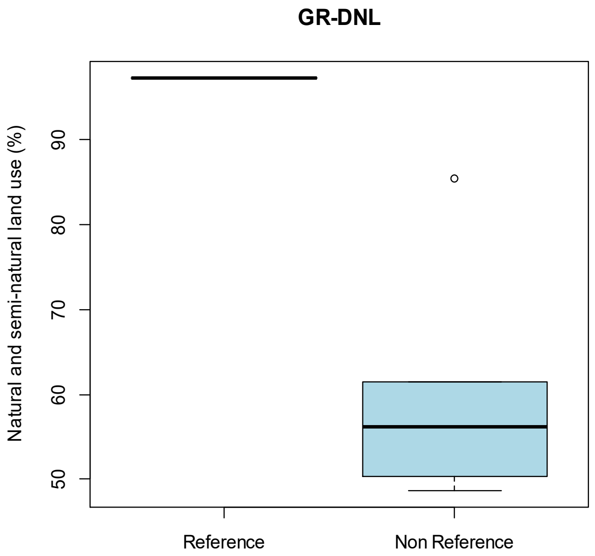
Table 3. Pressure criteria and their threshold limits for screening potential reference sites.

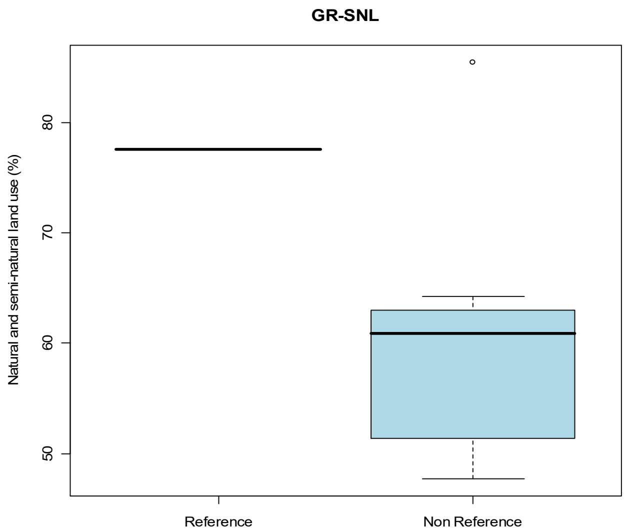
Lake national type	TP (μg/L)	ALU (%)	IA (%)	NASN (%)	PD (h/km^2)
Deep natural lakes (GR-DNL)	<12	<4	<25	>70	<30
Shallow natural lakes (GR-SNL)	<15	<4	<25	>70	<30

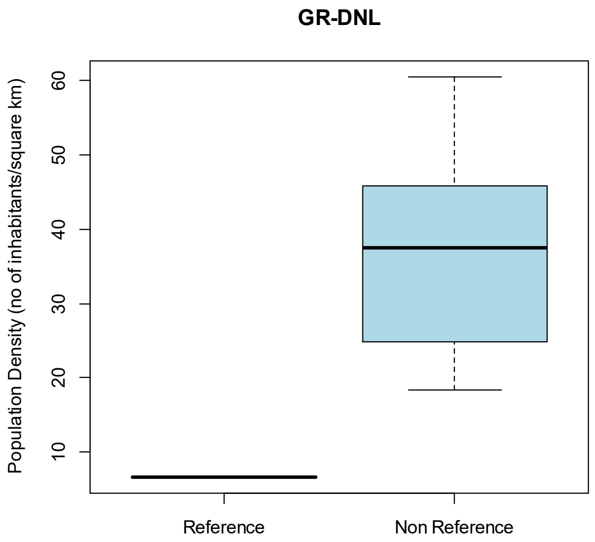
In the first step of screening for reference sites, pressure criteria were used (Table 3). Biological parameters (cyanobacteria biovolume, chl a) confirmed the screening for the selected reference sites. In total, 1 out of the 7 lakes of the GR-DNL type (3 out of 26 lake years) and 1 out of 8 lakes of GR-SNL type (4 out of 31 lake years) are considered as reference sites. Reference sites describe the national reference conditions and are used in the national boundary setting procedure. The box-plots below show the differences between reference and non-reference lakes.

