THE GOULANDRIS NATURAL HISTORY MUSEUM
GREEK BIOTOPE/WETLAND CENTRE

REPORT

ON THE DEVELOPMENT OF THE NATIONAL ASSESSMENT METHOD FOR THE ECOLOGICAL STATUS OF NATURAL LAKES IN GREECE, USING THE BIOLOGICAL QUALITY ELEMENT "MACROPHYTES" (HELLENIC LAKE MACROPHYTES-HeLM ASSESSMENT METHOD)

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1. INTRODUCTION

This report discusses the development of the national ecological assessment method for Greek natural lakes, based on the Biological Quality Element (BQE) "macrophytes".
Due to lack of a common natural lake type within the Mediterranean Lake Geographical Intercalibration Group, there has not been a Med GIG Intercalibration Exercise for macrophytes in natural lakes. As a result, there are neither proposed assessment methods with common boundaries within the Med GIG, nor proposed metrics for the assessment of lakes based on macrophytes. It is noted that at the Mediterranean Lake Phytoplankton GIG Intercalibration Report, Member States defined two common water body types (L-M5/7 and L-M8) for reservoirs but none for natural lakes.
The operation of the Greek water monitoring network started in 2012, following the publication of a Joint Ministerial Decision in 2011. The development of the current assessment method, as described in this report, is based on the data from this national water monitoring network. In particular, 50 lake water bodies (including 26 reservoirs) have been included in the monitoring network, out of which 16 have been monitored for macrophytes during the 3-year period of 2013-2015. Eight of them are warm monomictic, deep natural* lakes with mean depth >9m (GR-DNL), when the other eight are polymictic, shallow natural lakes with mean depth 3-9m (GR-SNL). In these 16 lakes, a total of 272 monitoring sites were established for sampling macrophytes, which resulted in an equal number of macrophytic sampling transects, the data of which have been added in the national dataset. Thirty six of these sites were revisited during the 3-year period, and a total of 308 measurements of maximum macrophytic colonization depth were made.
On this national dataset, the most suitable lake macrophyte based assessment components proposed by WISER deliverables D3.2-1 (Kolada et al., 2009), D3.2-2 (Dudley et al., 2011) and D3.2-3 (Kolada et al., 2011) were tested, in various combinations, so as to reach a final form that can be used as a national assessment method for Greece. As already mentioned, this is the first effort to establish a national method, which may need additions and improvements in the future, as well as intercalibration exercises among Member States in the Mediterranean GIG.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The Hellenic Lake Macrophytes (HeLM) assessment method, is a newly developed method to assess eutrophication and general degradation pressures in Greek natural lakes. It utilizes the results of the WISER deliverables, so as to become a relatively easy to use method, which at the same time delivers satisfactory results. Before 2013, macrophytes were not used in monitoring assessment in Greece. In order to develop this assessment system, the national dataset, resulted from macrophytic vegetation records during the 3-year period 2013-2015, in 16 lakes relevant to the WFD in Greece, was used.

2.1. METHODS AND REQUIRED BQE PARAMETERS

Metrics of the ecological assessment method

Table 1. Overview of the metrics included in the Hellenic Lake Macrophytes (HeLM) national assessment method

MS	Surface Water Category	Biological Quality Element	Taxonomic composition and Sensitivity/tolerance	Abundance
GR	Lakes	Macrophytes	TIHeLM: Total score of characteristic species, depending on species indication value and species abundance	Cmax: Depth limit of macrophytes

*Seven out of eight warm monomictic deep lakes are actually natural. The one remaining (Feneos lake) is in reality a reservoir for storage purposes, but due to a steady water level for many decades, a species-rich and abundant aquatic vegetation has been developed to such an extent that now the reservoir resembles a natural lake.

HeLM assessment method for Greek lake macrophytes consists of two different metrics:

- **Trophic Index HeLM (TIHeLM).** Essentially, this metric is a modified form of Intercalibration Common Metric for lake macrophytes (ICMLM), which is based on species trophic scores and was originally developed for the purpose of the pan-European intercalibration exercise (Kolada et al., 2011). As described by Birk & Willby (2010), national assessment results (EQRs) for macrophyte taxa were averaged, so as to result lake trophic ranks (LTRs), which grade each taxon in regards to its response to nutrient enrichment (Kolada et al., 2011). For each lake, an Intercalibration Common Index is calculated, by averaging the LTR values (ranging from -2.2 for *Tolypella canadensis* to 11.4 for *Lemna minuta*) of the taxa present in them (using only presence/absence data). Thus, theoretically, values can range between -2.2 for extreme oligotrophic lakes to 11.4 for hyper-eutrophic ones.

  For HeLM assessment method, ICMLM needed to be modified to TIHeLM so as to become more effective in evaluating the eutrophication pressure in Greek lakes. Firstly, in the WISER macrophyte dataset (Kolada et al., 2011), there are LTR scores for 135 taxa of hydrophytes. This dataset covered a good part of the number of taxa found in Greek lakes but there were enough and important taxa that needed grading. Most of them are helophytes, which in some cases are the only representatives of macrophytic vegetation in Greek eutrophic and degraded lakes. As Kolada (2016) concludes, they provide reliable information on ecosystem ecological conditions and can support assessment of the ecological status of lakes under eutrophication pressure. So, as preceded in Kolada et al. (2011), the missing LTRs were estimated from the LTR-Ellenberg N regression equation: $LTR = 1.395N - 0.6276$ (R^2=0.6430; R=0.8019; p=0.0000). The final dataset* with LTR scores for all the taxa in Greek lakes is included in Table 2.

  The second modification that was needed for the optimization of the metric, was to elaborate in the calculation of TIHeLM (Equation 1) the relative cover-abundance values of taxa (and not only their presence-basence data), so as to distinguish lakes with different abundance patterns in their floristic composition. Cover-abundance values are commonly used in calculations of trophic indices in many other member states' assessment methods (Hellsten et al., 2014; Pall et al., 2014; Portielje et al., 2014).

  The third and final modification, needed for the boundary setting procedure and the spatial monitoring of each lake, was to calculate the index for the data of each lake's sampling transect separately (Equation 1) and then calculate the final index for the lake, by averaging the values of its transects (Equation 2).

*At the current state, LTR scores for macrophytic taxa, as calculated during the pan-European intercalibration exercise are used, due to lack of available data for the development of a Mediterranean or a Greek specific taxa list. However, when the necessary data will become available, the LTR taxa list may be revised to represent in a more appropriate way, the macrophytic ranking in Mediterranean natural lakes.

