PHYMED: an ecological classification system for the Water Framework Directive based on phytoplankton community composition

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Abstract

The European Water Framework Directive (WFD) has as its objective the establishment of a framework for the protection of continental, groundwater and surface, transitional, and coastal water. The phytoplankton is one of the biological quality elements established for determining the ecological status within the Directive. Phytoplankton biomass, composition and abundance, together with frequency of blooms, are the metrics to be assessed according to the WFD.

For the Mediterranean coastal water only biomass (chlorophyll *a*) has been intercalibrated by the Mediterranean Geographical Intercalibration Group. In the present contribution, a multimetric index PHYMED for ecological classification of coastal water bodies in Valencia Region is developed based on biomass and phytoplankton community composition.

First, a conceptual model was established to develop a system of indicators based on the composition of the phytoplankton community. Formulating the model required a preliminary analysis of the existing correlations between the parameters related to the phytoplankton community composition and the variations in the pressure indicators in each coastal water body. After discarding those variables which did not readily allow a distinction between coastal water bodies to be discerned four phytoplankton variables were selected to constitute the PHYMED index. Reference conditions were determined based on the analysis of pressures and impacts and supplemented by a thorough territorial analysis.

To verify that this index responded well to the varying ranges in pressure, a statistically significant correlation was confirmed to exist between the index and the phosphorus values.

The results obtained from this index were compared with those derived using chlorophyll *a* finding that the existing deviations presently arising from the use of chlorophyll *a* as an ecological status indicator can be corrected with the proposed multimetric index.

Keywords Water Framework Directive, phytoplankton composition, coastal water body, Mediterranean Sea

1. Introduction

The European Union Framework Directive 2000/60/EC (WFD), aims to maintain and improve the aquatic environment within the European Community by providing a framework for the protection of ground, inland surface, transitional and coastal waters. The principal environmental objectives of the WFD are to achieve sustainable water-resource management, to attain good ecological quality in the water bodies, to prevent further deterioration of surface and ground waters, and to ensure sustainable functioning of aquatic ecosystems by 2015.

To achieve these proposed objectives, the WFD requires Member States (MS) to assess the ecological quality status of their respective water bodies through a series of steps, including: characterisation of surface waters, establishment of typology; establishment of monitoring programmes; definition of type-specific reference conditions (RC) for biological quality elements; classification of all surface water bodies using Ecological Quality Ratios based on biological quality elements; and, finally, intercalibration exercise (IC) of MS.

The Common Strategy Implementation (WFD CIS, 2004) established the Ecological Status (ECOSTAT) working group to carry out this intercalibration exercise. To that end, within ECOSTAT various Geographic Intercalibration Group (GIG) were formed to address the distinct categories of superficial waters.

The Spanish coast belongs to two different Geographic Intercalibration Groups; first, the entire north of Spain, extending west from the border with France until the border with Portugal, the southern coast of Spain from Portugal to the Strait of Gibraltar, and the Canary Islands belongs to the North East Atlantic GIG (NEA-GIG). The Mediterranean littoral from France to the Strait of Gibraltar comprises the second group, the Mediterranean GIG (MEDGIG).

Within Spain, the intercalibration process for phytoplankton as a Biological Quality Element (BQE) for coastal waters differs, depending on the GIG overseeing the process. In the case of the NEA-GIG three metrics were selected: chlorophyll, Indicator Taxa

(Frequency of *Phaeocystis* Cell counts) and Taxa Cell Counts (Frequency of phytoplankton taxa cells counts).

All MS have agreed to use chlorophyll-a as a metric within the intercalibration process applying the 90th percentile of data, sampling monthly during the growth season (Commission Decision 2088/915/EC of 30 October 2008).

Regarding to the other parameters, elevated cell counts and *Phaeocystis* blooms are not used uniformly across member states, in fact some countries are using the elevated count tool and other countries are using the *Phaeocystis* tool.

The Spanish regional governments belonging to the above-mentioned GIG adopted its proposed measures during the first phase of intercalibration and furthermore have developed, based on their phytoplankton data, the boundary values above which phytoplankton proliferations can be considered to exist (JRC Scientific and Technical Reports Water Framework Directive intercalibration- Technical report-Part 3: Coastal and Transitional Waters).

However, in the first phase of the MEDGIG intercalibration, only the BQE parameter for phytoplankton was agreed upon for use: Chlorophyll *a* concentration as parameter/indicator for biomass. Since a methodology based on a common data set, was not elaborated, different metrics of this parameter and different statistical approaches for setting the boundaries (derived from national methods) were analysed and compared. National methods adopted, in general, three kinds of metrics: 90th percentile, annual geometric mean and average. Depending on the MS the metrics were calculated using only surface data or water-column integrated data, covering different period (e.g. one year in case of geometric mean and 5 to 6 years when using 90th percentile) (Commission Decision 2088/915/EC of 30 October 2008).

Boundaries are in terms of Chlorophyll *a* concentrations and Ecological Quality Ratios (EQR) that have different status of implementation. The remaining phytoplankton metric (abundance, composition and blooms) are slated to be intercalibrated during the second phase of the exercise.

Chlorophyll *a* has been widely utilized as an estimator of phytoplankton biomass in the majority of monitoring programs (Kaas et al., 2005), as well as an indicator of nutrient enrichment (CSTT, 1997; Gowen et al., 1992; Painting et al., 2005).

There are studies which demonstrate the existence of a positive and predictable relation between chlorophyll *a* and nutrient growth (Kaas et al., 2005). Nevertheless, other authors such as Andersen et al. (2006) advise that chlorophyll *a* measurements must be interpreted for what they are: the concentration of chlorophyll *a* and nothing more. They are neither measurements of biomass, nor indicators of nutrient states or growth rates. These authors recommend measurements of primary production-and not chlorophyll *a*-to be used as growth indicators in evaluating a state of eutrophication.