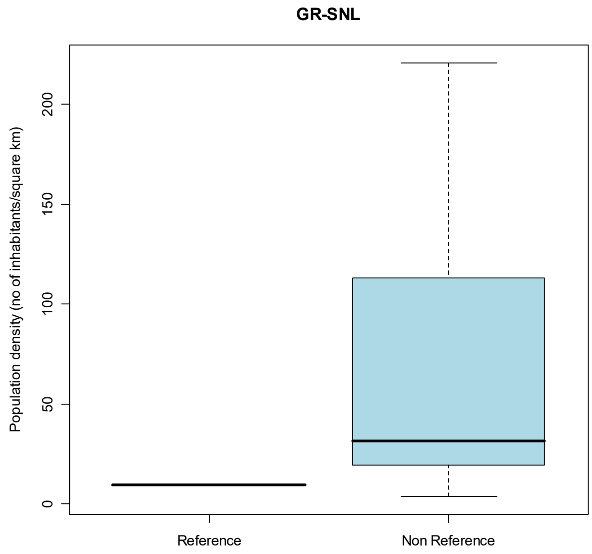

GR-DNL
Intensive Agriculture (%)
10
20
30
40
Reference
Non Reference

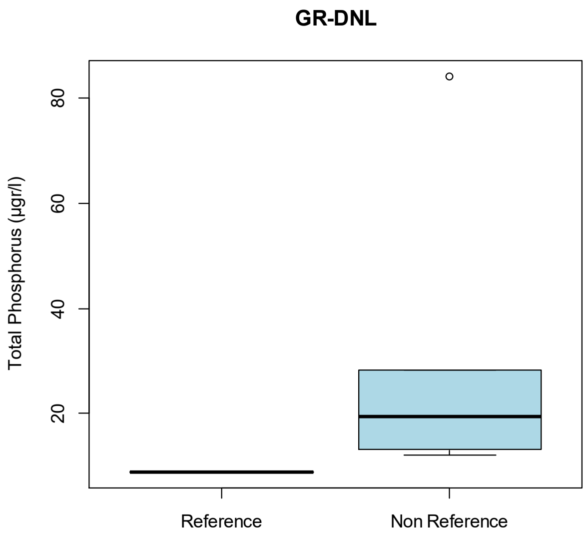

GR-SNL
Intensive Agriculture (%)
10
15
20
25
30
35
40
Reference
Non Reference

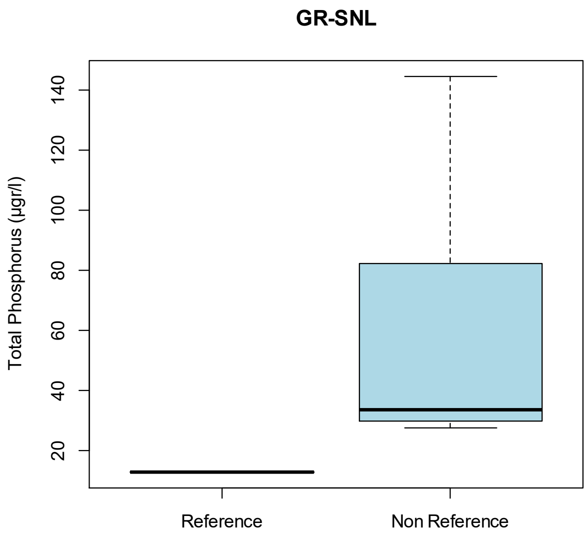

GR-DNL
Natural and semi-natural land use (%)
50
60
70
80
90
Reference
Non Reference


GR-SNL
Natural and semi-natural land use (%)
50
60
70
80
Reference
Non Reference


GR-DNL
Population Density (no of inhabitants/square km)
10
20
30
40
50
60
Reference
Non Reference


GR-SNL
Population density (no of inhabitants/square km)
0
50
100
150
200
Reference
Non Reference


