# ANNEX 1: NATIONAL METHODS INCLUDED IN THE INTERCALIBRATION

## SLOVENIA: National Methods for Phytoplankton

**Metric: annual geometric mean of Chl-*a* (µg/l) concentrations**
Type of data:
- water-column integrated data: depth of water column 16-21 m, 4-5 sampling depths
- frequency: monthly sampling
- period: all year round

**Reference conditions**
Reference conditions are the expression of high quality structure and function of aquatic ecosystems, that should have not suffered any impact on their natural state because of human activities and there is none or only very minor evidence of disturbance on each of the general physico-chemical, hydromorphological and biological quality elements. The average phytoplankton biomass in the high quality status is consistent with the type-specific physico-chemical conditions and is not such as to significantly alter the type-specific transparency conditions.
For the reference conditions an existing site was chosen on the basis of previous analysis on the long-term dynamic of Chl-*a* concentrations in Slovenian coastal waters (Mozetič et al., 2005). The statistical analysis on five stations of the Gulf of Trieste showed that the station designed as the reference site has statistically significantly the lowest mean biomass and that variations are due to seasonal more than to interannual fluctuations. Besides, concentrations of inorganic nutrients and of specific pollutants are such to reflect none or only minor evidence of anthropogenic disturbance, whereas transparency conditions are the best when considering mean values and $10^{th}$ percentile of Secchi disc's depth.
All these facts guided us towards the selection of one reference site with above-mentioned characteristics.
The annual geometric mean was chosen as metric in order to smooth large seasonal variations characteristic for the coastal waters belonging to Type II (all Slovenian water bodies belong to type II). Furthermore, statistical analysis for the definition of reference conditions was performed on a large data set of 19 yeas, bearing in mind the importance of long-term analysis in studying marine pelagic ecosystems (e.g. Harding & Perry, 1997; Wiltshire & Durselen, 2004)

Type of data:
- one reference site for Type II; frequency: monthly sampling, depths: 4 to 5, period: all year round, 1984-2002 (N=1100)
- water-column integrated data (N=254)
- annual means (use of geometric means due to log-transformed normal distribution of Chl-*a* concentrations) (N=19)

Statistics:
- median: indicating Reference condition's concentration
- $90^{th}$ percentile: Chl-*a* concentration at the High/Good boundary of ecological quality classes

Boundary setting

An attempt was made to apply the Boundary Setting Protocol to identify discontinues in the relationship between selected nutrient(s), i.e. pressure and biological response, i.e. increased Chl-*a* concentrations. Phosphate is considered as the limiting factor of phytoplankton production in some parts of the Mediterranean (Krom et al., 1991), including northern Adriatic (Vollenweider et al., 1992) and its generally very low concentrations indicate rapid utilization by phytoplankton (and also bacteria) and short turnover time. Since there is always a time-lag between increased concentrations of nutrients measured at certain time in the water and utilization of these nutrients by phytoplankton and building-up of the biomass, the relationship between inorganic phosphorus and Chl-*a* does not seem a plausible choice. We therefore performed a regression between log-transformed concentrations of total phosphorus and Chl-*a* on the data set of all stations that have ever been included in the past in some national monitoring programs, extending over a wide range of trophic conditions.

As already observed during the intercalibration exercise, there was no relationship between total phosphorus and Chl-*a*, meaning that no discontinuities on the regression line/curve were identified that would allowed to define a boundary between the two classes of ecological status (most important between Good and Moderate).

The following approach in the boundary setting protocol was therefore the expert judgement. We identified a site of good ecological status that conforms with the definitions of the WFD:

- The site is in the vicinity (0.5 NM off the coast) of local river inflow, to which also mechanically treated wastewaters are discharged meaning that this *"human activity could provoke low levels of distortion of the values of the biological quality elements…"*;
- Concentrations of nutrients, especially of phosphate and silicate, are significantly higher that those from the reference site, but they *"do not exceed the levels established so as to ensure the functioning of the ecosystem…"*;
- *"Temperature, oxygenation and transparency do not reach levels outside the ranges established so as to ensure the functioning of the ecosystem…"*;
- Concentrations of pollutants are slightly higher or equal to those from the reference site but not in excess of national normative;
- Average Chl-*a* concentration at this site is statistically higher than the average value of the reference site but *"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 quality of the water"*;
- Comparison of phytoplankton abundance and taxonomic composition between good site and reference site in the period 2005-2006 showed total abundance higher at the good site and Shanon-Wiener diversity index slightly higher at reference site thus indicating that *"the composition and abundance of planktonic taxa show slight signs of disturbance"*.

Our expert judgement for the selection of the good site was based on existing data and on several analyses of these data. Once the site was selected we performed the same statistic and used the same metric as for the definition of reference value and H/G boundary value.

Type of data:

- one good site for Type II; frequency: monthly sampling, depths: 4, period: all year round, 1989-2002 (N=656)
- water-column integrated data (N=164)
- annual means (use of geometric means due to log-transformed normal distribution of Chl-*a* concentrations) (N=14)

Statistics:

- $90^{th}$ percentile: Chl-*a* concentration at the Good/Moderate boundary of ecological quality classes

Table 1: Chl-*a* concentrations and EQRs derived from existing data of reference site and good site.

| | Chl-*a* (µg/l) | EQR |
|---|---|---|
| Ref. Cond. | 1.02 | 1 |
| H/G | 1.27 | 0.80 |
| G/M | 1.87 | 0.54 |

Values for the other class boundaries, either concentrations or EQRs, were defined by calculating EQRs for the remaining boundaries applying the equal distance between G/M, M/P and P/B EQRs. The corresponding Chl-*a* concentrations were calculated from the equation of the regression curve (power function)

$y = 1.0205x^{-0.9987}$

between known EQRs (x) and Chl-*a* values (y) at boundaries (see Table 1). Chl-*a* concentrations and EQRs for all class boundaries are presented in Table 2.

Table 2: Classification system: Chl-*a* concentrations and EQRs at class boundaries using water-column integrated data.

| | Chl-*a* (µg/l) | EQR |
|---|---|---|
| Ref. Cond. | 1.02 | 1 |
| H/G | 1.27 | 0.80 |
| G/M | 1.87 | 0.54 |
| M/P | 2.81 | 0.36 |
| P/B | 5.62 | 0.18 |

The highly significant relationship (R=1) between these values is described with power function (Figure 1) and the equation of the power function will be used for the classification of the water bodies in national monitoring programs in the future.