Chlorophyll *a* presents obvious difficulties when establishing eutrophic levels. This can be attributed to various scenarios in pelagic systems when biomass must not necessarily be present in an autrophic form. In areas with high contamination levels brought on by waste waters, for example, biomass bacterial levels can increase. In other cases, the herbivorous zooplankton can maintain a low biomass, which might not correspond to a system's nutrient content (Painting et al., 2005).

It must be remembered, then, that chlorophyll-a concentration, biomass and phytoplankton abundance are three distinct variables and, therefore, the use of the first to estimate the others can be problematic (Domingues et al., 2008; Kruskopf and Flynn, 2006). Therefore, a univariated focus (chlorophyll *a*), or a quality index derived from it, may not be sufficiently adequate to determine the trophic state of a marine system (Guidance Document No. 14, 2009; Primpas et al., 2009).

Moreover, phytoplankton composition is considered as a natural bioindicator because of its complex and rapid responses to fluctuations of environmental conditions (Brogueira et al., 2007). Moreover, phytoplankton composition provides further information about quality water than nutrients or chlorophyll *a* concentration (Vuorio et al., 2007).

For these reasons, the objective of the present study is to develop a multimetric index based on biomass and phytoplanktonic communities' composition which will enable an ecological classification of coastal water bodies in the Valencian Region (VR).

2. Material and Methods

2.1 Typology

The water body typologies present in the VR have been established and defined using a landscape approach, as required by the WFD; hence, an analysis of the entire region was undertaken, taking into account not only coastal waters, but also littoral coastline influences. The following factors were considered: geomorphology, littoral transport, dominant winds, rainfall, area of the fluvial basins and continental inputs and wet zones. The outcome of the analysis revealed that San Antonio Cape effectively divides the VR into two areas (Hermosilla, 2009).

In the area north of the Cape's delimitation, the coastline is regular and almost rectilinear, predominated by sand deposits. Sand particles originating from the Ebro

River are easily found in this area (Díez, 1996; Serra, 1986). Prevailing winds are north-easterly with an eastern component and rainfall data reveals that this area receives more rainfall than in areas located south of the Cape (CEAM, 2008). Furthermore, in this northern partition the river basins are larger and receive continental inputs from rivers and irrigation channels. Finally, the area's wetlands possess oligohaline waters.

In contrast, at the south of the Cape high and low cliffs predominate (BEACHMED, 2003; Serra, 2002); an Ebro River influence is undetectable (Esteban, 1988); winds are south-easterly (Serra and Medina, 1996); and the river basins are smaller, with predominantly dry riverbeds en route to the sea (Serra, 2002). Moreover, wetlands to the south are generally hypersaline with some brackish water influences (GVA, 2002). This global landscape approach indicates that in the VR there are two distinct zones with different characteristics: one to the north of the Cape, with a moderate influence by freshwater input, (Type II-A)), found in the waters extending immediately north from San Antonio Cape to the Region's northernmost border with Tarragona and another one to the south until the border with the Murcia province, with a limited continental influence (Type III-W).These typologies offers the advantage of stability, as types do not change substantially over a period of years, despite temporary shifts in pluviometry or other climatic circumstances (Fig. 1). Since August 2005, the network has been comprised of a total of 100-110 sampling stations spanning 18 coastal water bodies (WB).

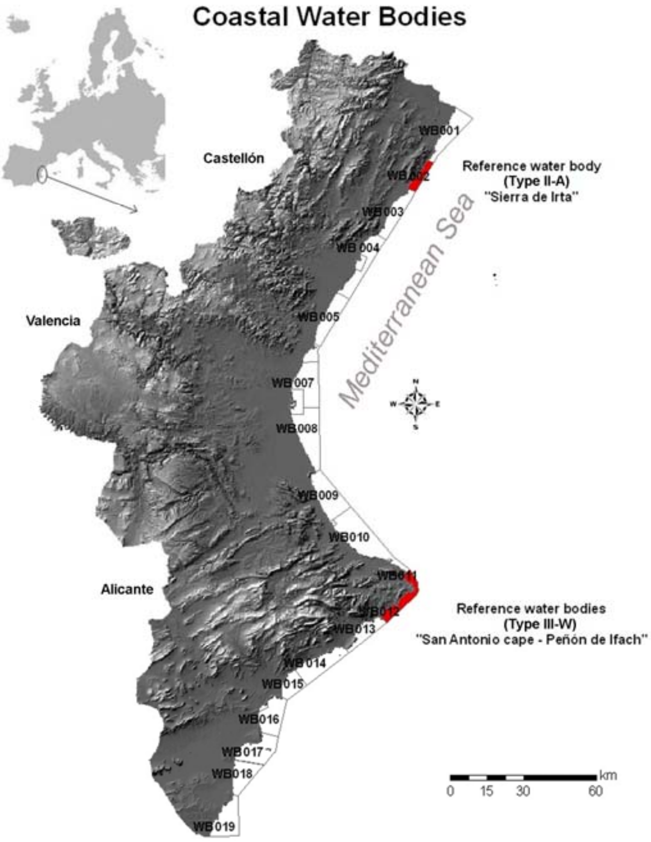

Fig.1: Coastal Water Bodies and Reference Water Bodies for Type II-A and III-W in the Valencian Region

2.2 Sampling procedure

Though all of the decisions in the IC exercise were predicated on nearshore measurements, the MEDGIG accepted that the established values would be doubled for those countries with inshore measurements. Adhering to the stipulations of the MEDGIG for the BQE of phytoplankton monitoring of the water bodies was performed monthly.

Water samples were taken from the water column at a 10 cm-depth, and from beyond the wave breakpoint to avoid extracting samples from areas where the effect of wave motion might generate sediment resuspension and, in turn, affect the biochemical quality of the water.