Table 2. The list of macrophytes in the Greek national dataset, used for calculating TIHeLM metric. LTR stands for Lake Trophic Rank scores as elaborated by Willby (Kolada et al., 2011). LTR scores derived from the regression of LTR / Ellenberg N values relationship, are marked with asterisks

Taxon Name	LTR	Taxon Name	LTR
Agrostis stolonifera	*6.35	*Najas minor*	*4.96
Alisma gramineum	*4.96	*Nitella gracilis*	4.17
Alisma lanceolatum	*6.35	*Nitella hyalina*	3.81
Alisma plantago-aquatica	*10.54	*Nitella syncarpa*	3.81
Arundo donax	*7.75	*Nitellopsis obtusa*	6.13
Azolla filiculoides	*10.54	*Nuphar lutea*	7.05
Berula erecta	*7.75	*Nymphaea alba*	6.02
Bolboschoenus maritimus	9.14	*Nymphoides peltata*	7.76
Butomus umbellatus	8.73	*Paspalum dilatatum*	*9.14
Carex sp.	*4.96	*Paspalum distichum*	*9.14
Ceratophyllum demersum	7.82	*Persicaria amphibia*	8.07
Ceratophyllum submersum	7.85	*Phalaroides arundinacea*	*9.14
Chara aspera	4.70	*Phyla nodiflora*	*6.35
Chara corfuensis	6.03	*Phragmites australis*	*9.14
Chara globularis	6.80	*Potamogeton berchtoldii*	5.73
Chara hispida	4.48	*Potamogeton compressus*	5.43
Chara tomentosa	5.27	*Potamogeton crispus*	8.02
Chara vulgaris	6.53	*Potamogeton gramineus*	3.17
Elatine triandra	5.21	*Potamogeton lucens*	6.01
Eleocharis mitracarpa	*4.96	*Potamogeton nodosus*	*6.35
Eleocharis palustris	*4.96	*Potamogeton perfoliatus*	4.95
Eleocharis parvula	*6.35	*Potamogeton pusilus*	9.10
Elodea canadensis	7.42	*Potamogeton trichoides*	7.19
Epilobium lanceolatum	*3.56	*Ranunculus rionii*	3.81
Epilobium parviflorum	*7.75	*Ranunculus trichophyllus*	3.81
Filamentous macroalgae	8.78	*Rorippa amphibia*	*10.54
Hydrocharis morsus-ranae	7.09	*Rumex palustris*	*10.54
Iris pseudacorus	*9.14	*Salvinia natans*	*9.14
Juncus articulatus	*2.16	*Samolus valerandi*	*6.35
Juncus inflexus	*4.96	*Schoenoplectus lacustris*	*7.75
Juncus subnodulosus	*3.56	*Schoenoplectus litoralis*	*7.75
Juncus tenuis	*6.35	*Scirpoides holoschoenus*	*10.54
Lemna gibba	9.63	*Sparganium angustifolium*	2.69
Lemna minor	8.82	*Sparganium erectum*	*9.14
Ludwigia peploides	*4.96	*Sparganium neglectum*	*7.75
Lycopus europaeus	*9.14	*Spirodela polyrhiza*	9.57
Lysimachia vulgaris	*6.35	*Stuckenia pectinata*	8.64
Lythrum salicaria	*6.35	*Trapa natans*	*10.54
Mentha aquatica	*6.35	*Trichophorum cespitosum*	*0.77
Mentha pulegium	*3.56	*Typha angustifolia*	*9.14
Fontinalis antipyretica	5.48	*Typha domingensis*	*10.54
Myriophyllum spicatum	7.30	*Typha latifolia*	*10.54
Myriophyllum verticillatum	5.74	*Utricularia vulgaris*	3.86
Najas gracillima	*10.54	*Vallisneria spiralis*	*7.75
Najas graminea	*6.35	*Vitex agnus-castus*	*3.56
Najas marina	6.78	*Zannichellia pedunculata*	9.53

TIHeLM calculation for each transect (Eq. 1):

Equation 1

$$\text{TIHeLM}_{\text{TRANS}} = \sum_{i=1}^{n} (RAb_i \times LTR_i)$$

$\text{TIHeLM}_{\text{TRANS}}$: HeLM Trophic Index value calculated for a specific transect;
n : Number of observed taxa in the specific transect;
RAb_i : Relative abundance of taxon i in the specific transect;
LTR_i : Lake trophic rank of taxon i.

Lake TIHeLM calculation as an average of transect values (Eq. 2):

Equation 2

$$\text{TIHeLM}_{\text{LAKE}} = \frac{\sum_{i=1}^{n} TIHeLM_{TRANSi}}{n}$$

$\text{TIHeLM}_{\text{LAKE}}$: HeLM Trophic Index value calculated for a specific lake;
n : Number of transects calculated for this lake;
$\text{TIHeLM}_{\text{TRANSi}}$: HeLM Trophic Index value calculated for transect i.

- **Maximum depth of colonization (Cmax).** This is a widely used metric of abundance macrophyte metrics and it simply expresses the maximum observed depth of a lake where submerged rooted macrophytes are present. Values can range from zero meters for hypereutrophic lakes with no submerged aquatic vegetation, to many meters of depth for oligotrophic lakes with extensively developed submerged vegetation. Submerged macrophytes abundance metrics seem to respond significantly to eutrophication stressors (Kolada et al., 2011). Changes in the abundance of submerged macrophytes may be expressed either by their relative mean percent coverage of the total lake area or the maximum depth of colonization by submerged rooted macrophytes. As recommended by Kolada et al. (2011), mean macrophytes coverage is used only in very shallow lakes (mean depth <3m), but in the case of the current national database, all lakes monitored of macrophytes have mean depth >3m, so Cmax is chosen.
  As Kolada et. al remarked, Cmax presents annual variations, which should be taken into account to reduce the risk of misclassification of a lake. For that reason, the value that is used in the HeLM assessment method for each lake, is the mean average of all annual Cmax values measured in the 3-year period (Eq. 3):

  Equation 3

$$\text{Cmax}_{\text{LAKE}} = \frac{\sum_{i=1}^{n} Cmax_i}{n}$$

  $\text{Cmax}_{\text{LAKE}}$: Calculated maximum depth of colonization for a specific lake in a 3-year period;
  n : Number of annual maximum depth of colonization values available for this lake;
  Cmax_i : Annual value for maximum depth of colonization for this lake.

Conversion to Ecological Quality Ratios (EQRs)

In order to allow the combination of the two metrics to a total Biological Quality Element assessment, for each metric an Ecological Quality Ratio should be calculated. (Eq. 4 and 5):

Equation 4

$$\mathrm{EQR_{TIHeLMi}} = \frac{TIHeLM_{REF}}{TIHeLM_{LAKEi}}$$

$EQR_{TIHeLMi}$: Ecological Quality Ratio of TIHeLM metric for lake i;
$TIHeLM_{REF}$: TIHeLM value in reference conditions;
$TIHeLM_{LAKEi}$: TIHeLM value as calculated for lake i.

Equation 5

$$\mathrm{EQR_{Cmaxi}} = \frac{Cmax_{LAKEi}}{Cmax_{REF}}$$

EQR_{Cmaxi} : Ecological Quality Ratio of Cmax metric for lake i;
$Cmax_{LAKEi}$: Cmax value as calculated for lake i;
$Cmax_{REF}$: Cmax value in reference conditions.