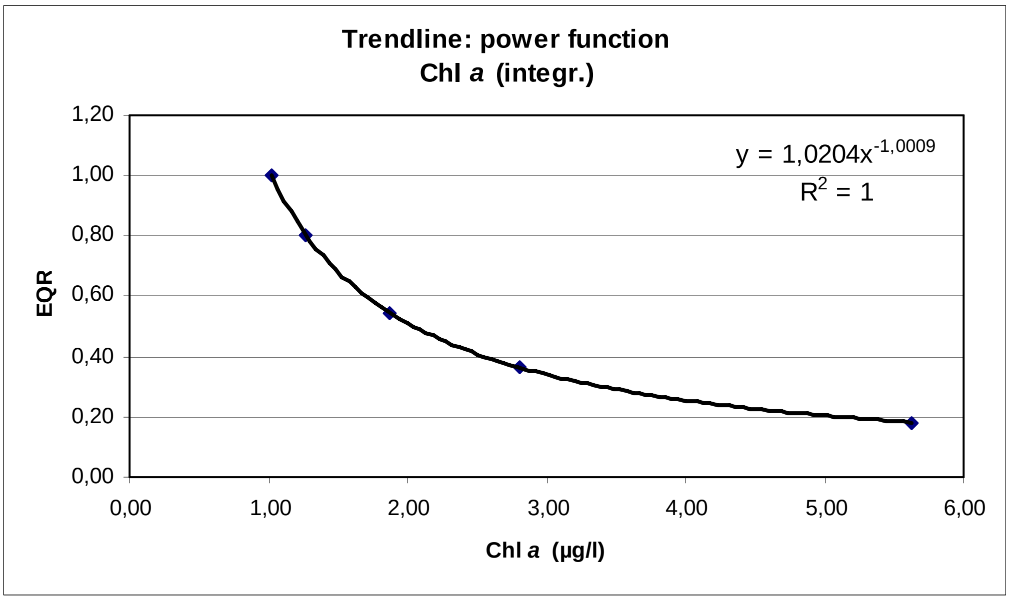

Figure 1: Relationship between Chl-*a* concentrations and EQRs at class boundaries.

**Relationship water-column integrated data *vs.* surface data**

Since other member states, which participated at the intercalibration exercise have considered only surface data we had to compare our classification system with the one based on surface data only. The relationship between geometric means of water-column integrated data and geometric means of surface data is best described (R=0.93) with logarithmic regression curve (Figure 2).

This justifies us to work with water-column integrated Chl-*a* concentrations and to be well comparable to other metrics metric based on surface data.

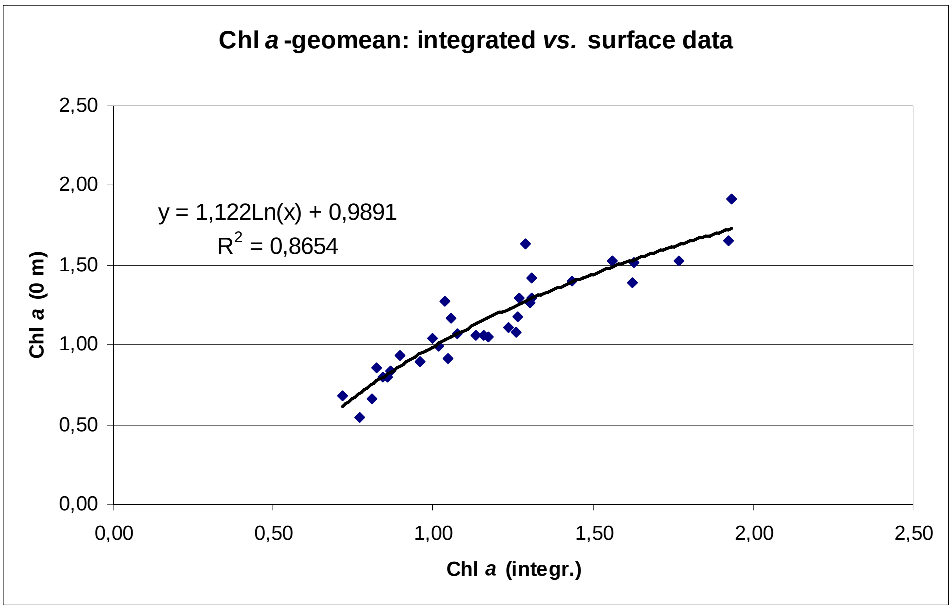


Figure 2: Relationship between annual geometric means of water-column integrated Chl-*a* and surface Chl-*a* concentrations.

Thereupon we performed exactly the same procedure as described above (statistic and setting of the boundaries) and results are presented in Table 3.

Table 3: Classification system: Chl-*a* concentrations and EQRs at class boundaries using surface data.

| | Chl-*a* (µg/l) | EQR |
|---|---|---|
| Ref. Cond. | 0.99 | 1 |
| H/G | 1.28 | 0.78 |
| G/M | 1.62 | 0.61 |
| M/P | 2.42 | 0.41 |
| P/B | 4.96 | 0.20 |

**Intercalibration between two metrics: 90th percentile and annual geometric mean**

Since different metrics were used in different national methodologies we had to compare Chl-*a* concentrations and EQRs at boundaries of these methodologies.
Other member states have chosen another metric for the Chl-*a*: 90$^{th}$ percentile of surface data, calculated over the period of 5 to 6 years.
In order to compare the final results based on two different metrics – annual geometric mean and 90$^{th}$ percentile – we firstly considered only surface data (see Figure 2 and Table 3) and then we calculated, besides annual geometric means, annual 90$^{th}$ percentiles on a data set of Chl-*a* concentrations from reference and good site (N=33).
Relationship between the two metrics can be described by linear regression (Figure 3) with high coefficient of correlation (R=0.83).