At each sampling station, the water for nutrient, salinity and chlorophyll *a* analysis was collected in 2-litre plastic bottles, kept refrigerated at 4ºC and carried to the laboratory (always within the first 12 hours after collected). For phytoplankton samples were stored in glass bottles and fixed in situ with glutaraldehyde (2% final concentration) according to Sournia (1978) for phytoplankton analyses.

Once in the laboratory, the samples were divided into several proportional parts, following the conservation procedures suggested by APHA (1998). The samples were filtered through cellulose acetate membrane filters of 0.45μm (Millipore HAWP) for nutrients and chlorophyll *a* analyses.

For the analysis of nutrients (ammonium, nitrite, nitrate, reactive soluble phosphorus, total phosphorus and orthosilicic acid) an Alliance Instruments Integral Futura auto-analyzer was used, following the methodology described by Treguer and Le Corre (1975), and taking into account Kirkwood et al. (1991) and Parsons et al. (1984).

On the determination of chlorophyll *a* the trichromatic method was used, based on spectrophotometry (APHA, 1998).

Salinity was measured with Portasal Guildline 8410 A, calibrated with the suitable standards (I.A.P.S.O. Standard Seawater, Ocean Scientific International Ltd, K15= 0.99986, S= 34.995‰).

Phytoplankton analyses were carried out by filtering the samples through a 0.2μm membrane filter, later drying the filtered material. Then salt was removed by adding 5ml of distilled water and the samples were again filtered and dried. After this, the material on the filter was dehydrated by successive washings with 50%, 80%, 90% and 99% aqueous ethanol. Each dried filter was placed onto a drop of immersion oil in the centre of a slide and 2 more drops were added on the top side of the filter (Paches, 2010). Finally, a coverglass was placed on the top of the filter (Fournier, 1978). Algal counts were made by epifluorescence microscopy (Vargo, 1978) with a Leica DM2500 microscope, using a 100× oil-immersion objective. A minimum of 300 cells were counted and at least 100 cells of the most abundant species or genera were counted with an error below 20% (Lund et al., 1958).

3. Results and Discussion

3.1 Reference Conditions

The selection of reference conditions was carried out with an integrated analysis of the pressures and impacts of the VR, accounting for anthropic pressures (point sources of pollution and their morphological alterations), and an analysis of population density and physicochemical parameters.

As stipulated in Article V of the WFD, a study must be undertaken of the repercussions of human activity on the status of superficial waters which, for the VR, was carried out following the manual published by the Ministry of the Environment (Manual for the Identification of Pressures and the Analysis of Impact on Superficial Waters, Ministry of the Environment- 14 February, 2005).This manual instructs that the analysis of anthropic pressures should be centered primarily on the significant pressures which, for a coastal water body, are: Point sources pollution, those for which the supply of total phosphorous (TP), limiting phytoplankton nutrient in the Mediterranean Sea, and priority pollutants have been taken into account, and the morphological alterations which can be attributed to the anthropization of the coastal morphology. With the different pressures enumerated above, the global pressure is estimated using a criterion similar to that adopted by the WFD for the indicators, assuming the global pressure will be significant when one of the pressure types is.

Accounting for all the information regarding anthropic pressures, territorial analysis and the analysis of the existing population in each water body (GVA, 2009), WB002 “Sierra de Irta” for Type II-A and WB011 “San Antonio Cape-Moraira” and WB012 “Moraira-Peñon de Ifach” for Type III-W were selected as reference WB. The WB002 is the most extensive undeveloped littoral area in the VR (GVA, 2009), exhibits the lowest population density and furthermore, the sea area around this strip has been declared a marine reserve (Decreto 108/2002). In the other hand, WB011 “San Antonio Cape-Moraira” and WB012 “Moraira-Peñón de Ifach” present low population density and the area surrounding likewise enjoys marine reserve status (Decreto 212/1993) as well (Fig.1).

It should be noted that, for the Mediterranean region, the possibility has been raised of utilizing the marine reserve areas as reference conditions, since they afford the best ecological conditions (Casazza et al., 2004).

However, Guidance Document No. 14 (2009) states that when selecting reference conditions for a biological quality element, the values of that quality element must be excluded. Thus, during the selection of the reference stations (in the aforementioned water bodies), consideration was made for the values of chemical indicators affecting biological indicators (salinity and nutrients, WFD Annex V, 1.1.4.) obtained for the three-year series.

The principal statistical characteristics (10th, 20th, 50th, 80th and 90th percentile) of these variables for the sampling stations of WB002 named as: DP010, DP134, DP135, DP136 and DP137; WB011: DP076, DP078 and DP080 and WB012: DP082, DP084 and DP086 are summarized in the box-whisker diagrams (Fig.2).