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 High-Good (H/G), Good-Moderate (G/M), Moderate-Poor (M/P) and Poor-Bad (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. 6):

Equation 6

$$\text{If } \mathrm{EQR_i} \geq 1 \quad : \quad \mathrm{nEQR_i} = 1$$

$$1 \geq \mathrm{EQR_i} \geq \mathrm{EQR_{H/G}} \quad : \quad \mathrm{nEQR_i} = \frac{(\mathrm{EQR_i} - \mathrm{EQR_{H/G}})}{(1 - \mathrm{EQR_{H/G}})} \times 0.2 + 0.8$$

$$\mathrm{EQR_{H/G}} \geq \mathrm{EQR_i} \geq \mathrm{EQR_{G/M}} \quad : \quad \mathrm{nEQR_i} = \frac{(\mathrm{EQR_i} - \mathrm{EQR_{G/M}})}{(\mathrm{EQR_{H/G}} - \mathrm{EQR_{G/M}})} \times 0.2 + 0.6$$

$$\mathrm{EQR_{G/M}} \geq \mathrm{EQR_i} \geq \mathrm{EQR_{M/P}} \quad : \quad \mathrm{nEQR_i} = \frac{(\mathrm{EQR_i} - \mathrm{EQR_{M/P}})}{(\mathrm{EQR_{G/M}} - \mathrm{EQR_{M/P}})} \times 0.2 + 0.4$$

$$\mathrm{EQR_{M/P}} \geq \mathrm{EQR_i} \geq \mathrm{EQR_{P/B}} \quad : \quad \mathrm{nEQR_i} = \frac{(\mathrm{EQR_i} - \mathrm{EQR_{P/B}})}{(\mathrm{EQR_{M/P}} - \mathrm{EQR_{P/B}})} \times 0.2 + 0.2$$

$$\mathrm{EQR_{P/B}} \geq \mathrm{EQR_i} \geq 0 \quad : \quad \mathrm{nEQR_i} = \frac{\mathrm{EQR_i}}{\mathrm{EQR_{P/B}}} \times 0.2$$

EQR_i : Ecological Quality Ratio value as calculated for TIHeLM or Cmax 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 TIHeLM or Cmax metric of lake i.

Rule of combination to a final score

The final lake assessment, according to the HeLM assessment method, is determined using the principle of equal weight for taxonomic composition and abundance metrics. After the calculation of

EQRs for both metrics and their normalization procedure, the final lake score is calculated by averaging the normalized EQRs of the above two metrics (Eq.7):

Equation 7

$$\mathrm{HeLM_i} = \mathrm{nEQR_{HeLMi}} = \frac{nEQR_{TIHeLMi} + nEQR_{Cmaxi}}{2}$$

$HeLM_i$ and $nEQR_{HeLMi}$: Final value of HeLM assessment method, which is a normalized EQR for the assessment of lake i;
$nEQR_{TIHeLMi}$: Normalized EQR value of TIHeLM metric for lake i;
$nEQR_{Cmaxi}$: Normalized EQR value of Cmax metric for lake i.

WFD compliance

Overall, HeLM assessment method meets the criteria needed for WFD compliance. Both taxonomic composition and abundance parameters are being assessed by the metrics described above. Both 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 3. Overview of sampling and data processing for HeLM national assessment method

Parameter	Description
Sampling	Based on I.S. EN 15460:2007, CEN EN 14184:2003, XP T90-328:2010 Standard and WISER Deliverable D3.2-1
Sampling methods	Belt Transect method; Number of transects depending on land use variability; From shoreline to maximum depth of macrophyte occurrence; Sampling in five depth zones (0-1 m, 1-2 m, 2-4 m, 4-8 m, >8m); By rake and bathyscope survey; All macrophytic species and their abundance
Level of identification	Species level for vascular plants; species level for bryophyta and charophyta; genus level for green macroalgae
Frequency	For taxonomic composition: Once per three years, at the peak of macrophytic vegetation season (Lakes in South Greece: late May - July; Lakes in North Greece: July – early September) For abundance: Once per year, during the peak of macrophytic vegetation season
Data processing	For TIHeLM: Estimation of mean percent cover of each taxon in a transect and then calculation of their relative abundance by dividing their transformed ($x^{0.2}$) abundance to the sum of transformed abundance of all taxa in the transect. For Cmax: No data processing needed.

Rationale

The sampling method chosen for HeLM assessment method is the most commonly applied method for aquatic vegetation surveys and monitoring methodologies in many European countries, the belt transect-based method. This method is also recommended by the European Committee for Standardization CEN (*Comite Europeen de Normalisation)* (CEN, 2003; Kolada et al., 2009), as it provides at the same time abundance, frequency and depth distribution data of different species in a lake (Kolada et al., 2009).

Preparatory phase – Number of samples

Before beginning fieldwork, the position and the number of sampling sites in each lake are decided. The multitude and the geographic positioning of the sampling sites depend on the size of the lakes, their morphology, as well as the habitats diversity and the land use variability along their perimeter. By applying the Jensen's method (1977) and taking into consideration (if possible) bathymetric data, habitat maps and land use maps of the specific lake and its catchment area, these sites are selected in order to acquire the best representation of the aquatic vegetation of a lake with minimal effort.
More specifically, as described at XP T90-328 French standard (Pall et al., 2014), the perimeter of each lake is divided in four different types of riparian zones:

1. Natural, typical wetland riparian types (bogs, fringing reeds, boggy heaths, marshes, water meadows, hygrophilous forests / wet woodlands e.g. Alnus-Salix, etc.);
2. Natural riparian zone colonized by terrestrial shrubs and bushes (mixed deciduous forests, coniferous forests, bushes and shrubs, heathlands, etc.);
3. Natural riparian zone not colonized by dry-land shrubs and bushes (scrublands, tall plants, rocky shorelines, beaches, etc.);
4. Artificial areas or areas visibly subjected to human pressure (docks, moorings, shore vegetation clearances, walls, artificial beaches or parks, roads and tracks, etc.).

For each of the four different types of riparian zones, at least three sampling sites are established. This number is increased in some cases, depending on the in-type variability and the lake morphology and bathymetry.
In total, the number of sampling sites for each lake, according to the criteria described above, fluctuates from the minimum of 10 to the maximum of 20 transects per lake, so as to cover the full diversity of lake's vegetation patterns.

Sampling strategy - Equipment

The sampling method consists in establishing belt transects perpendicular to the lake's shoreline, of a length covering from the shoreline to the maximum depth of plant growth and of the width of approximately 5m, to enable boat maneuvering and the handling of the sampling tools. This sampling method described in this section, is developed for lakes with mean depth > 3m (GR-SNL and GR-DNL lake types), in which there is a depth limit of macrophytic colonization and thus Cmax values are being gathered. In the case of very shallow lakes, with mean depth <3m (GR-VSNL lake type), in which the macrophytic abundance is assessed by estimating the relative mean per-cent coverage of the whole lake area, the sampling method is modified.
At each sampling site, one belt transect is established from the shore to the maximum boundary of macrophytic occurrence. In these transects, the taxonomic composition of macrophytic vegetation is recorded in five depth zones 0-1 m, 1-2 m, 2-4 m, 4-8 m, >8m (Janauer, 2002; C.E.N., 2003) and their abundance is estimated on the semi-quantitative five-point DAFOR scale (Dominant >75%, Abundant 25-75%, Frequent 10-25%, Occasional 1-10%, Rare <1%) (Palmer et al., 1992; CEN, 2003). In each depth zone, five sampling points, evenly distributed along the increasing depth gradient, are determined.
Sampling starts at the shoreline for the first depth zone (0-1m) on foot, by wading. Macrophytic vegetation is assessed by the use of a rake (with a scaled handle) and a bathyscope. For the other depth zones, sampling is made by boat, with the use of a bathyscope and a double-headed rake attached to a rope. A Secchi disc, a GPS device and a bathymetric device are also used. Going along the transect, in each sampling point, two different samples (one sample from each side of the boat) are taken. Based on these two samples in each sampling point, all macrophytic species are identified and their abundance is estimated. Sampling bags are used to store samples for species identification by using a stereoscope and identification keys. To assure that the maximum depth of plant growth is defined properly, at the end of each transect, some samples with no vegetation are taken.