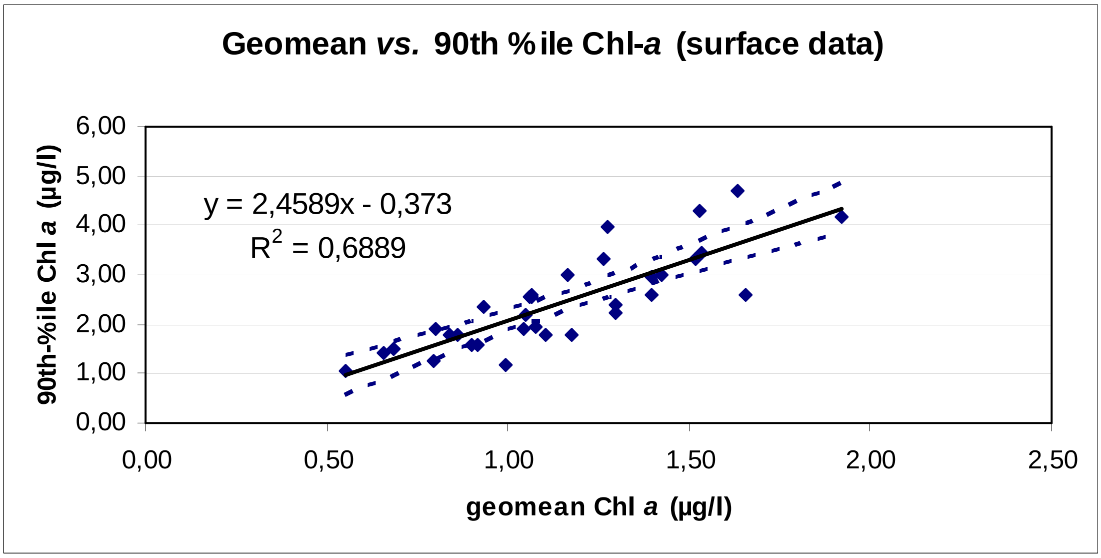


Figure 3: Relationship between annual geometric means and annual 90$^{th}$-%ile of Chl-*a* concentrations, surface data only. Linear regression line and 95% confidence limits are shown.

Using the above equation that describes the linear relationship between the two metrics, Chl-*a* values at boundaries based on annual geomeans are translated to values based on 90$^{th}$-%ile as follows (Table 4). These values are then compared to values agreed during the intercalibartion exercise for Type II water bodies where 90$^{th}$-%ile as metric was applied.

Table 4: Chl-*a* concentrations of the reference condition and H/G and G/M class boundaries based on two metrics (using the equation from Figure 3) and comparison with the outcome of the intercalibration exercise (metric: 90$^{th}$-%ile) for the Type II water bodies.

| | Geomean Chl-*a* | 90$^{th}$-%ile Chl-*a* | Intercalibration (Type II) |
|---|---|---|---|
| Ref. Cond. | 0.99 | 2.1 | 1.9 |
| H/G | 1.28 | 2.8 | 2.4 |
| G/M | 1.62 | 3.6 | 3.6 |

# DESCRIPTION OF NATIONAL METHODS INCLUDED IN THE INTERCALIBRATION

*(ANNEX TO TECHNICAL REPORT)*

Spain Member State Report for the Phytoplankton Element:

Coastal Waters MED M1/M2/M3/M4 Types

*June 2007*

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**CONTENTS**

## 1. INTRODUCTION

According to the Water Framework Directive (WFD), the classification of the water quality by means of phytoplankton should be based on phytoplankton biomass, composition and abundance and phytoplankton bloom frequency and intensity. For the Mediterranean waters, chlorophyll-a (Chl-a) has been the parameter chosen as indicator of phytoplankton biomass and it has been considered as the phytoplankton metric for the WFD intercalibration (IC) process. Additional parameters or metrics should be included in the future to complete the assessment of this BQE.

Several regional governments (*Comunidades Autónomas*) as well as the Central Government are involved in the application of the WFD in Spain. This annex is the result of a coordinated work among experts of the Spanish regional administrations MED M1/M2/M3/M4 types. The regional governments (Fig. 1) involved in the application of the WFD in the Spanish Mediterranean waters are: Catalonia, Balearic Islands, Valencia, Murcia and Andalusia.

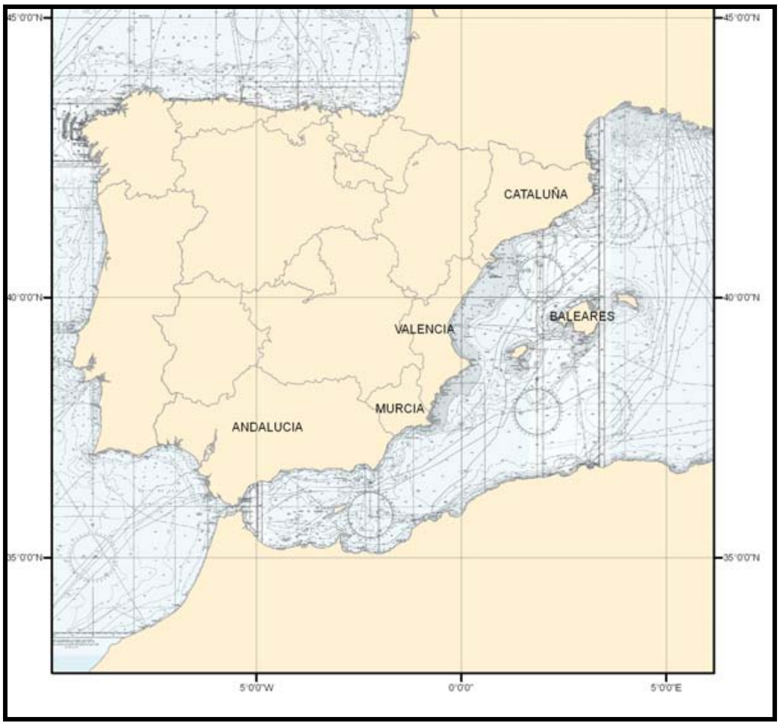



**Figure 1.** Regional Governments involved in WFD for Mediterranean Coastal Waters.

The following report is based on the methodologies and monitoring networks developed by the different regions and described in the different sections.

## 2. TYPOLOGY

In the MED-GIG meetings of phytoplankton working group, experts agree that the previous defined 4 Mediterranean Coastal IC types (M1, M2, M3 and M4) cannot be applied to the IC for phytoplankton quality element within the Mediterranean basin.

For this reason a new typology has been developed, mainly focused on hydrological parameters characterizing water bodies, dynamics and circulation (see Technical Report of Phytoplankton). As a result of the Med-GIG meetings, countries agreed to adopt surface density as a proxy indicator for static stability in order to distinguish three typologies: sites highly influenced, moderately influenced or not affected by freshwater inputs coming from the continent (Table 1).

In Spanish Mediterranean coastal waters, the three density thresholds were also calculated into salinity ones using the annual mean temperature which is 18 ºC (Table 1). After that, all the stations were classified by both indicators (surface mean density and surface mean salinity) into the three types and the resulting classification was the same. Furthermore, salinity involves direct relations with nutrients inputs which are strictly related with phytoplankton growth, and their changes should be closer related with the River Basin Influence (ecological definition of types).