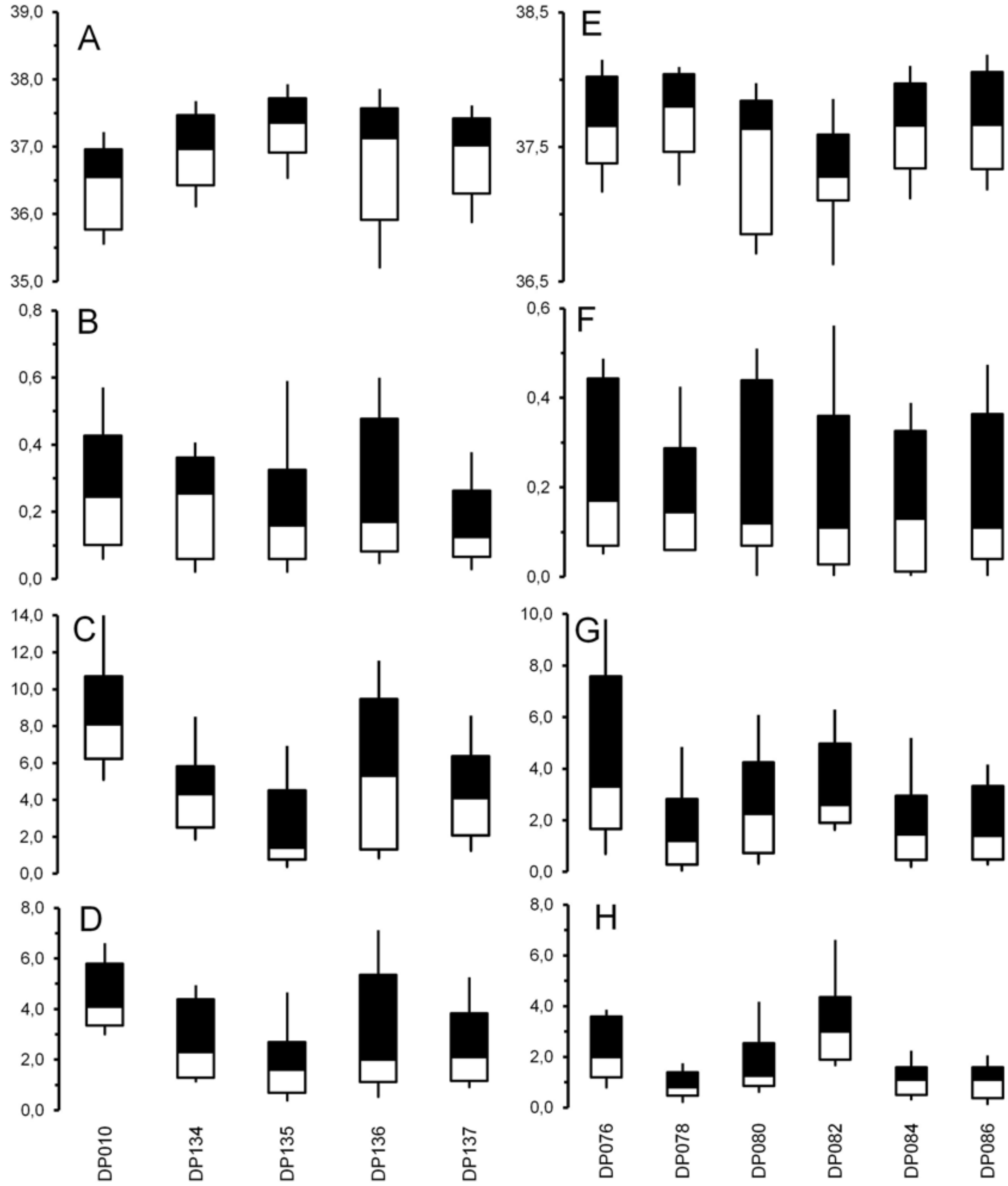

Fig.2: Box-whisker diagram of Salinity (A-E), Total Phosphorous (B-F), Nitrate (C-G) and Orthosilicic acid (D-H) in WB002 (DP010, DP134, DP135, DP136 and DP137), WB011 (DP076, DP078 and DP080) and WB012 (DP082, DP084 and DP086)

sampling station; the ends (whiskers) represent 10th and 90th percentile values, the edges of the boxes are 20th and 80th percentile and the interior line the 50th percentile

Sampling stations DP135, DP136 and DP137 (for Type II-A) and DP078, DP084 and DP086 (for Type III-W) were selected, based on their 50th percentile of salinity and phosphorous values, which were the highest and lowest, respectively, of all stations. With regards to orthosilicic acid, the selected stations exhibited the lowest 50th percentile values, indicating little continental influence. These stations likewise reflect the natural variability within the typology of coastal water bodies to which they belong.

3.2 Statistical parameters

As it is mentioned before, the criterion used by NEA-GIG to establish the ecological status of coastal water bodies based on phytoplanktonic biomass has been the 90th percentile of chlorophyll *a* between the growth season. The use of the 90th percentile seems appropriate because this statistical parameter adequately represents the conditions of phytoplankton blooms in spring, since the limited irradiance these marine ecosystems receive impedes phytoplankton growth during winter months.

However, the Mediterranean Sea is different in many features from other European seas. The limited light conditions characteristic of other seas are not found in the western Mediterranean, while minimum winter temperatures are about 16ºC. The seasonal evolution of chlorophyll *a* content and the episodes of phytoplankton blooms differ from those seas; allowing high values of chlorophyll *a* and including phytoplankton bloom process to be found during winter months. Fig. 3 shows the 50th percentile chlorophyll *a* for four coastal WB from the VR. As it can be seen there are two peaks of chlorophyll *a*, the first in August-October and the second in January-February.

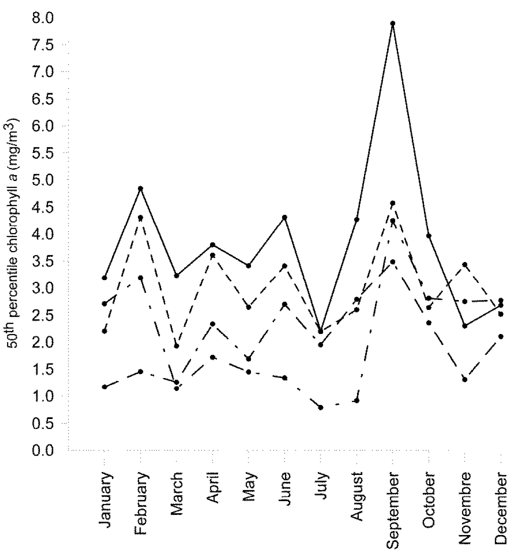

Fig.3: 50th percentile of chlorophyll *a* for 4 coastal WB (008-009-010 and 016) of VR, showing elevated values during winter months (Valencian Monitoring Network, 2005-2008)

Based on all of the aforementioned it is necessary to include biological data (biomass, composition and cell density) from the whole year. Furthermore, it is necessary to revise which statistical parameter fits better with the Mediterranean Sea conditions.