GR-DNL
Total Phosphorus (μgr/l)
20
40
60
80
Reference
Non Reference


GR-SNL
Total Phosphorus (μgr/l)
20
40
60
80
100
120
140
Reference
Non Reference

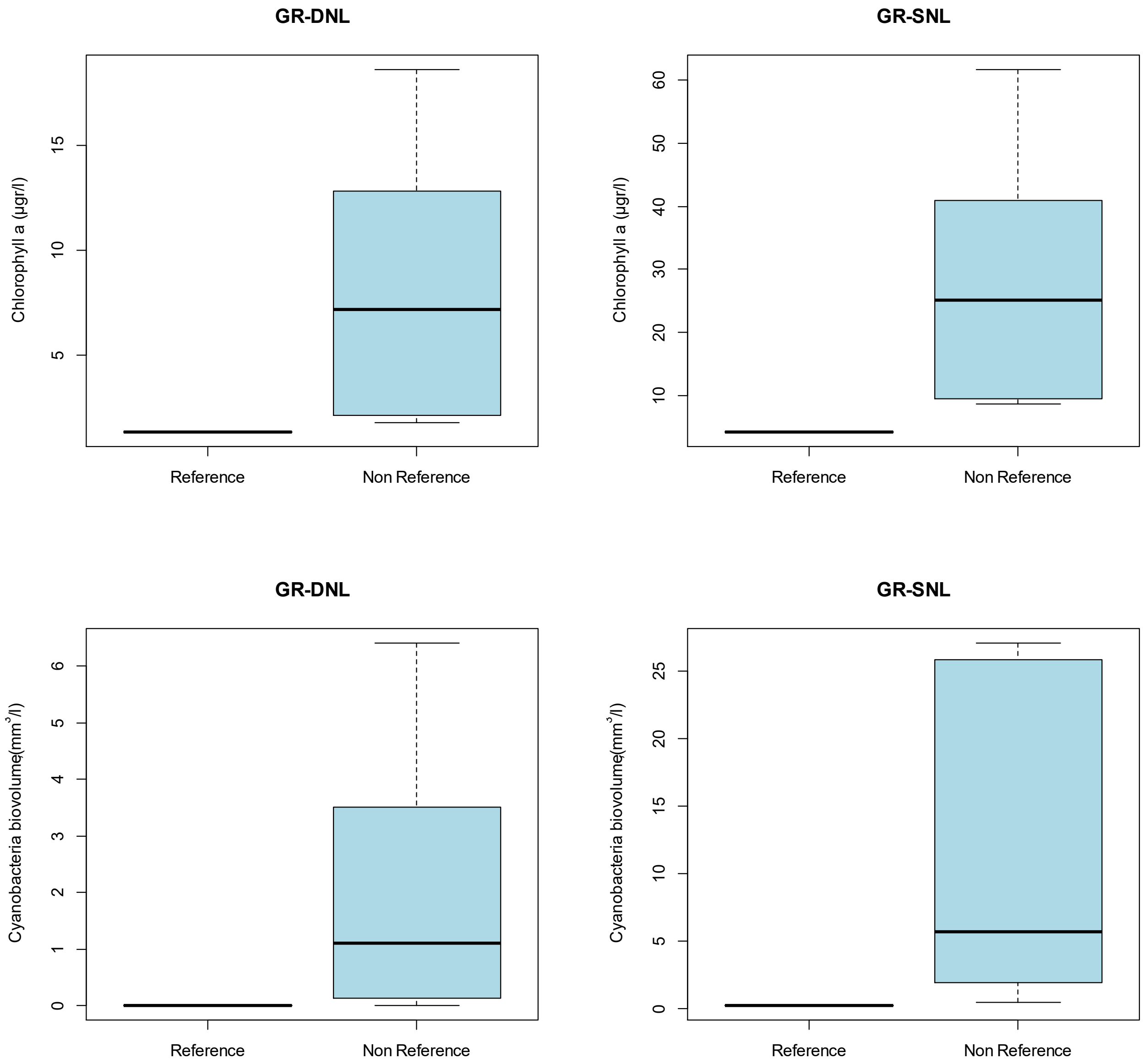


Figure 1. Distribution of intensive agriculture (IA), natural and semi-natural land use cover (NASN) and population density (PD), total phosphorus, Chlorophyll α, Cyanobacteria Biovolume, in reference and non-reference lakes of the two national types (GR-DNL and GR-SNL).

2.4. NATIONAL BOUNDARY SETTING

Type specific reference values and class boundaries for the individual metrics are calculated as recommended by the REFCOND Guidance document No. 10 (European Commission 2003). The base for these calculations are existing near-natural reference sites, as selected after the pressure screening process, using data from monitoring sites (Section 2.3.).

Reference values were determined as the median values of all four metrics in the reference sites, except for cyanobacteria biovolume in type GR-SNL, the value of which was set at 0.01, replacing the median zero value of the reference site. High/Good (H/G) boundaries for each metric were determined at a percentile distribution of their values in reference sites, as follows: GR-DNL – 90% percentile for all metrics except total biovolume (60%) and GR-SNL - 90% percentile for all metrics.

Good/Moderate (G/M) boundaries were determined using data distributed in sites that belong to the 20-50 μg/L TP group. This method was previously applied for the development of G/M boundaries of the New Mediterranean Assessment System for Reservoirs Phytoplankton NMASRP during the MED GIG Intercalibration (de Hoyos et al. 2014). The G/M boundaries for the metrics were determined at 75^{th} percentile of the distribution of the values of each metric for both types, except for total biovolume and modNygaard metrics of GR-DNL type, where G/M boundaries were set at 80% percentile. The G/M boundary location will be further checked in terms of species, or composition metric, changes with data available from 2016-17 sampling campaigns.