**Table 1.** Type threshold definition based on the annual Spanish Mediterranean mean salinity or density (other Med-GIG countries).

| | Type I (Highly influenced by freshwater inputs) | Type II | | Type III (Not affected by freshwater inputs) |
|---|---|---|---|---|
| | | A (Not directly affected by freshwater inputs) | B (Influenced by Atlantic waters) | |
| Annual Mean Salinity | < 34.5 | 34.5 – 37.5 | | > 37.5 |
| Density | < 25 | 25 - 27 | | > 27 |

NOTE: this new typology is not in agreement with the one previously sent by Mediterranean MS to the Commission (i.e. corresponding to WFD articles 5 and 8).

In addition, as a proposal of the Andalusia government and agreed at national level, type II was divided into two sub-types (A and B). type II-A is comparable with the other Med-GIGs type II (not directly affected by freshwater inputs), whereas type II-B was defined by the same salinity range (34.5-37.5) but from different origin (influenced by Atlantic waters).

The Andalusian coastal waters comprises from the mouth of the Guadalmesí river (Cádiz) at the Strait of Gibraltar (Estrecho de Gibraltar) to the limit with the Murcia region, at Punta Parda (Fig. 2).

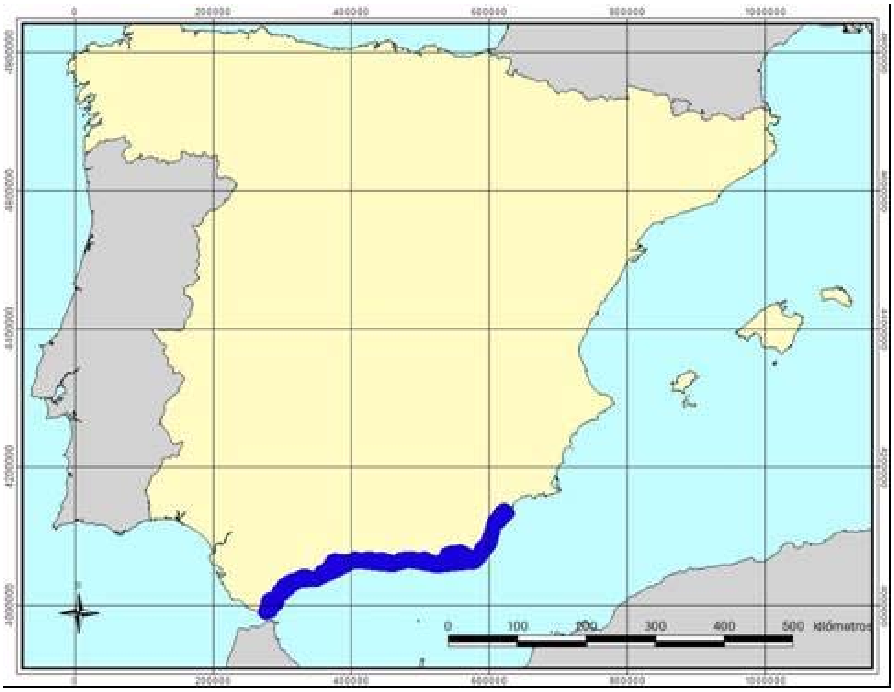



**Figure 2.** Andalusian coastal waters in the Mediterranean Sea Ecoregion.

It is interesting to remark that an important part of the Andalusian Mediterranean coastal waters is strongly influenced by the surface currents from the Alborán sea, that flow to the east, contributing with Atlantic water to the Mediterranean. The deep currents flow to the west, moving the warm, salty Mediterranean waters to the Atlantic. Even more it must be taken into account the wind surface effect, that at the sea surface level are East dominant (from East to West).

In this important zone of exchanging water it can be proved the creation of several gyres (some researcher suggest for the seasonality), one located in the western basin and two, less powerful, in the eastern basin, that determine the formation of surface currents that reach the coastal zone of the region (Fig. 3).

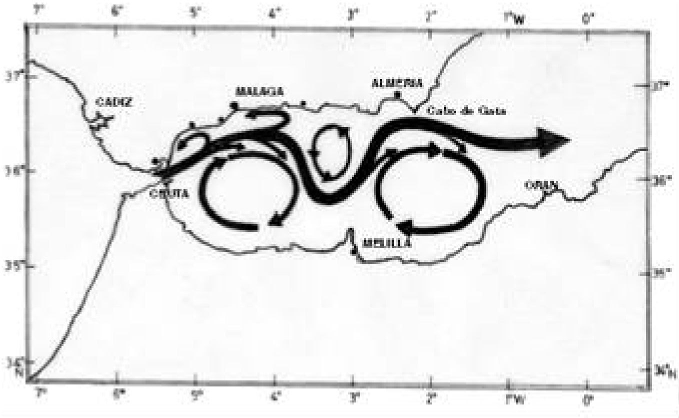



**Figure 3.** Circulation patterns in the Alborán Sea.

This distribution of mixing waters has an important effect in the biologic characteristics of the Andalusian coastal waters.

By taking a look at the chlorophyll-a values from the satellite imagery along a year (2004, Fig. 4), it can be observed the influence of the mixing waters Atlantic-Mediterranean, with seasonal changes, that sets the existence of an specific type (type II-B) for this zone.