It must also be noted that phosphorous is the limiting element in Mediterranean waters, most likely due to the elimination of this element by iron-rich particles from the Sahara (Agius et al., 1982; Estrada, 1996; Krom et al., 1991; Vaulot, 1996). Thus, the coefficient of determination (r2) is calculated for the TP (as a measurement of eutrophic pressure) and the chlorophyll *a* content for various statistical parameters, as seen below.

r^2	Mean	50^{th} percentile	80^{th} percentile	90^{th} percentile
TP *vs* Chl *a*	0.74*	0.80*	0.78*	0.64*

**denotes a significant correlation at α 0.01 level*

Table 1: Coefficient of determination for TP versus Chl a for the 50th, 80th, 90th percentile and mean values of the coastal water bodies of the VR

As seen in Table 1, the highest correlations are established with the 50th and 80th percentiles, while the lowest with the 90th percentile. Therefore, it was decided hereafter to employ the 50th percentile as the most suitable statistical parameter for the Mediterranean.

Fig.4 shows the principal statistical characteristics (10th, 20th, 50th, 80th and 90th percentile) of chlorophyll *a* and the main phytoplankton groups in coastal water bodies from Valencian Monitoring Network 2005-2008.

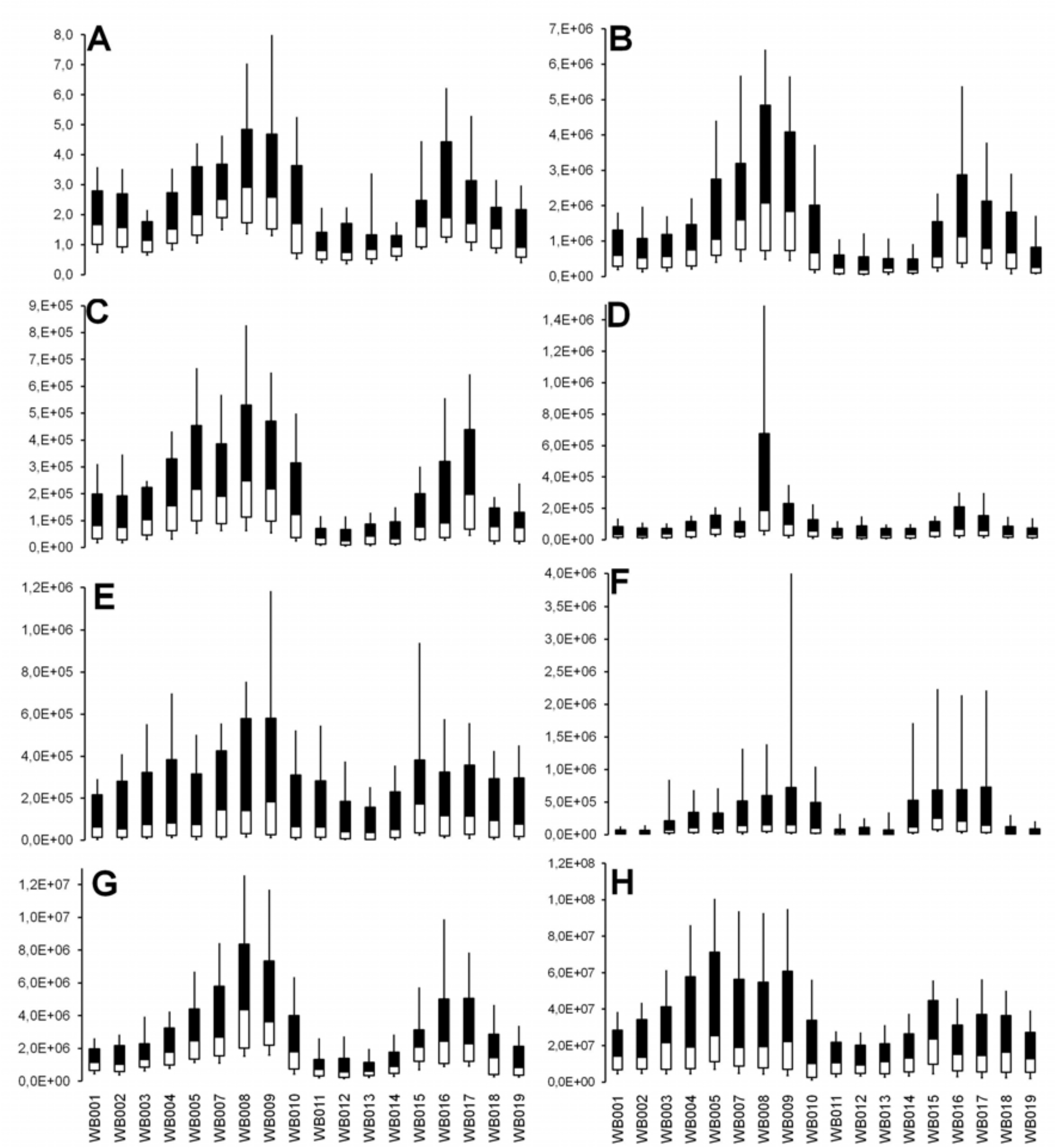

Fig.4: Chlorophyll *a* (mg/m³) (A), Diatoms (cell/L) (B), Cryptophyceae (cell/L) (C), Chlorophyceae (cell/L) (D), Prymnesiophyceae (cell/L) (E), Prasinophyceae (cell/L) (F), Total Eukaryotic (cell/L) (G) and picocyanobacteria (cell/L) (H) for coastal water bodies (Valencian Monitoring Network, 2005-2008)

3.3 PHYMED Index

Guidance Document No. 14 states that all biological indicators utilized to establish water quality must always be referenced to a determined pressure level. Hence, prior to the establishment of any index in which the BQE established by the WFD might be involved, it is necessary to check that these elements are correlated to pressure levels. The Levantine basin of eastern Mediterranean is characterized as nutrient-deficient and therefore ultra-oligotrophic in comparison to the Atlantic Ocean (Berman et al., 1984).