Species identification

During the survey, a list of all macrophytic taxa and their relative abundance in each point of the transect, is recorded. That includes all:

- Angiosperms (helophytes, hydrophytes, amphiphytes, aquatic forms of land species);
- Pteridophyta;
- Bryophyta;
- Charophyta;
- Other green filamentous macroalgae (*Cladophora* spp.).

Angiosperms, pteridophyta, bryophyta and charophyta are commonly determined to species level. Most of them are identified in the field and validated afterwards in the laboratory, by using a stereoscope and identification keys. Other filamentous macroalgae are determined to genus level.

Time and frequency of sampling

Total macrophytic vegetation survey for each natural lake, is carried out as recommended, once per three years (E.C., 2003a). During that survey, the taxonomic composition of all macrophytic vegetation (presence and abundance of each species) in a lake is assessed, as well as the macrophytic vegetation's abundance by estimating its maximum colonization depth.
Sampling period needs to be during the peak of the vegetation season, so it is chosen by expert judgment, taking into consideration the geographical position of each lake and the climatological conditions prevailing at the given year. For lakes in southern Greece, early summer months (late May to early July), are usually ideal for vegetation sampling, while lakes in northern Greece need to be assessed later in the summer (late July to early September).
For macrophytic abundance monitoring, annual values of maximum colonization depth are needed. So for the next two years after the main vegetation survey, two additional surveys are made again at the peak of the vegetation season. During these additional surveys, the three transects with the maximum colonization depth values are visited and new annual Cmax values for the lake are being measured.

Data processing

For the calculation of TIHeLM metric, the values needed are the relative abundance values of each taxon in the specific transect. The abundance of each taxon, during sampling, is estimated at each sampling point on the semi-quantitative five-point DAFOR scale, as already described. These values are transformed to class average percent coverage as follows: 1=0.5%; 2=5.5%; 3=17.5%; 4=50%; 5=87.5%. Percent coverage of a taxon in all sampling points in a specific transect is averaged, so as to calculate a mean per cent coverage of each taxon in the specific transect. To avoid over-dominance of frequent species and under-presentation of rare ones, the calculated coverage of each taxon in the transect is transformed by raising its abundance to the 0.2 power. Finally, the transformed coverage of each taxon in a specific transect is divided by the total transformed coverage of all taxa in this transect, so as to calculate a modified relative abundance of each taxon in a transect (Eq. 8):

Equation 8

$$\mathrm{RAb_i} = \frac{Ab_i^{0.2}}{\sum_{i=1}^{n} Ab_i^{0.2}}$$

RAb_i : Relative abundance of taxon i in a specific transect;
Ab_i : Mean per cent coverage of taxon i in the specific transect;
n : Number of taxa found in the specific transect.

For the calculation of the Cmax metric, no data processing is needed as it simply expresses the maximum observed depth of a lake where submerged rooted macrophytes are present. The values

used for the calculations are the absolute maximum values observed among the transects, during sampling of a lake for each year.

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 (E.C., 2003b). 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 (E.C., 2003b) and the pressure indicators used commonly in the bibliography (Poikane et al., 2015):

- Total phosphorus concentration (TP), calculated as annual mean for each lake;
- Chlorophyll a concentration (CHLA), calculated as summer (June-August) mean for each lake;
- Secchi depth (SD), calculated as summer (June-August) 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 on one of these pressure criteria, then it is not considered as reference. These threshold values (Table 4), were derived from bibliographical data (values adopted from other member states or values proposed in publications) and were supported by expert judgment.

Table 4. Pressure criteria and their threshold limits for screening potential reference sites

Lake national type	TP (µg/L)	CHLA (µg/L)	SD (m)	ALU (%)	IA (%)	NASN (%)	PD (h/km²)
Deep natural lakes (GR-DNL)	<12	<2	>6	<4	<25	>70	<30
Shallow natural lakes (GR-SNL)	<15	<5	>2	<4	<25	>70	<30

The distribution of the values that these pressure criteria have for the lakes in the national dataset, can be seen in Figure 1. These distributions clearly present the differences between non reference and reference lakes.