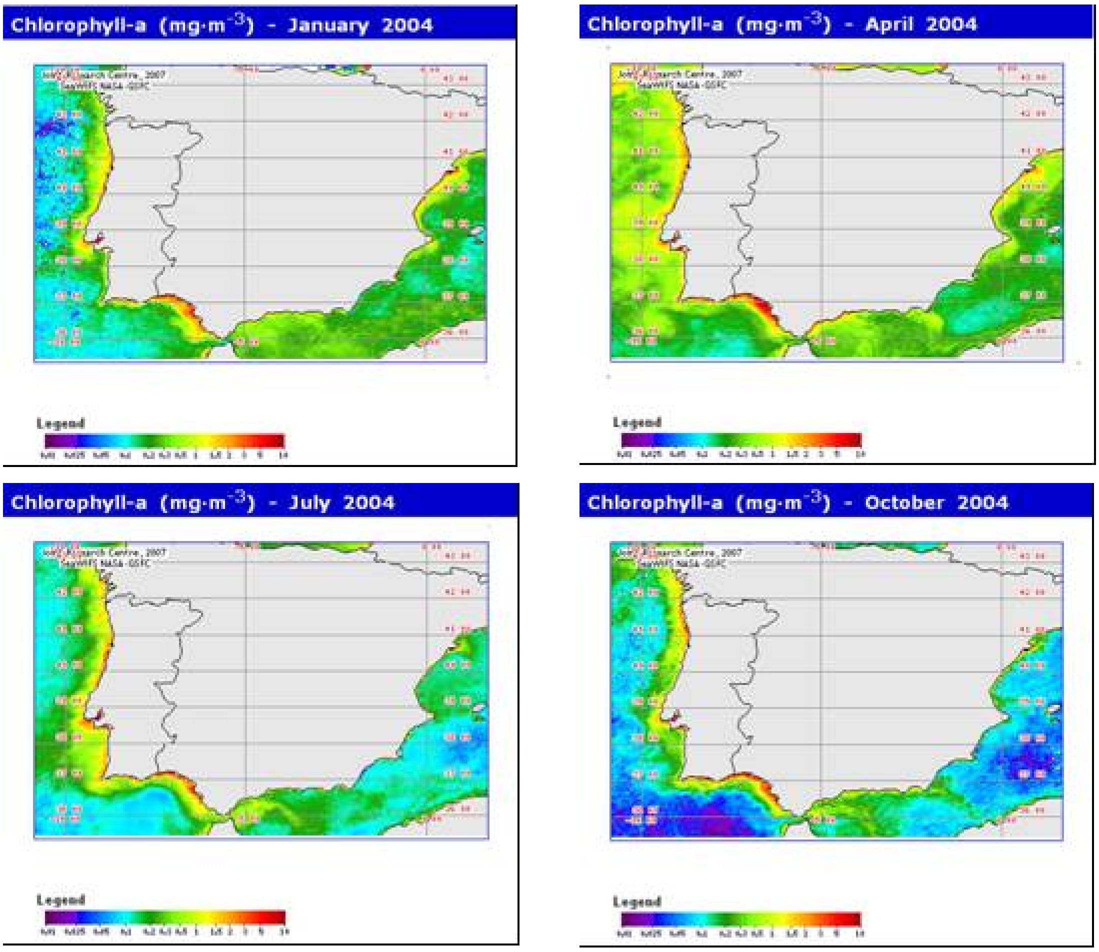



**Figure 4.** Chlorophyll-a values in Andalusian coastal waters.

The satellite imagery referred to the period 2000-2004, with the 90th percentile and mean chlorophyll values, are useful to delimit in a specific way the influence zones of the Atlantic coastal waters within the Andalusian Mediterranean coastal waters (Fig. 5).

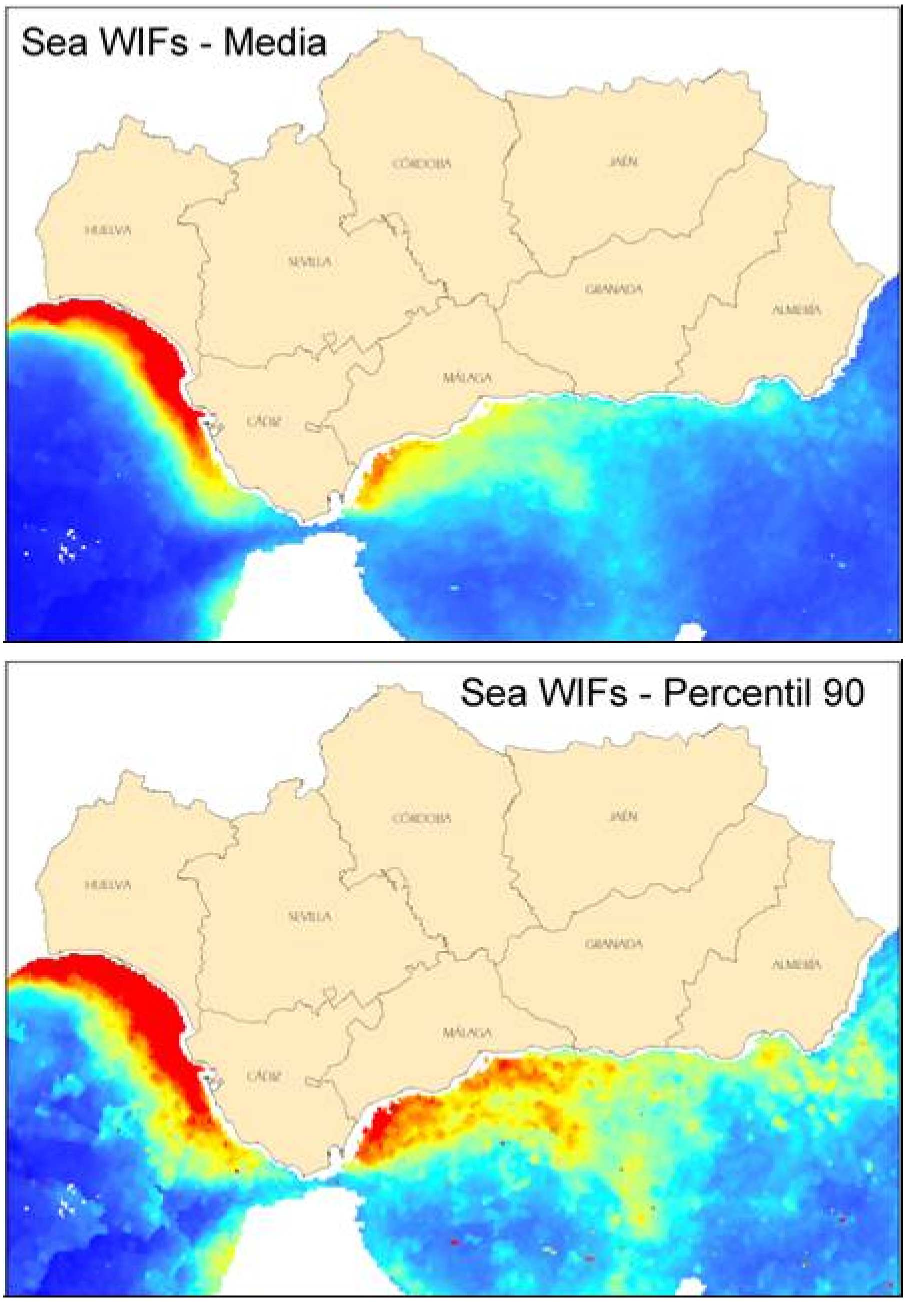



**Figure 5.** Chlorophyll values from satellite imagery (2000-2004).

The salinity values of this mixing zone are similar to the ones of previously defined type II. Due to that, this type was subdivided into two subtypes A and B, the first related with freshwater inputs the second related with mixed Atlantic waters (Table 1).