Below G/M boundary, boundaries in raw data are divided equally between the lowest value and the G/M value to form the Moderate/Poor (M/P) and Poor/Bad (P/B) boundaries. M/P boundary values of cyanobacteria biovolume were adjusted to form a percentage of total phytoplankton biovolume.

Conversion to Ecological Quality Ratios (EQRs)

In order to allow the combination of the four metrics to a final Biological Quality Element assessment, for each metric an Ecological Quality Ratio is calculated. (Eq. 2):

Equation 2

$$EQR_i = \frac{REF}{LAKE_i}$$

All boundary values, as calculated for each metric, are presented in Tables 4 and 5.

Table 4. Summary of individual metrics boundary values (original values and EQRs) of HeLPhy assessment method for GR-DNL type.

Metric	Total Phytoplankton Biovolume		Cyanobacteria Biovolume		modNygaard Index		Chlorophyll a	
National lake types	GR-DNL		GR-DNL		GR-DNL		GR-DNL	
Boundary	EQR	Value	EQR	Value	EQR	Value	EQR	Value
Reference	1.00	1.29[2]	1.00	0.01	1.00	1.03	1.00	1.56
H/G	0.96	1.34	0,3513	0.04	0.79	1.30	0.53	2.98
G/M	0.37	3.43	0.0120	1.13	0.36	2.82	0.19	8.29
M/P	0.07	17.97	0.0013	10.78	0.06	16.13	0.05	33.37
P/B	0.04	32.01	0.0005	28.30	0.03	29.44	0.03	58.45

Table 5. Summary of individual metrics boundary values (original values and EQRs) of HeLPhy assessment method for GR-SNL type.

Metric	Total Phytoplankton Biovolume		Cyanobacteria Biovolume		modNygaard Index		Chlorophyll a	
National lake types	GR-SNL		GR-SNL		GR-SNL		GR-SNL	
Boundary	EQR	Value	EQR	Value	EQR	Value	EQR	Value
Reference	1.00	0.74	1.00	0.01	1.00	1.11	1.00	3.59
H/G	0.51	1.46	0.03	0.29	0.88	1.25	0.74	4.86
G/M	0.12	6.25	0.004	2.70	0.30	3.70	0.25	14.52
M/P	0.024	30.91	0.0003	23.18	0.007	155.80	0.06	63.74
P/B	0.013	55.56	0.0002	54.78	0.002	307.90	0.03	112.96

Normalization of EQRs

The next step for the combination of the two metrics is to convert each metric's Ecological Quality Ratio to a normalized scale with equal class widths and standardized class boundaries, where the H/G, G/M, M/P and P/B boundaries are 0.8, 0.6, 0.4 and 0.2, respectively. This normalization is based on a linear interpolation between each class's upper and lower boundaries (Eq. 3):

Equation 3

$$\text{If } EQRi \geq 1 \quad : nEQRi = 1$$

$$1 \geq EQRi \geq EQR_{H/G} \quad : nEQRi = \frac{(EQRi - EQR_{H/G})}{(1 - EQR_{G/M})} x0.2 + 0.8$$

$$EQR_{H/G} \geq EQRi \geq EQR_{G/M} \quad : nEQRi = \frac{(EQRi - EQR_{H/G})}{(EQR_{H/G} - EQR_{G/M})} x0.2 + 0.6$$

$$EQR_{G/M} \geq EQRi \geq EQR_{M/P} \quad : nEQRi = \frac{(EQRi - EQR_{M/P})}{(EQR_{G/M} - EQR_{M/P})} x0.2 + 0.4$$

$$EQR_{M/P} \geq EQRi \geq EQR_{P/B} \quad : nEQRi = \frac{(EQRi - EQR_{P/B})}{(EQR_{M/P} - EQR_{P/B})} x0.2 + 0.2$$

[2] Although this reference value is higher than the one set in the previous version of the report (0.29 mm^3/l), it is closer to the ones proposed by expert judgment in Katsiapi et al. (2016).

$$EQR_{P/B} \geq EQRi \geq 0 \qquad : \; nEQRi = \frac{EQRi}{EQR_{P/B}} x0.2$$

EQR_i: Ecological Quality Ratio value as calculated for all metrics for a lake i;
$EQR_{H/G \text{ or } G/M \text{ etc.}}$: EQR values for the corresponding boundaries, as calculated during boundary setting;
$nEQR_i$: Normalized EQR value for the corresponding EQR value of the metrics of lake i.