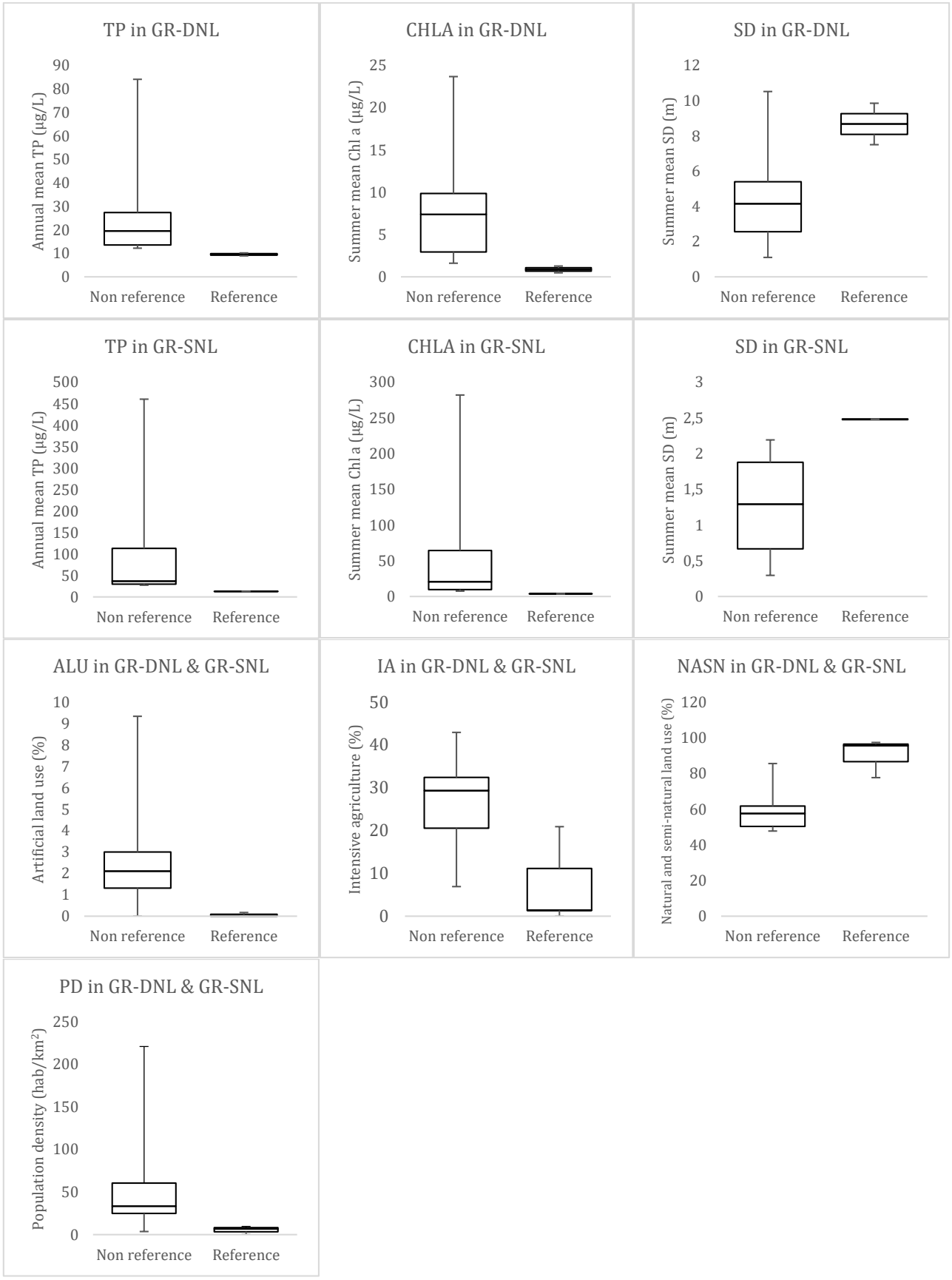

Figure 1. Distribution of total phosphorus (TP), chlorophyll a (CHLA), Secchi depth (SD), artificial land use (ALU), intensive agriculture (IA), natural and semi-natural land use cover (NASN) and population density (PD) in reference and non-reference lakes of the two national types (GR-DNL and GR-SNL)

As can be seen above, in the first step of screening reference sites, the pressure criteria used consist of land use, physicochemical and chlorophyll a. Biological parameters were excluded to avoid circularity (use of the same parameter to filter possible reference conditions and then validate them) and bias (different persons may have different opinions of what reference conditions may represent). After this first step, biological criteria are used to validate or disqualify sampling sites in the potential reference lakes. If sampling results show that parameters of the BQE macrophytes (taxonomic composition and abundance) deviate a lot from what is expected to occur under reference conditions, but no known human-generated pressures are evident (e.g. substratum restrictions), then these sites are removed. The sites that remain are the ones that describe the national reference conditions and are used in the national boundary setting procedure.

2.4. NATIONAL BOUNDARY SETTING

Reference values and class boundaries for the TIHeLM and Cmax metrics are calculated as recommended by the REFCOND Guidance document No. 10 (E.C., 2003b). The base for these calculations are existing near-natural reference sites, as selected after the pressure screening process, using data from monitoring sites.

For the taxonomic composition metric (TIHeLM), common boundaries for both national lake types (GR-DNL & GR-SNL) are estimated, since taxonomic composition is not affected by their difference in maximum depth (there are no deep lake specific taxa and no more depth zone divisions after 8m of depth). For the abundance metric though (Cmax), different boundaries are calculated, since the potential maximum colonization depth in GR-SNLs is limited by their maximum depth, in contrast to GR-DNLs which do not have the same limitations. Values calculated for each metric, are transformed to Ecological Quality Ratios (EQRs) as described at Section 2.1 (Equations 4 & 5).

Reference values are determined as the median values of TIHeLM and Cmax metrics, as calculated for all near-natural reference sites in the potential reference lakes.

High/Good (H/G) boundaries for each metric are determined at the 90th percentile (P90) of the distribution of their values in reference sites.

For Good/Moderate (G/M) boundaries, the results of data statistical distribution in different TP groups, as calculated for the New Mediterranean Assessment System for Reservoirs Phytoplankton NMASRP (de Hoyos et al., 2014), were adopted. Therefore, G/M boundaries are determined at 75th percentile (P75) of the distribution of the values of each metric, in sites that belong to the 20-50μg/L TP-group.

Below G/M boundary, the EQRs range to their minimum values, are divided equally to form the Moderate/Poor (M/P) boundary and Poor/Bad (P/B) boundary.

All boundary values as calculated for each metric, are presented in Table 5.

Table 5. Summary of HeLM assessment method's macrophyte metric boundary values

Metric	TIHeLM		Cmax		Cmax	
National lake types	GR-DNL & GR-SNL		GR-DNL		GR-SNL	
Boundary	EQR	Value	EQR	Value	EQR	Value
Reference	1	7.14	1	12.2	1	6.1
High	>0.94	<7.60	>0.89	>10.86	>0.69	>4.21
Good	0.90-0.94	7.60-7.93	0.36-0.89	4.39-10.86	0.58-0.69	3.54-4.21
Moderate	0.82-0.90	7.93-8.71	0.24-0.36	2.93-4.39	0.39-0.58	2.38-3.54
Poor	0.75-0.82	8.71-9.52	0.12-0.24	1.46-2.93	0.19-0.39	1.16-2.38
Bad	<0.75	>9.52	0-0.12	0-1.46	0-0.19	0-1.16

After the calculation of EQRs for both metrics and their normalization procedure (see Section 2.1, Equation 6), the final lake score ($HeLM_i$) is calculated by averaging the normalized EQRs of the two

metrics (Section 2.1, Equation 7). As a result, the final score of $HeLM_i$ can be assigned to an ecological status class according to Table 6.

Table 6. Final boundary values of HeLM assessment method

$HeLM_i$	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

HeLM assessment method, as already mentioned, addresses eutrophication and general degradation pressures in Greek natural lakes. The main pressure indicators used for the evaluation of the metrics are total phosphorus concentration (Annual mean; TP), chlorophyll a concentration (Summer mean; CHLA) and Secchi depth (Summer mean; SD).
To improve data distribution, TIHeLM metric values were log-transformed, while Cmax metric values were square root-transformed. The transformation of HeLM final values (HeLM), did not improve the distribution, thus the values remained untransformed. Pressure indicators TP, CHLA and SD values were all log-transformed. For linear relationships, a linear regression model was applied and the resulting coefficient of determination (R^2), Pearson's correlation coefficient (R) and p-value (p) of the model, were assessed. As proposed by Kolada et al. (2011), the values of the coefficients $R^2>0.30$ and $R>0.55$, for statistically significant models ($p<0.05$) are assumed as sufficient to accept a metric as a well performing one. For the relationship between HeLM and all three pressure indicators, a multivariate regression model was applied and the same coefficients were assessed.
At the following table (Table 7) and graphs (Figures 2 and 3), all relationships between these pressure indicators and HeLM assessment method's metrics are presented.

Table 7. Overview of the relationships between HeLM metrics (TIHeLM and Cmax) and HeLM final values (HeLM) and pressure indicator values (total phosphorus concentration - TP, chlorophyll a concentration - CHLA and Secchi depth - SD), after linear regression and multivariate regression analysis. For $R^2>0.30$ (coefficient of determination), $R>0.55$ (Pearson's correlation coefficient) and $p<0.05$ (p-value of significance), it was considered that there are significant relationships