Furthermore, the eastern Mediterranean is more P-limiting to the growth of phytoplankton; in contrast to the general dogma that N is the more limiting nutrient in marine systems (Krom et al., 1991). Thereby, Mediterranean anthropic pressure is measured as a concentration of total phosphorous (Correll, 1998). For that, every available phytoplankton variable has been correlated to the TP, with the aim of observing which of these variables fulfill the WFD requirements (to be correlated with pressure). This analysis was done using both, variables of cellular density and percentages of abundance, from the main phytoplankton groups in the statistics program SPSS 16.1 at the 0.01 level. The correlations with the 90th percentile of the TP are likewise shown to attest that the highest correlations in the Mediterranean occur with the 50th percentile.

Total Phosphorous (μM)	50^{th}	90^{th}	Total Phosphorous (μM)	50^{th}	90^{th}
Diatoms (cell/L)	0.76*	0.67*	picocnb/(TE+cnb)	0.82*	0.42*
Cryptophyceae (cell/L)	0.67*	0.57*	picocnb/TE	0.84*	0.36*
Dtm+Crypt (cell/L)	0.81*	0.67*	picocnb/(TE – Pras)	0.72*	0.43*
Chlorophyceae (cell/L)	0.64*	0.30	picocnb/(Dtm+Crypt)	0.61*	0.50*
Dinophyceae (cell/L)	0.64*	0.38*	(picocnb+ Pras)/(TE -Pras)	0.72*	0.41*
Prasinophyceae (cell/L)	0.34	0.16	(Dtm +Crypt)/Prymn	0.77*	0.41*
Prymnesiophyceae (cell/L)	0.52*	0.21	Dtm/Crypt	0.10	0.30
colonial Cyanophyceae (cell/L)	0.36*	0.19	Dtm/Prymn	0.75*	0.37*
picocyanobacteria (cell/L)	0.19	0.21	(TE+ cnb)/picocnb	0.67*	0.34
Total Eukaryotic (cell/L)	0.80*	0.58*	TE/picocnb	0.79*	0.36*
%Diatoms	0.34	0.12	(TE-Pras)/picocnb	0.82*	0.36*
%Cryptophyceae	0.02	0.03	(TE -Pras)/(picocnb+Pras)	0.82*	0.39*
%Dtm+%Crypt	0.35	0.27	(Dtm+Crypt)/picocnb	0.82*	0.38*
%Chlorophyceae	0.19	0.02	Prymn/(Dtm+Crypt)	0.61*	0.39*
%Dinophyceae	0.11	0.08	Crypt/Dtm	0.03	0.04
%Prasinophyceae	0.02	0.00	Prymn/Dtm	0.61*	0.29*
%Prymnesiophyceae	0.71*	0.46*	cnb+Chlorophyceae	0.46*	0.19
inverse % prymnesiophyceae	0.79*	0.41*			

** denotes a significant correlation at the 0.01 level*

Table 2: Statistical correlation between TP and phytoplankton variables for the 50^{th} and 90^{th} percentile *(dtm: diatoms, Crpt: Cryptophyceae, TE: total eukaryotic cells, picocnb: picocyanobacteria, Pras: Prasinophyceae, Prim: Prymnesiophycae)*

As the Table 2 clearly demonstrates, the correlations between phytoplanktonic variables and statistically significant pressure levels are consistently higher with the 50th percentile than with the 90th. In fact, some variables only present statistically significant correlations with the 50th percentile.

Index development continued only with those phytoplankton variables presenting high and significant correlation with TP as an indicator of pressure level (Pachés, 2010). These variables are: Diatoms, (picocnb+Pras)/(TE-Pras), (TE+cnb)/picocnb, TE/picocnb, (TE-Pras)/picocnb, (Dtm+Cript)/picocnb, Prymnesiophyceae /(Dtm+Cript), inv % Prymnesiophyceae.

First, the data derived from phytoplankton recounts of these variables were normalized applying log (x+1). Some variables were inverted so that the behaviour versus the pressure was equal to the multimetric index; in other words, to increase the value of these variables as the anthropic pressure levels decreased.

Following the criteria laid out in the WFD (RC/WB; WB/RC), individual indices were then defined for each of the variables VR coastal water body typologies. The aforementioned indices represent the relationship between the value of these phytoplankton variables observed in the water body and the values in the reference conditions (Annex V, 1.4.1 of the WFD).