Rule of combination to a final score

The final lake assessment, according to the HeLPhy assessment method, is determined using the principle of equal weight for taxonomic composition and abundance metrics. After the calculation of EQRs for all metrics and their normalization procedure, the final lake score is calculated by averaging the normalized EQRs of the above metrics (Eq.4):

Equation 4

$$HeLPhy = \frac{\left(\frac{nEQR_{Chl} + nEQR_{BV}}{2} + \frac{nEQR_{\text{mod}\,Nygaard} + nEQR_{CyanoBV}}{2} \right)}{2}$$

$HeLPhy_i$:	Final value of HeLPhy assessment method, which is a normalized EQR for the assessment of lake i;
$nEQR_{Chli}$:	Normalized EQR value of Chl a for lake i;
$nEQR_{BVi}$:	Normalized EQR value of Total biovolume for lake i.
$nEQR_{modNygaardi}$:	Normalized EQR value of modified Nygaard for lake i.
$nEQR_{CyanoBV}$:	Normalized EQR value of Cyanobacteria biovolume for lake i.

As a result, the final score of HeLPhy can be assigned to an ecological status class according to Table 6.

Table 6. Final boundary values of HeLPhy assessment method.

HeLPhy	Ecological status class
0.80-1.00	High
0.60-0.80	Good
0.40-0.60	Moderate
0.20-0.40	Poor
0.00-0.20	Bad