# 3. DESCRIPTION OF AREAS AND TYPES

## 3.1. Catalonia

All the Catalan Chl-a data for the intercalibration exercise to obtain quality boundaries and reference conditions (RCs) has been provided by two Monitoring Programmes “Nuisance and Toxic Phytoplankton Control Network” and “Littoral Water Quality Control Network” (*Agència Catalana de l’Aigua - Institut de Ciències del Mar*).

In these two programmes, working since 1994, 107 inshore (beaches and rocky areas) and 17 nearshore stations have been sampled along the Catalan coast. Inshore stations, located between 0 and 25 m from the coastal line and where the water column is around 1 m depth, have been sampled monthly along the whole year (Fig. 6). Nearshore stations, located at 1500 m from shore, have been sampled seasonally. All the stations have been sampled at surface and distributed into the 34 water bodies (WB) defined in Catalonia for the internal hydrologic basins. Within inshore waters the three WB types are present; 3 WB designated as type I, 17 as type II-A and 14 as type III. As it has described above there are only 17 nearshore stations, which are located into 16 WB. In these WB only type II-A (4 WB) and type III (13 WB) are present. The rest of WB (18 of 34) have not a nearshore station associated, thus they have been provisionally classified into types II-A or III, based on inshore data (Table 2).

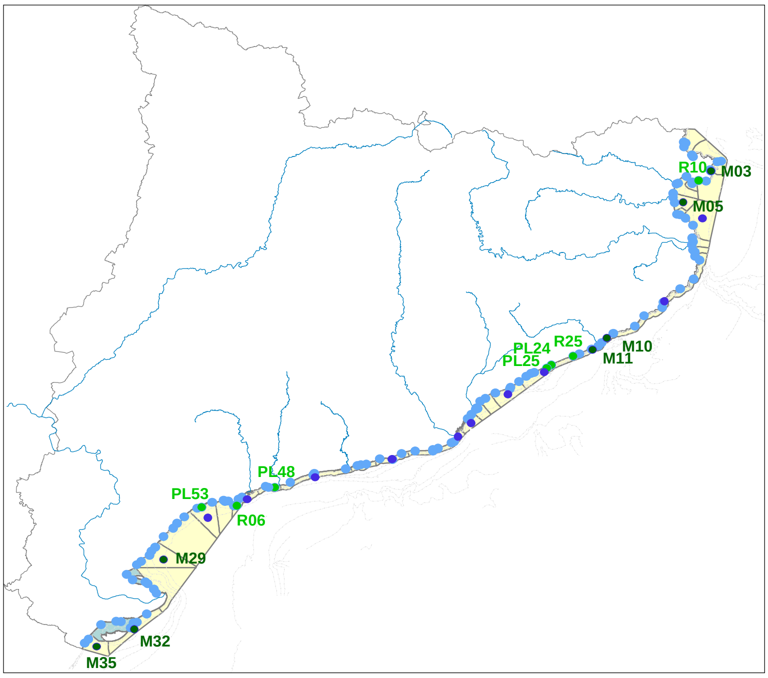



**Figure 6.** Sampling points of Catalan phytoplankton monitoring (inshore stations in light blue and nearshore stations in dark blue) and reference stations (inshore reference stations in light green and nearshore reference stations in dark green).

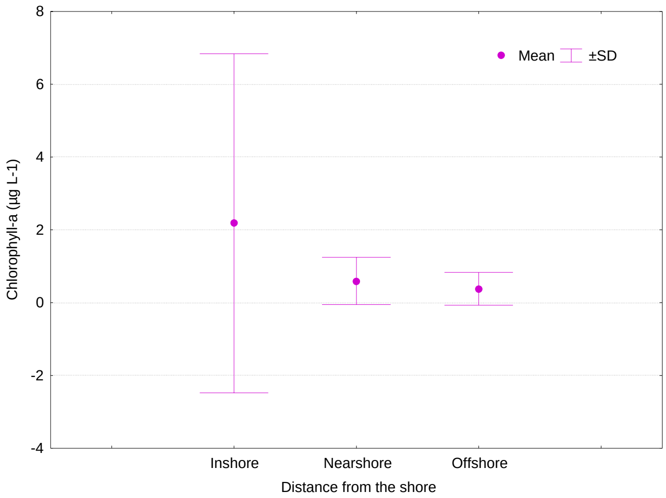
**Table 2.** Catalan WBs, inshore and nearshore mean salinity, and designated type (see text).

| WB Code | WB Name | Inshore Mean Salinity | Inshore Type | Nearshore Mean Salinity | Nearshore Type |
|---|---|---|---|---|---|
| C01 | Portbou-Llançà | 36.18 | Type II | - | Type III |
| C02 | Badia de Port de la Selva | 36.92 | Type II | - | Type III |
| C03 | Cap de Creus | 37.66 | Type III | - | Type III |
| C04 | Badia de Cadaqués | 37.45 | Type II | 37.69 | Type III |
| C05 | Cap Norfeu | 37.74 | Type III | - | Type III |
| C06 | Canyelles – Roses | 37.87 | Type III | - | Type III |
| C07 | Roses – Castelló | 35.62 | Type II | - | Type III |
| C08 | Sant Pere Pescador – Fluvià | 34.26 | Type I | 37.48 | Type II |
| C09 | L'Escala | 37.16 | Type II | - | Type III |
| C10 | Montgrí – Illes Medes | 37.56 | Type III | 37.59 | Type III |
| C11 | Torroella de Montgrí – Ter | 36.53 | Type II | - | Type III |
| C12 | Pals – Sa Riera | 36.64 | Type II | - | Type III |
| C14 | Begur – Blanes | 37.83 | Type III | 37.82 | Type III |
| C15 | Blanes – Pineda | 36.80 | Type II | 37.89 | Type III |
| C16 | Pineda – Mataró | 36.45 | Type II | 37.90 | Type III |
| C17 | Cabrera – Montgat | 36.81 | Type II | 37.92 | Type III |
| C18 | Montgat – Badalona | 37.82 | Type III | - | Type III |
| C19 | Sant Adrià de Besòs | 36.58 | Type II | - | Type III |
| C20 | Barcelona | 37.63 | Type III | 37.85 | Type III |
| C21 | Llobregat | 37.14 | Type II | 37.65 | Type III |
| C22 | El Prat de Llobregat – Castelldefels | 37.60 | Type III | - | Type III |
| C23 | Sitges | 37.94 | Type III | 37.98 | Type III |
| C24 | Vilanova i la Geltrú | 37.39 | Type II | - | Type III |
| C25 | Cubelles – Altafulla | 37.78 | Type III | 37.97 | Type III |
| C26 | Tarragona Nord | 37.91 | Type III | - | Type III |
| C27 | Tarragona – Vila-seca | 36.83 | Type II | 37.88 | Type III |
| C28 | Cap de Salou | 37.96 | Type III | - | Type III |
| C29 | Salou – Cambrils | 37.85 | Type III | - | Type III |
| C30 | Cambrils – Mont-roig del Camp | 37.12 | Type II | 37.95 | Type III |
| C31 | Vandellós i l'Hospitalet de l'Infant | 37.72 | Type III | - | Type III |
| C32 | L'Ametlla de Mar | 36.87 | Type II | 37.32 | Type II |
| C33 | Delta Nord | 29.68 | Type I | - | Type II |
| C34 | Delta Sud | 33.35 | Type I | 36.68 | Type II |
| C35 | Alcanar | 35.81 | Type II | 36.20 | Type II |