Individual EQR		Chl*a*	Diatoms	(picocnb+Pras)/(TE-Pras)	(TE+cnb)/picocnb	TE/picocnb	(TE-Pras)/picocnb	(Dtm+Crypt)/picocnb	Prymnesiophyceae /(Dtm+Crypt)	inv % Prymnesiophyceae
II-A	RC	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	WB001	0.87	0.98	0.99	0.92	0.96	0.94	0.76	0.87	0.72
	WB002	0.90	0.99	0.98	1.00	0.97	0.94	0.85	1.24	0.99
	WB003	1.12	0.98	1.04	1.00	0.99	1.12	0.99	1.32	0.90
	WB004	0.92	0.96	0.97	0.82	0.82	0.86	0.82	1.13	0.87
	WB005	0.78	0.94	0.94	0.81	0.81	0.83	0.67	0.77	0.61
	WB007	0.68	0.91	0.85	0.67	0.65	0.65	0.53	0.60	0.54
	WB008	0.63	0.89	0.72	0.26	0.41	0.42	0.37	0.73	0.61
	WB009	0.67	0.90	0.84	0.54	0.52	0.53	0.41	0.74	0.53
	WB010	0.86	0.97	0.78	0.41	0.44	0.49	0.41	0.81	0.61
III-W	RC	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	WB011	0.85	0.96	0.93	0.81	0.87	0.82	0.78	1.38	1.11
	WB012	0.90	0.98	0.99	0.93	0.93	0.99	0.95	0.95	0.92
	WB013	0.82	0.96	0.91	0.78	0.76	0.72	0.66	0.72	0.67
	WB014	0.77	0.98	1.03	0.79	0.75	1.11	1.19	1.11	0.66
	WB015	0.53	0.90	0.90	0.79	0.70	0.77	0.61	0.85	0.60
	WB016	0.47	0.85	0.68	0.30	0.31	0.33	0.26	0.55	0.51
	WB017	0.50	0.87	0.73	0.33	0.35	0.37	0.24	0.50	0.49
	WB018	0.54	0.89	0.87	0.81	0.74	0.67	0.48	0.64	0.76
	WB019	0.77	0.95	0.91	0.77	0.78	0.74	0.71	1.09	0.92

Table 3: EQR of various phytoplankton variables for the Reference Conditions and WB

Some of the variables do not allow a discrimination between coastal water bodies due to their similar values e.g. diatom variable, whose values oscillate between 0.85 and 0.98 across all WB.

Another phenomenon observed in the individual indices is some of the phytoplankton variables present greater variability in the stations selected to represent reference conditions than in the actual WB, thereby leading to the exclusion of these variables. This occurs with the variable "(Diatoms+Cryptophyceae)/picocyanobacteria".

The remaining variables ((TE+cnb)/picocnb; TE/picocnb; TE-Prasinophyceae)/picocnb; (TE- Prasinophyceae)/(picocnb+pras); Prymnesiophyceae /(Dtm+ Cryptophyceae); % Prymnesiophyceae) show themselves to be adequate for inclusion in the multimetric index. The variable Prymnesiophyceae/(Dtm+Cryptophyceae) represents the relation between a typical oligotrophic group (Prymnesiophyceae) and the groups most adapted to eutrophic environments, such as diatoms and Cryptophyceae. Finally, the three variables expressing the relation between nano and microplankton forms and the picoplankton forms of the community were adequate for inclusion in the index. To decide which of the three was optimal, the correlation of the index and TP was established (Table 4).

TP (µM) P_{50}		TYPE	
		II-A	III-W
Index	Cl *a*+(TE+cnb/picocnb)+Prymnesiophyceae/(Dtm+ Cryptophyceae)+inv%prim	0.88*	0.85*
	Cl *a*+(TE/picocnb)+ Prymnesiophyceae /(Dtm +Cryptophyceae)+ inv%prim	0.87*	0.82*
	Cl *a*+(TE-Prasinophyceae/ picocnb)+ Prymnesiophyceae /(Dtm+Cryptophyceae)+ iv%prim	0.85*	0.79*
	Cl *a*+(TE-Prasinophyceae/picocnb +Prasinophyceae)+Prymnesiophyceae/(Dtm+Cryptophyceae)+inv%prim	0.85*	0.79*

**denotes a significant correlation at α 0.01 level*

Table 4: Coefficient of determination of the different index with pressure 50th percentile of TP

A multimetric index was thus formulated:

PHYMED =[Index (Chla) + Index ((TE+cnb)/picocnb) + Index (Prymnesiophyceae /(Dtm+Cryptophyceae))+ Index(inv% Prymnesiophyceae)] /4

The variable (TE+cnb)/picocnb was selected for inclusion in the multimetric index because it reflects the greatest correlation with the anthropic pressure levels. Applying this multimetric index to the VR coastal WB yields the PHYMED index for each.
As the MEDGIG has not yet developed the aforesaid IC for the variables related to phytoplankton community composition, the boundaries between ecological classes was achieved by dividing the index into five equidistant intervals. Thus, applying the multimetric PHYMED index and the boundaries between classes the ecological status for coastal WB is shown in Table 5. As shown in the table, the VR presents eight WB with “High” ecological status, which corresponds to the north of the province of Castellón and Alicante (Fig.1). These bodies are coastal areas receiving less anthropic pressure. Seven WB yield “Good” ecological status while only three WB “Moderate” status.

3.4 Classification of Ecological Status using Chlorophyll *a*

As mentioned earlier, the single parameter corresponding to phytoplankton intercalibrated by the MEDGIG is biomass (chlorophyll *a*). In the Mediterranean, it was decided to use chlorophyll *a* as biomass estimator and, following the IC, the 90th percentile for 5-6 years was determined to be the most adequate parameter.

The ecological status of the coastal WB of the VR was calculated using the criteria established in the IC, compiled in the Official Journal of the European Union (Commission Decision, October 2008) along with the boundary between status classes. Shown below are each of the coastal WB and their corresponding ecological status with both index for the three-year series (August 2005- July 2008). The values of the TP 50th percentile are also included as a measurement of pressure.