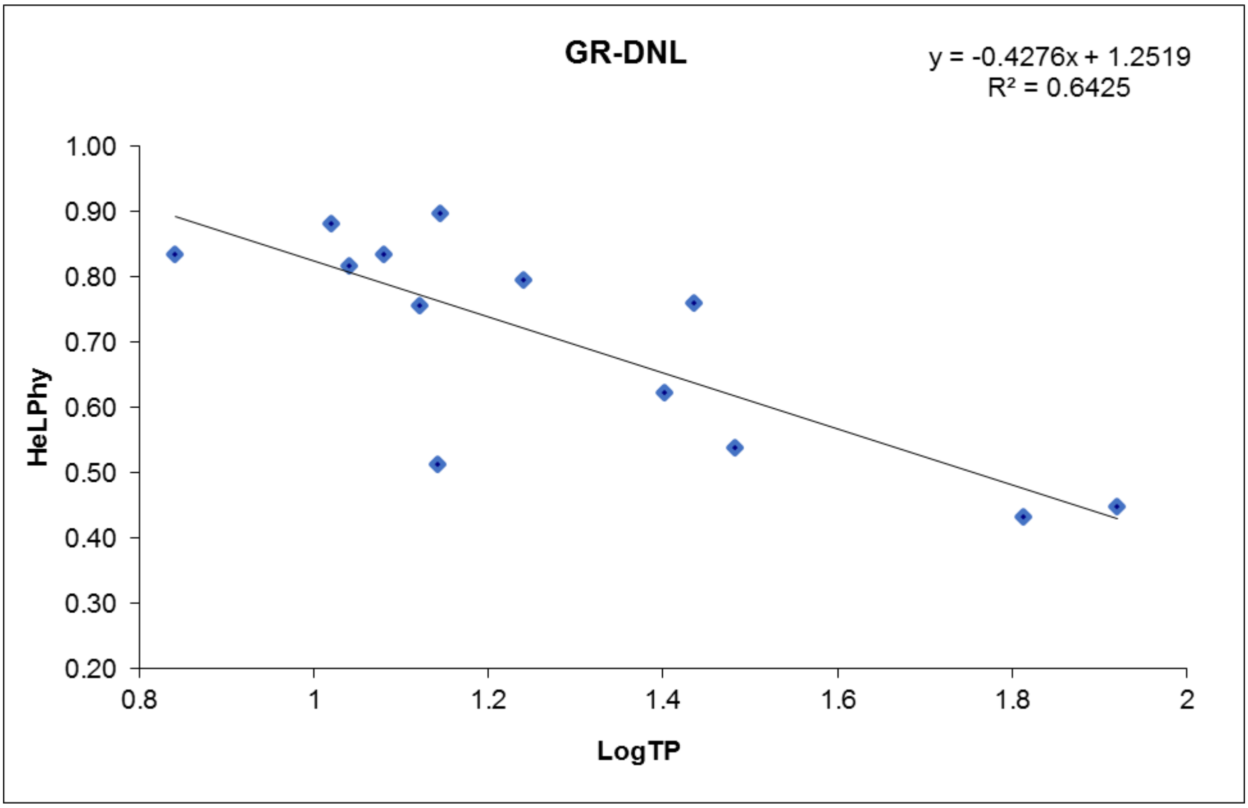
2.5. PRESSURES ADDRESSED

The HeLPhy assessment method, as already mentioned, addresses eutrophication pressure in Greek natural lakes. In order to evaluate the performance of the method in assessing eutrophication, total phosphorus concentration (Annual mean; TP) is used as the main proxy. For the regression, values of phytoplankton and TP for years 2014 – 2015 were used, except for one lake in each type, where values only for 2014 were available. A linear regression model was applied and the resulting coefficient of determination (R^2), Pearson's correlation coefficient (r) and p value (p) were assessed. At the following table (Table 7) all relationships between TP and HeLPhy metrics (individual metrics and overall index, all expressed in nEQRs) for GR-DNL and GR-SNL types are given. Pressure-response curves between TP and HeLPhy are given in Figure 2.

Table 7. Overview of the relationships between nEQR values and pressure indicator values (total phosphorus concentration - TP,), after linear regression. Significant relationships are indicated in bold.

Lake Type	Relationship	n	r	R^2	p	Regression Equation
GR-DNL	nEQR TotalPhytoplankton Biovolume-TP	13	-0.419	0.175	0.154	nEQR = -0.3001 LogTP + 1.0907
GR-DNL	nEQR Cyanobacteria Biovolume -TP	13	-0.637	0.406	**0.019**	nEQR = -0.4706 LogTP + 1.279
GR-DNL	nEQR Chlorophyll_a-TP	13	-0.843	0.711	**0.000**	nEQR = -0.5987 LogTP + 1.5026
GR-DNL	nEQR modNygaard Index-TP	13	-0.576	0.331	**0.039**	nEQR = -0.341 LogTP + 1.1353
GR-DNL	HeLPhy-TP	13	-0.801	0.642	**0.001**	HeLPhy = -0.4276 LogTP + 1.2519
GR-SNL	nEQR Total Phytoplankton Biovolume -TP	15	-0.872	0.760	**<0.001**	nEQR = -0.5489 LogTP + 1.5514
GR-SNL	nEQR Cyanobacteria Biovolume -TP	15	-0.895	0.800	**<0.001**	nEQR = -0.5031 LogTP + 1.426
GR-SNL	nEQR Chlorophyll_a-TP	15	-0.906	0.821	**<0.001**	nEQR = -0.4856 LogTP + 1.3653
GR-SNL	nEQR modNygaard Index-TP	15	-0.822	0.676	**<0.001**	nEQR = -0.3695 LogTP + 1.2676
GR-SNL	HeLPhy-TP	15	-0.906	0.821	**<0.001**	HeLPhy = -0.4768 LogTP + 1.4026

Current results show (Table 7) that relationships between TP and all individual metrics as well as the overall HeLPhy index are strong and statistically significant in GR-SNL. It is noted that although the dataset of GR-SNL is small, it comprises lakes from the whole trophic spectrum, giving high and statistically significant correlations. In the case of GR-DNL, where the lakes do not span the whole trophic spectrum, one correlation with TP was not strong and statistically significant, i.e. total phytoplankton biovolume. Integration of monitoring data of the following years in the dataset is expected to make the results of the pressure-response curve more robust.