Thirteen years (1994-2006) of available data set -salinity, Chl-a and dissolved inorganic nutrients- has been used to establish quality boundaries and reference conditions.

In Catalan coastal waters, the highest concentrations of phytoplankton are observed at the inshore stations. Whereas at these stations the mean Chl-a is 2.18 µg/l, at the nearshore stations the mean Chl-a is more than 50% lower (0.6 µg/l; Fig.7a). For this reason, the monitoring programmes are mainly established at coastal line (inshore stations) where most of the problems concerning phytoplankton are found (Fig. 7b). That is why most of the work done for setting quality boundaries and RCs in Catalan waters is based on the information obtained from inshore waters.

However, as most of the working groups, which are participating in the intercalibration exercises (Med-GIG), are working at nearshore waters, quality boundaries and RCs have been also obtained for these waters.




a

b

**Figure 7.** **a)** Mean Chl-a (± SD) values for the inshore (N = 6267), nearshore (N=916) and offshore waters (sampled at 5000 m from the coast, N=696); **b)** Phytoplankton bloom along Catalan coastal line (Photo: ACA).

### 3.2. Valencia

Basins of rivers Júcar and Segura are spread out along 477 km of coastal waters of Valencia. In order to implement WFD at the Valencian Coast, littoral of Valencia has been divided into 16 water bodies. To identify water body types (due to influence of fresh water inputs) annual mean salinity values of inshore samplings are used (Table 3).

**Table 3.** Annual mean salinities.

| water body | salinity (mean values) |
|---|---|
| 001 | 36,84 |
| 002 | 36,58 |
| 003 | 36,34 |
| 004 | 36,58 |
| 005 | 35,96 |
| 006 | 37,77 |
| 007 | 28,80 |
| 008 | 36,59 |
| 009 | 36,90 |
| 010 | 37,88 |
| 011 | 37,65 |
| 012 | 37,43 |
| 013 | 37,65 |
| 014 | 37,55 |
| 015 | 37,62 |
| 016 | 37,47 |

Even though salinity values of water bodies 6 and 7 are not between 34.5 and 37.5, we have included them in type IIA because:

- Water bodies of Valencian Community were recently reorganized. Due to these last changes, water body number 7 has passed to have one station instead of previous 5. The remained station and its related salinity values do not represent the global and real condition of the water body due to the direct continental influence of the Carraixet creek. Do not consider significantly this mean salinity to define the water body type but it was the only data available up to now. By expert criteria this WB should be included into the WB type II-A.

- Water body 6 has a very reduced size, and it is clearly influenced by Sagunto Port and therefore this area is considered heavily modified. We do not believe adequate to differentiate this water body from rest of water bodies of Valencia Gulf (north of Valencian Community), by expert criteria.

Although water bodies 12 and 16 have salinity values <37.5, we have included them into type III. Both water bodies have salinity values closer the threshold. They are located in the coastal areas of minimal continental influence and we need a larger data set (more than one year) to establish properly the type for this water body. These WB have been classified as type III by expert criteria and due to the geographic and climatologic ambient in which they are settled.

There are nine water bodies designated as water bodies type II-A (Not directly affected by freshwater inputs) and 7 water bodies classified as type III (Not affected by freshwater inputs).

**Figure 8.** Valencian coast, water body types (type II-A (light blue) and type III (dark blue).

On the Fig. 8, it is easily observed that the area affected by freshwater inputs is located on the north coast, bordering Catalan coastal waters and on the south Cape of San Antonio. Part of the coast with water bodies designated as a type III on the south is bordering Murcia coastal waters.

The Valencian phytoplankton monitoring is based on inshore network which started to be operative in August 2005 with the first pilot cruise which included 180 stations. After the pilot sampling, spatial coverage was reduced to approximately 90 inshore stations (Fig. 9). In every station, one sample is taken approximately 10-15 meters from the shore.

Field samplings are carried out 12 times per year (monthly) from August 2005 with slightly changes on the number and the position of the stations to improve the monitoring network.