Relationship	n	R^2	R	p	Regression equation
TIHeLM-TP	16	0.494	0.703	0.002	$\log TIHeLM = 0.049 \times \log TP + 0.821$
TIHeLM-CHLA	16	0.454	0.674	0.004	$\log TIHeLM = 0.031 \times \log CHLA + 0.865$
TIHeLM-SD	16	0.475	-0.689	0.003	$\log TIHeLM = 0.912 - 0.051 \times \log SD$
Cmax-TP	16	0.686	-0.828	0.000	$\sqrt{Cmax} = 4.379 - 1.383 \times \log TP$
Cmax-CHLA	16	0.808	-0.899	0.000	$\sqrt{Cmax} = 3.247 - 0.994 \times \log CHLA$
Cmax-SD	16	0.751	0.867	0.000	$\sqrt{Cmax} = 1.528 \times \log SD + 1.764$
HeLM-TP	16	0.682	-0.826	0.000	$HeLM = 1.276 - 0.39 \times \log TP$
HeLM -CHLA	16	0.655	-0.809	0.000	$HeLM = 0.931 - 0.253 \times \log CHLA$
HeLM -SD	16	0.580	0.762	0.000	$HeLM = 0.38 \times logSD + 0.556$
HeLM-TP & CHLA & SD	16	0.694	-0.833	0.002	$HeLM = 1.256 - 0.29 \times \log TP - 0.112 \times \log CHLA - 0.068 \times \log SD$

Results show (Table 7) that all metrics and HeLM final values, relate significantly with all three common pressure indicators (all $R^2>0.3$ and all $p<0.05$). Specifically, TIHeLM metric shows a relatively high positive correlation with TP and CHLA, and an equal negative correlation with SD (R^2=0.494, 0.454, 0.475; R=0.703, 0.674, -0.689 respectively). On the other hand, Cmax metric shows high

negative correlation with TP and CHLA and an equal positive one with SD (R^2=0.686, 0.808, 0.751; R=-0.828, -0.899, 0.867 respectively). More importantly, final HeLM values show high negative correlation with TP and CHLA individually, and a high positive one with SD (R^2=0.682, 0.655, 0.580; R=-0.826, -0.809, 0.762 respectively). Finally, multivariate regression analysis results show also a strong negative correlation (R^2=0.694; R=-0.833) between final HeLM values and all pressure indicators evaluated.

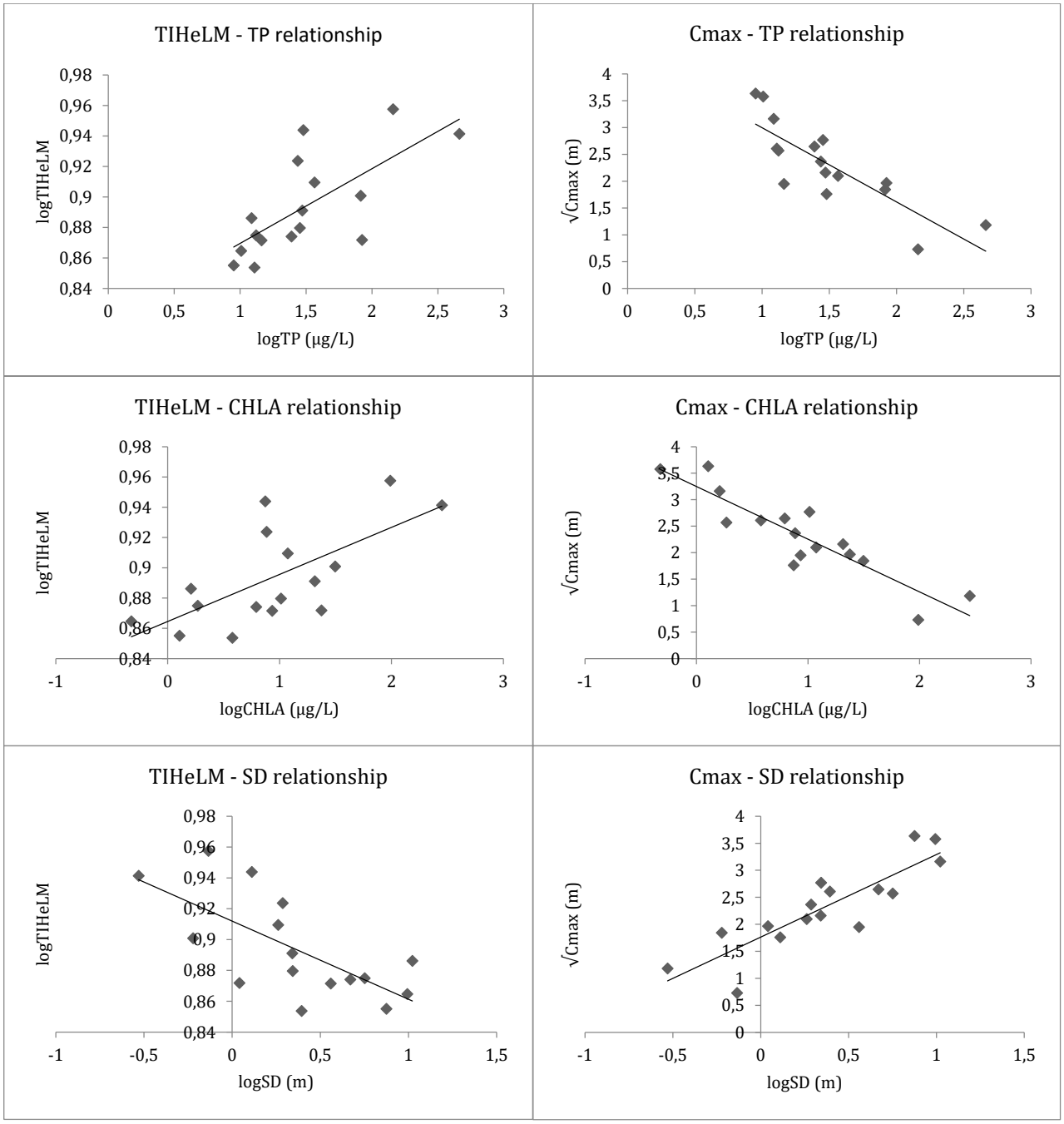

Figure 2. Pressure-response curves of metrics TIHeLM and Cmax, in regards to total phosphorus (TP), chlorophyll a (CHLA) and Secchi depth (SD) pressure indicators. Best linear fits' equations and coefficients, can be seen at Table 7.