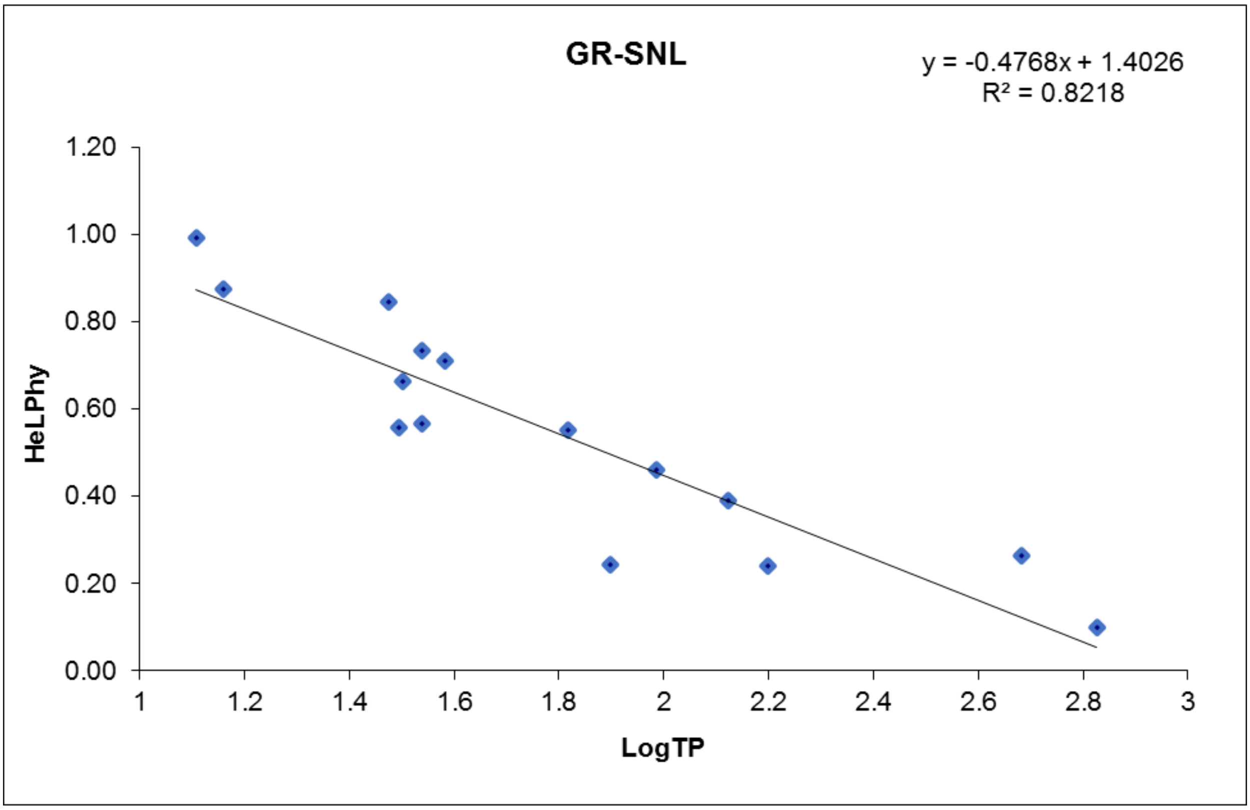


Figure 2. Pressure-response curves of HeLPhy assessment method (nEQR), in relation to Total Phosphorus.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria. The table below (Table 8) summarizes in which aspects HeLPhy assessment method complies with the criteria needed according to WFD.

Table 8. List of the WFD compliance criteria and the WFD compliance checking process and results of HeLPhy assessment method.

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES (Table 5)
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	YES (Section 2.4)
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 (Section 2.1)
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	NO, there are no intercalibration common types for MED-GIG natural lakes yet
The water body is assessed against **type-specific near-natural reference conditions**	YES (Section 2.3)
Assessment results are expressed as **EQRs**	YES (Tables 4, 5, 6)
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES (Section 2.2)
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES (Section 2.2)
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES (Section 2.2)

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.

The two national lake types that HeLPhy assessment method addresses are:

- GR-DNL: Deep (mean depth >9m), natural warm monomictic lakes;
- GR-SNL: Shallow (mean depth 3-9m), natural polymictic lakes.

There are no common intercalibration types for MED-GIG natural lakes. The two national types used for HeLPhy assessment method (GR-DNL & GR-SNL), may occur in other members of the same Intercalibration Group, so they may be used as common types. If not, broader types could be used, however, it is noted that the issue of the number of lakes still remains.

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 HeLPhy assessment method addresses eutrophication. To our knowledge, the French IPLAC method and the Italian method for natural lakes also address eutrophication pressure.

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.

The national assessment concept is similar with other IC countries and follows the same strategies.

Method	**Assessment concept**	**Remarks**
Method GR	Chlorophyll a, Total biovolume, BV of cyanobacteria, Modified Nygaard Index (species composition metric)	2-4 sampling during growing season (May – Oct), integrated sample from euphotic zone (2.5*Secchi depth).
Method FR	Chlorophyll a MCS (species composition metric)	Survey at each season (winter to autumn) with 3 during the growing season (May to October). Integrated samples from euphotic zone.
Method IT	Chlorophyll a, Total biovolume, PTI index (species composition metric)	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. Integrated samples from the euphotic zone, (2.5* Secchi depth).