	WB	TP 50th percentile	90th percentile Chl *a*	PHYMED index
TYPE II-A	001	0.21	High	High
	002	0.18	High	High
	003	0.16	High	High
	004	0.19	High	High
	005	0.32	Good	Good
	007	0.29	Good	Good
	008	0.38	Moderate	Moderate
	009	0.33	Moderate	Good
	010	0.30	Moderate	Good
TYPE III-W	011	0.13	Good	High
	012	0.13	Good	High
	013	0.18	Good	Good
	014	0.17	High	High
	015	0.19	Moderate	Good
	016	0.33	Moderate	Moderate
	017	0.26	Moderate	Moderate
	018	0.17	Good	Good
	019	0.14	Good	High

Table 5: Ecological status of coastal WB applying the 90th percentile of Chl a, the PHYMED index and 50th percentile of TP for three years

The following step involved comparing the classification system using exclusively chlorophyll *a* with that derived from the multimetric index PHYMED. As seen in Table 5, several WB have the same ecological status applying the two indexes. However, in other WB a different ecological status was generated. WB009 and 010 (Cullera Cape-San Antonio Cape) yield an ecological status of "Moderate" under the chlorophyll *a*-based Index, but they rise to "Good" status with the PHYMED index. These WB, in particular, receive fresh water from multiple channels and irrigation channels; rainfall or irrigation runoff, might therefore account for the sporadic increases in the chlorophyll *a* content, and the following rise in 90th percentile values of chlorophyll *a* over the three-year series. Nevertheless, these occasional charges do not significantly modify the composition of the phytoplankton community, as accurately reflected in the water quality status assigned by the PHYMED index.

Similarly, WB011 and WB012 (San Antonio Cape-Peñón de Ifach) which present a "High" ecological status under the PHYMED index given these WB were selected as references, yield a lower status of "Good" when only chlorophyll *a* is considered. Establishing ecological classifications with indices based exclusively on one parameter may create situations where WB with higher 50th percentile values of TP (WB 014), which also presents greater population density, receive a higher ecological status using chlorophyll *a* than those selected as references. Therefore, it becomes clear that the PHYMED index is more sensitive to changes in coastal trophic levels and responds better to the TP gradient.

A similar instance was observed with WB015, which possess 50th percentile, TP values similar to those of WB013, classified by both indices as "Good". However, with the chlorophyll *a*-based index, its ecological status is only "Moderate", while the PHYMED index yields the higher classification of "Good".

As a final example, WB019 (Cervera Cape –Valencia Regional Border) presented 50th percentile TP values similar to WB011 and WB012. Employing only chlorophyll *a*, the two bodies yield a "Good" status. However, WB019 does not present significant pressures (neither pointed nor diffused) and, moreover, its population density is low. According to the PHYMED index, its ecological status is "High".

Thus, the utilization of an index that takes into account both biomass and phytoplankton composition reveals itself to be more sensitive to pressure levels that littoral areas are subject to. Moreover, this index minimizes the occasional spiking linked to discharges of continental fresh water which can enhance chlorophyll *a* 90th percentile values, thereby falsely yielding a lower classification for the ecological status of a water body, despite the phytoplankton community composition remains unaffected.

Furthermore, it can be confirmed how using only a single parameter such as chlorophyll *a* to establish ecological status can create instances where bodies with similar pressures (TP) are classified with different ecological status, though the phytoplankton community is not significantly affected.

3.5 PHYMED index with 90th percentile

Finally, the value of the PHYMED index was calculated with the 90th percentile of chlorophyll *a*, as opposed to the 50th, as this is the statistical parameter which was accepted in the IC for the Mediterranean.

WB	Phymed Chl *a* 90th percentile	Phymed Chl *a* 50th percentile
001	0.84	0.84
002	1.03	1.03
003	1.09	1.01
004	0.92	0.93
005	0.74	0.74
007	0.64	0.62
008	0.56	0.55
009	0.60	0.62
010	0.64	0.67
011	1.11	1.04
012	0.98	0.92
013	0.77	0.75
014	0.96	0.83
015	0.76	0.69
016	0.51	0.46
017	0.51	0.45
018	0.78	0.69
019	0.93	0.89

Table 6: Ecological classifications using the 50th and 90th percentiles of chlorophyll *a* in the PHYMED index

As seen in Table 6, the ecological status classifications did not vary since the remaining parameters related to the composition and which comprise the index minimize these changes.

4. Conclusion

The main goal of the coastal monitoring network is the establishment of a coherent ecological status for coastal water bodies, thereby enabling water management measures to be taken if the conditions deteriorate due to the human activities.

A close study of the data clearly demonstrates that in the Mediterranean the most adequate statistical parameter to establish the level indicator of eutrophic conditions is the 50th percentile. In ecosystems with limited light and clearly demarcated seasonal periods, the phytoplankton growth is limited to the summer months and the 90th percentile values accurately reflect these growth periods. However, in the Mediterranean Sea the light conditions are profoundly different and phytoplankton growth occurring throughout the year and it depends fundamentally on nutrient discharges issuing from the coast. In these cases, the 90th percentile values might point to sporadic nutrient discharges from the coast which induces sporadic phytoplankton abundance (cell density, biomass). However, the 50th percentile values represent more appropriately the general pattern of phytoplankton behaviour.

Based on the pressures and impacts of the VR, the territorial analysis and the physicochemical parameters, WB002 (Sierra de Irta) and WB011 (San Antonio Cape-Moraira) and WB012 (Moraira–Peñón de Ifach) were selected as reference WB for Types II-A and III-W, respectively. Additionally, the ecosystems of these three WB enjoy varying degrees of legal protection instituted by the regional government.

Upon studying all of the phytoplankton variables showing a correlation with TP, an ecological classification system was developed for coastal water bodies based on the composition and biomass of the phytoplankton community. This system furthermore facilitates corrections to current and ongoing deviations derived from utilizing chlorophyll *a* as an ecological status indicator, attributed to the sporadic continental influxes and not to the characteristics intrinsic to the water body.

The PHYMED index should be applied to the values taken from five years of monitoring network (as stipulated by the MEDGIG for chlorophyll *a*) to determine the previous ecological status.

Moreover, the index should incorporate the other BQR parameters which are also required by the WFD such as abundance and bloom frequency to enable a global consideration of the phytoplankton.

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