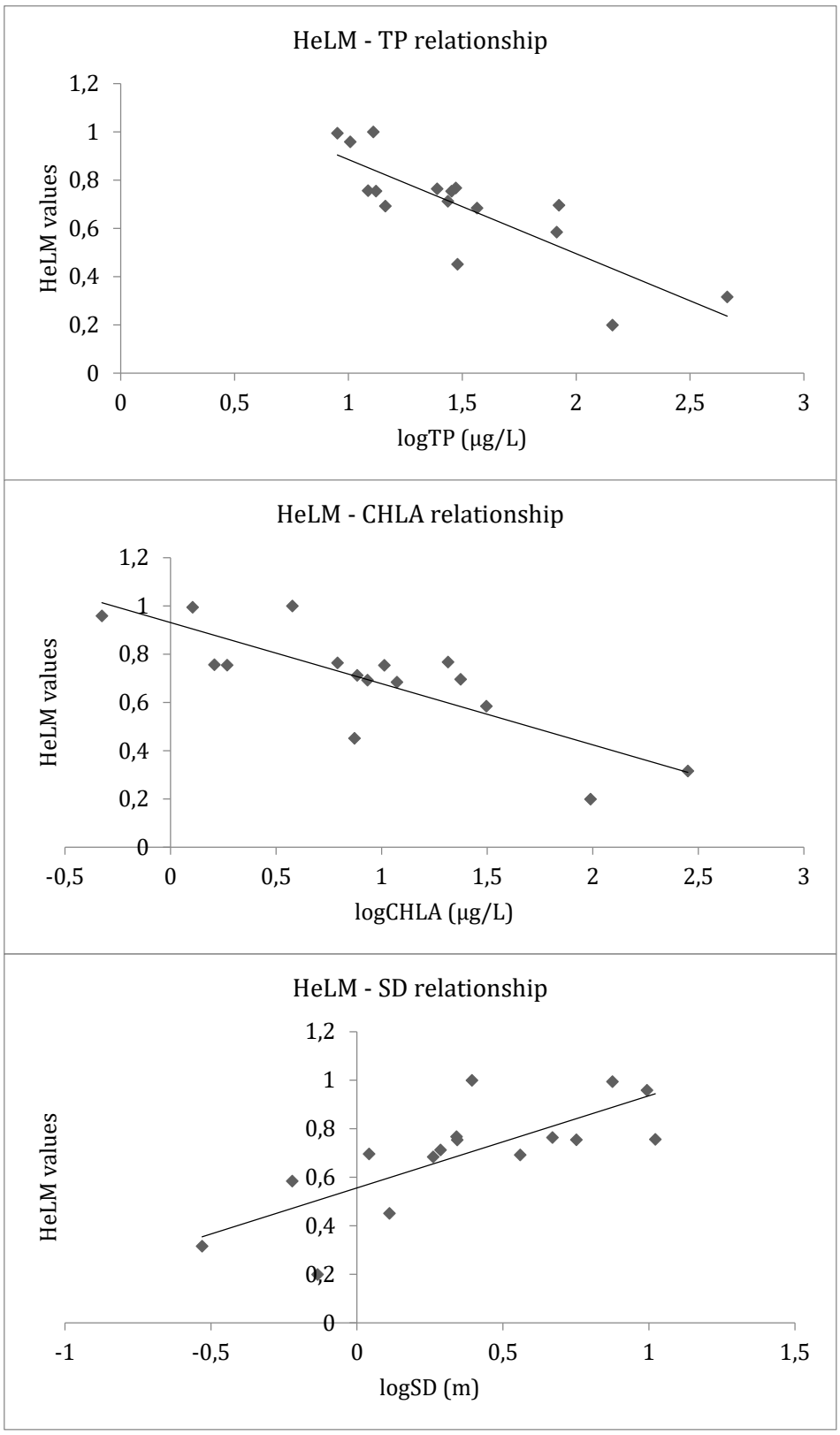

Figure 3. Pressure-response curves of HeLM assessment method's final values, in regards to total phosphorus (TP), chlorophyll a (CHLA) and Secchi depth (SD) pressure indicators. Best linear fits' equations and coefficients, can be seen at Table 7.

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 HeLM 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 HeLM assessment method

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES (Table 6)
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 (Equation 7 and Table 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 HeLM 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 yet and there is no information on which types other members may base their assessment methods. The two national types used for HeLM 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, types in HeLM assessment method can change according to the needs, so as to proceed to an intercalibration analysis.

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 HeLM assessment method addresses eutrophication and general degradation pressures. To our knowledge, the Aquatic Flora Spanish Assessment Method (CEDEX, 2010ab) and the French macrophytes assessment method IBML (Bertrin et al., 2012) do also address these pressures.

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.

We have no final information about the assessment concepts of other methods in the MED-GIG Intercalibration Group. Initial information reports from Spain and France (CEDEX, 2010ab; Bertrin et al., 2012), show that the French method follows a same assessment concept for taxonomic composition only (Trophic Index of all macrophytic taxa, weighted by their relative abundance), whereas the Spanish method uses a different concept (Coverage of eutrophication indicator species).

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Since there are no fully developed assessment methods for Mediterranean Lake Macrophytes reported in MED-GIG level, there is no way to check HeLM 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 macrophytes towards designing intercalibration exercises at least at a MS to MS level.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

In order to describe the biological communities of macrophytes in lakes of different ecological status, the national dataset with the monitoring data from the 16 studied lakes was used. The taxonomic composition metric TIHeLM, as mentioned previously, is based on all macrophytic taxa found in a lake, which contribute to the metric's calculation by their lake trophic ranks (LTRs – grades of response to nutrient enrichment). Thus, these LTR values (Table 2), are indicators of the preference of each taxon in lakes of oligotrophic or eutrophic status. For a better overview of the species turnover, along an eutrophication gradient, all taxa in the national dataset were classified in biotic forms. Relative abundance of each biotic form in a lake was calculated and plotted against taxonomic composition metric (TIHeLM) and the final assessment method's (HeLM) values (Figure 4 and 5).

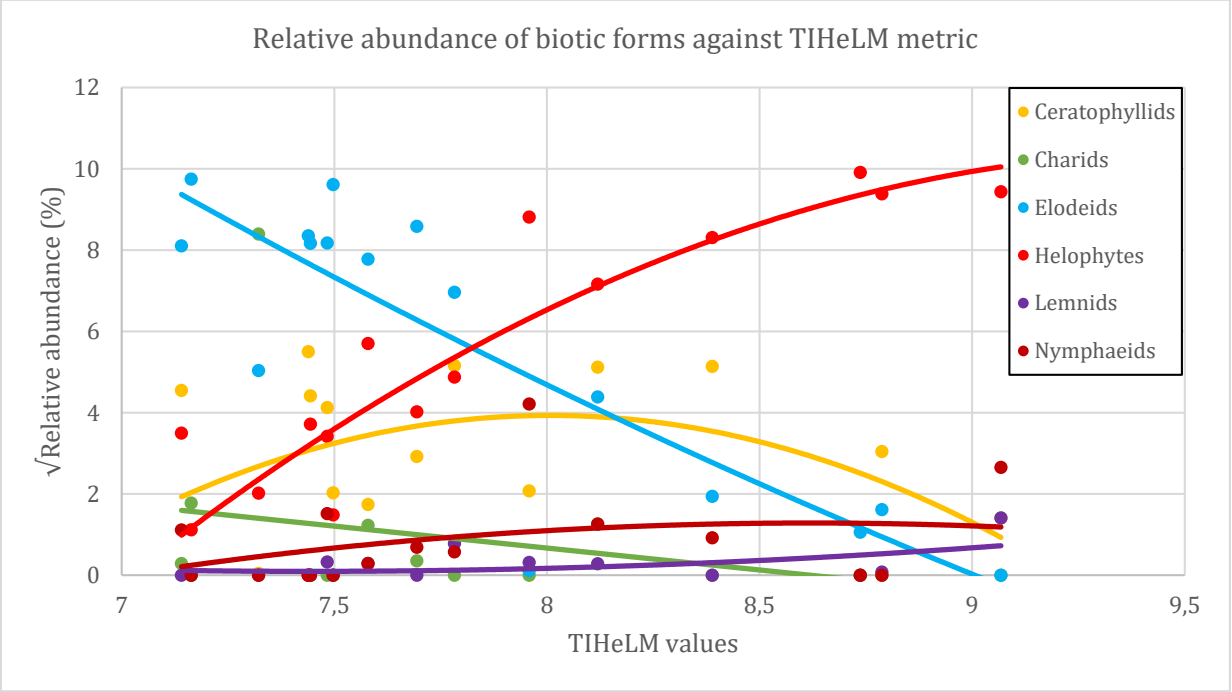

Figure 4. Scatter-plot between TIHeLM values as calculated for lakes in the Greek National Dataset and the relative abundance (square-root transformed) of macrophytes in different biotic forms. The lines represent polynomial adjustments.