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

During the Intercalibration exercise, MED-GIG countries had tried to find a statistically robust set of natural lakes belonging to the same type, but failed to do so. Thus, currently we are not able to check HeLPhy assessment method for its intercalibration feasibility. However, we plan to contact the other MED-GIG members in the immediate future, in order to exchange data and information on the BQE towards designing intercalibration exercises for natural lakes at least at a MS to MS level.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

GR-DNL

Phytoplankton communities at high status are mainly composed by diatoms (mostly *Cyclotella*), chlorophytes (such as *Closterium, Staurastrum, Elakatothrix, Monoraphidium*, *Oocystis*) and cryptophytes (*Cryptomonas*, *Rhodomonas*). Small numbers of abundance and biomass of dinophytes (e.g. *Ceratium*, *Peridiniopsis*) and chrysophytes (*Dinobryon* sp.) are present. Very low numbers (abundance and biovolume) of cyanobacteria may be present.

GR-SNL

Phytoplankton communities at high status are mainly composed by diatoms (mostly *Cyclotella*, *Synedra* and *Aulacoseira*), chlorophytes (such as *Staurastrum*, *Cosmarium, Sphaerocystis, Monoraphidium*, *Oocystis*), dinophytes (e.g. *Ceratium*, *Peridiniopsis*, *Peridinium*), cryptophytes (*Cryptomonas*, *Rhodomonas*) and a small number of chrysophytes (*Dinobryon*). Cyanobacteria may also appear (e.g. *Microcystis*), however in very small numbers of abundance and biovolume.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

There are slight changes in the composition and abundance of planktonic taxa compared to the type specific communities. Such changes do not indicate any accelerated growth of algae resulting in undesirable disturbance to the balance of organisms present in the water body or to the physico-chemical quality of the water.

GR-DNL

Chlorophytes (e.g. *Planktonema*, *Tetraedron*, *Coelastrum*, *Cosmarium*, *Oocystis*, *Sphaerocystis*) are becoming more dominant in the phytoplankton community, along with cryptophytes (mostly *Rhodomonas*). Dinophytes (e.g. *Ceratium*, *Peridiniopsis*, *Peridinium*) are still present. Diatoms (mostly *Cyclotella* but also *Fragilaria*) also contribute to the community but less. Prymnesiophyceae such as *Chrysochromulina* sp. are getting present. Cyanobacteria are starting to increase in abundance and biovolume (e.g. *Aphanizomenon*, *Planktothrix*, *Planktolyngbya*, *Anabaena*, *Limnothrix*). *Dinobryon* sp. (Chrysophytes) are getting scarcer.

GR-SNL

In good status, chlorophytes (such as *Oocystis*, *Pediastrum*, *Monoraphidium*), are getting more abundant in the phytoplankton community. Diatoms (mostly *Aulacoseira*, *Fragilaria*, *Synedra* and *Melosira*) are also present. Cryptophytes (mostly *Cryptomonas*, *Rhodomonas*) and dinophytes (e.g. *Ceratium*, *Peridiniopsis*, *Peridinium*) contribute in the community. Prymnesiophyceae such as *Chrysochromulina* sp. are getting present. Species of genus Dinobryon are getting much scarcer. Cyanobacteria are increasing in abundance and biomass (e.g. *Planktolyngbya*, *Microcystis*, *Aphanizomenon*, *Anabaena*).

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

The composition and abundance of planktonic taxa differ moderately from the type specific communities. Biomass is moderately disturbed and may be such as to produce a significant undesirable disturbance in the condition of other biological quality elements and the physico-chemical quality of the water.

GR-DNL

Cyanobacteria (e.g. *Limnothrix*, *Plantkolyngbya*, *Aphanizomenon*, *Anabaenopsis*, *Anabaena, Cylindrospermopsis*, *Microcystis*) increase their dominance in the phytoplankton community. Chlorophytes (e.g. *Tetraedron*, *Planktonema*, *Scenedesmus*) are still present. Diatoms are an important part of the community (*Nitzchia* is getting dominance over *Cyclotella* and *Synedra*). Cryptophytes (mostly *Rhodomonas*) and Prymnesiophyceae such as Chrysochromulina, are still present. Dinophytes (e.g. *Peridinium*) are scarcer.

GR-SNL

Less diverse phytoplankton community, with cyanobacteria (mostly *Anabaena*, *Microcystis, Aphanizomenon*, *Planktolyngbya*) increasing their dominance, in terms of both abundance and biovolume. Phytoplankton blooms can be seen in summers. Diatoms (mostly *Nitzchia* and *Aulacoseira*) and cryptophytes (e.g. *Rhodomonas*, *Cryptomonas*) form part of the community. Chlorophytes and Dinophytes (e.g. *Ceratium*) are still present.

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