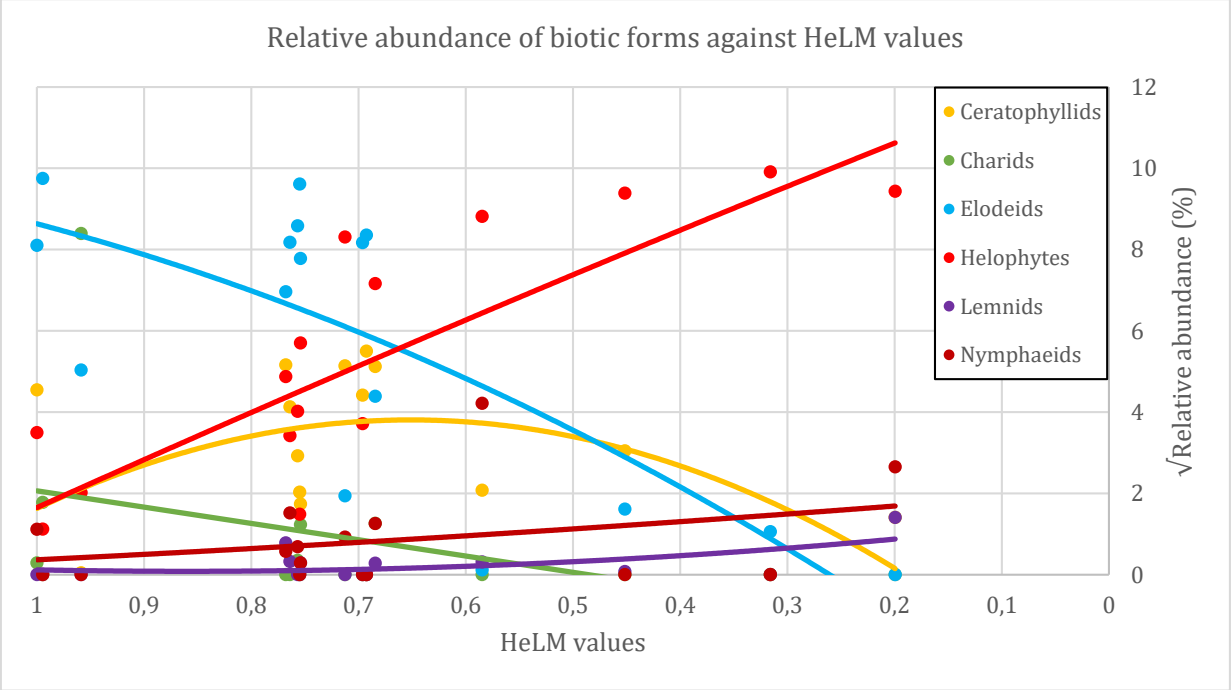

Figure 5. Scatter-plot between HeLM values as calculated for lakes in the Greek National Dataset and the relative abundance (square-root transformed) of macrophytes in different biotic forms. The lines represent polynomial adjustments.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

For high status lakes, TIHeLM values are <7.60 (Table 5) and final HeLM values are 0.8-1.0 (Table 6). This means that biological communities mostly consist of taxa with low LTR values (Table 2). As depicted in Figures 4 and 5, elodeids are the dominant life form in biological communities at high status. This result is reasonable enough, since high status sites provide great water clarity (Cmax values are also at their peak in high status sites), which allow elodeids (a submerged life form) to develop and expand in greater depths. The genera *Potamogeton, Myriophyllum, Najas and Ranunculus* are typical for high status sites. Charids (another submerged life form) and mostly the genera *Chara* and *Nitella*, are also found with maximum abundance in high status sites. The biotic forms that rely on direct solar radiation at the surface of the water, helophytes, lemnids and nymphaeids, can be found in high status sites in relatively low abundance numbers, due to the competitive advantage that is given to submerged macrophytes. Most common genera of emergent life forms, found in high status lakes, are *Alisma, Eleocharis, Juncus, Mentha* and the species *Nymphaea alba*. Some ceratophyllids (genus *Ceratophyllum)* can also be found at these sites, but in relatively low numbers.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

According to the normative definitions in WFD of ecological status classifications for lakes, sites are classified at good status when there are slight changes in the composition and abundance of macrophytic taxa, compared to the type-specific communities. For good status lakes, TIHeLM values are 7.60-7.93 (Table 5) and final HeLM values are 06-0.8 (Table 6). This means that in lakes at good status, compared to high status ones, either new taxa with higher LTR values (Table 2) appear or the relative abundance of taxa with higher LTR values, which are also present at high status ones, increases substantially. As depicted in Figures 4 and 5, in the biological communities at good status, the dominant macrophytic life form starts to change from elodeids to helophytes. Slightly decreased water clarity compared to that of high status sites, has as a consequence a slight decrease in the abundance of the rooted submerged life forms (elodeids and charids) and therefore space is created for the development of the non-rooted submerged ceratophyllids and the emergent life forms (helophytes, lemnids and nymphaeids). Composition changes are also apparent. Species *Vallisneria spiralis* and *Nitellopsis obtusa* become more frequent in elodeids and charids, respectively. *Phragmites australis* and *Typha* spp. start to dominate in the helophytes group. *Hydrocharis morsus-ranae, Nuphar lutea* and *Nymphoides peltata* are becoming more often in nymphaeids. Also lemnids, with *Lemna minor* and *Spirodela polyrhiza* start to make their appearance. Ceratophyllids (genus *Ceratophyllum*), as already mentioned, increase a lot in the water column.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

Based on the interpretations of normative definitions for the biological quality elements, macrophytic communities are classified at moderate status when their taxonomic composition and abundance differ significantly from the type specific reference conditions. That means that taxa that cannot be found at reference conditions (particularly pollution tolerant taxa) may dominate the flora. For moderate status lakes, TIHeLM values are 7.93-8.71 (Table 5) and final HeLM values are 0.4-0.6 (Table 6). In these ranges, as depicted at Figures 4 and 5, due to even smaller water clarity values, the composition of macrophytic vegetation changes a lot. Elodeids have lost the dominance of macrophytic vegetation from helophytes. Ceratophyllids have increased in abundance and begin to dominate over elodeids also. Charids begin to disappear completely from the macrophytic vegetation, when nymphaeids and lemnids show a steady increase. In short, there is a rapid decrease in rooted submerged life forms (elodeids and charids), non-rooted submerged ceratophyllids are at their peak of their expansion and the emergent life forms (helophytes, lemnids and nymphaeids) steadily increase to become the only remaining life forms of macrophytic vegetation in fully degraded sites. *Phragmites australis*, among other helophytes (*Typha* spp., *Paspalum* spp. and *Schoenoplectus* spp.), becomes the

overall dominating species in these sites. Submerged in the water column, elodeid species are becoming more scarce (*Vallisneria spiralis*, *Najas marina, Potamogeton pusilus, Stuckenia pectinata*), with ceratophyllid *Ceratophyllum demersum become the* dominating species. Nymphaeids and lemnids increase in abundance and diversity with species like *Persicaria amphibia, Salvinia natans, Azolla filiculoides* and *Lemna gibba*.

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