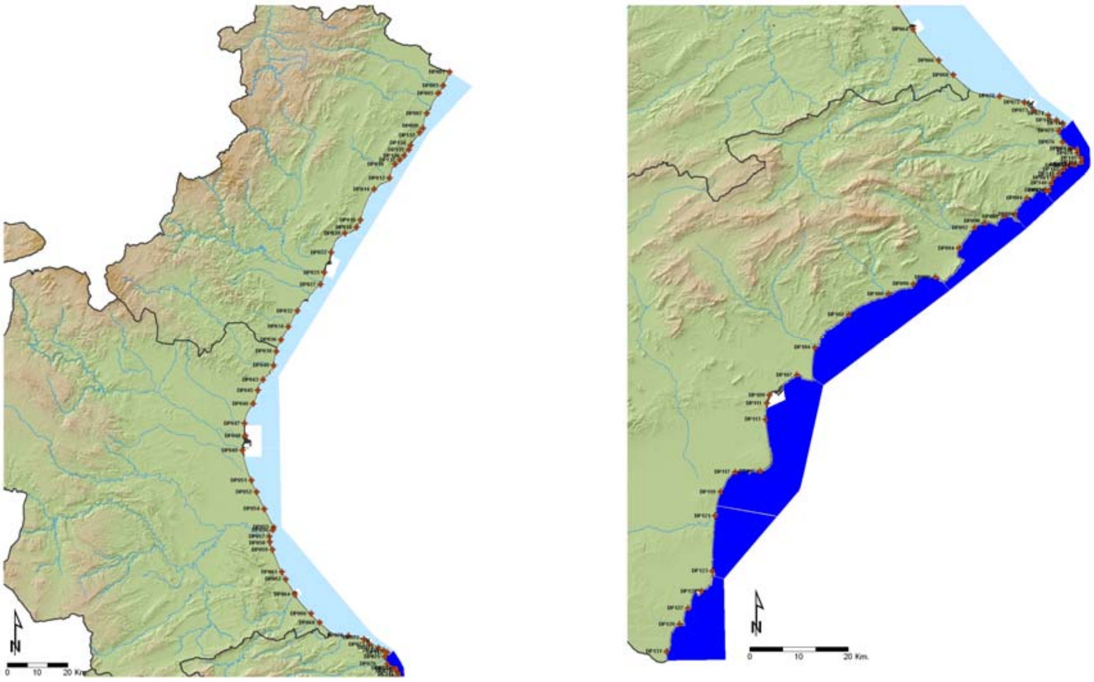


**Figure 9.** Monitoring network for the water bodies: type II-A (light blue) and type III (deep blue).

Mean values of chlorophyll-a for the type II-A stations vary from 0,60 to 5,45 µg/l and for type III from 0,41 to 3,20 µg/l. 90$^{th}$ percentile values of chlorophyll-a for the water bodies of type II-A vary from 1,19 to 5,02 µg/l and for type III from 0,77 to 2,06 µg/l.

### 3.3. Andalusia

In the Mediterranean coast, as well as in the rest of the Andalusian coastal waters, there are several monitoring networks, focused on the water quality knowledge. From these, two surveys have been selected that are considered more suitable, taking into account the parameters analysed, frequency of samplings, and quality of data and boundaries. These are the following:

- Results from the chlorophyll-a observations corresponding to the samples from the eutrophication network of the Andalusian coast (2000-2007).
- Results of the quality phytoplankton analysis from the monitoring of the shellfish production zones, performed by the *Consejería de Pesca y Agricultura* (1999-2005).

The sampling methodology has been applied in the same way for the Atlantic and Mediterranean coastal waters. Stations were established 1500 m from the shoreline and chlorophyll-a was measured at the surface layer and salinity values were obtained as an average of three measurements (surface, light extinction depth and bottom).

These works have made possible to define for the Andalusian Mediterranean coastal waters (with around 450km of coast) the existence of 22 coastal water masses, from which mostly (19 masses) are identified with the type II- B, meanwhile only three ones are included in the type III.

The type distribution is related with the definition of type II-B (see Section 2) and the Atlantic influence along the coast.

The eastern zone of the Andalusia coast, comprised between the Cape Gata and Punta Parda (regional boundary with Murcia), can be included in the type III. The type II-B is limited by the mouth of the Guadalmesí river (Cádiz province), at the strait of Gibraltar, and by “Punta de la Testa”, at Cape Gata (Almería province) (Fig. 10).

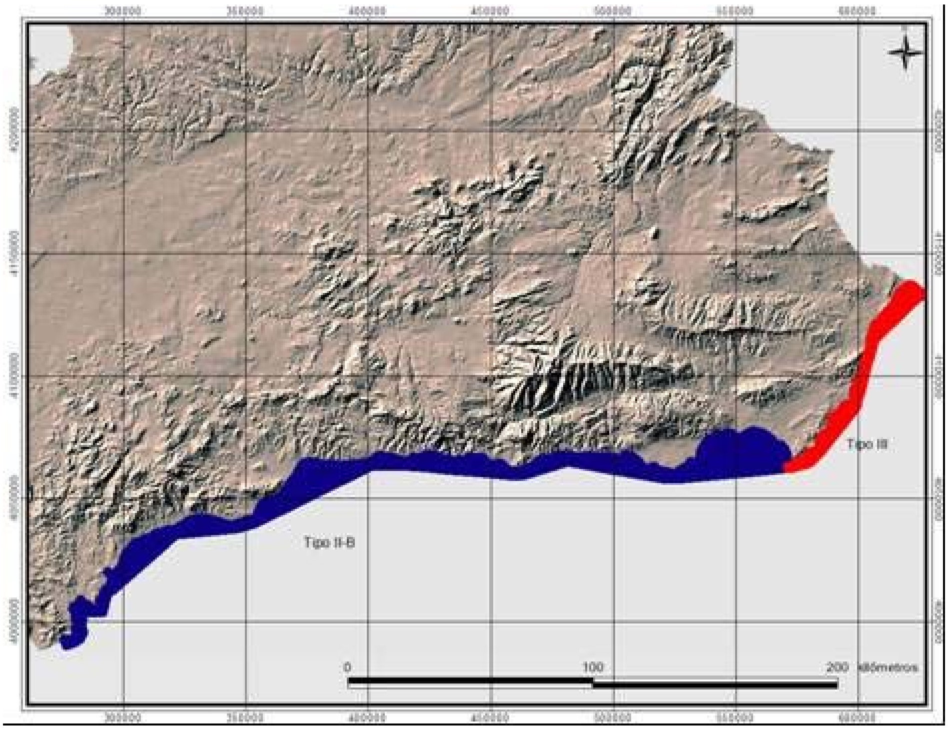



**Figure 10.** Distribution of coastal water types in Andalusia.

Andalusian coastal waters classified as type III are not influenced by freshwater inputs and are only affected by occasional riverine discharges where mean salinity values are about 37.5 .

Monitoring sites (Table 4) are showed in Fig. 11 where no impacts have been detected except in some local urban or industrialised areas resulting in chlorophyll-a mean values around de 0.5 µg/l (0.8 µg/l for $90^{th}$ percentile values).

The reference stations were established at the beginning of the intercalibration exercise: 14A and 15A, both in the "rada de Almería". As a consequence of the application of the new type proposal a revision of the reference sites was undertaken. The following reference stations were established:

a. Type III: stations 11A, 12A and 13A.
b. Type II-B: stations 18G, 20M and 16C.

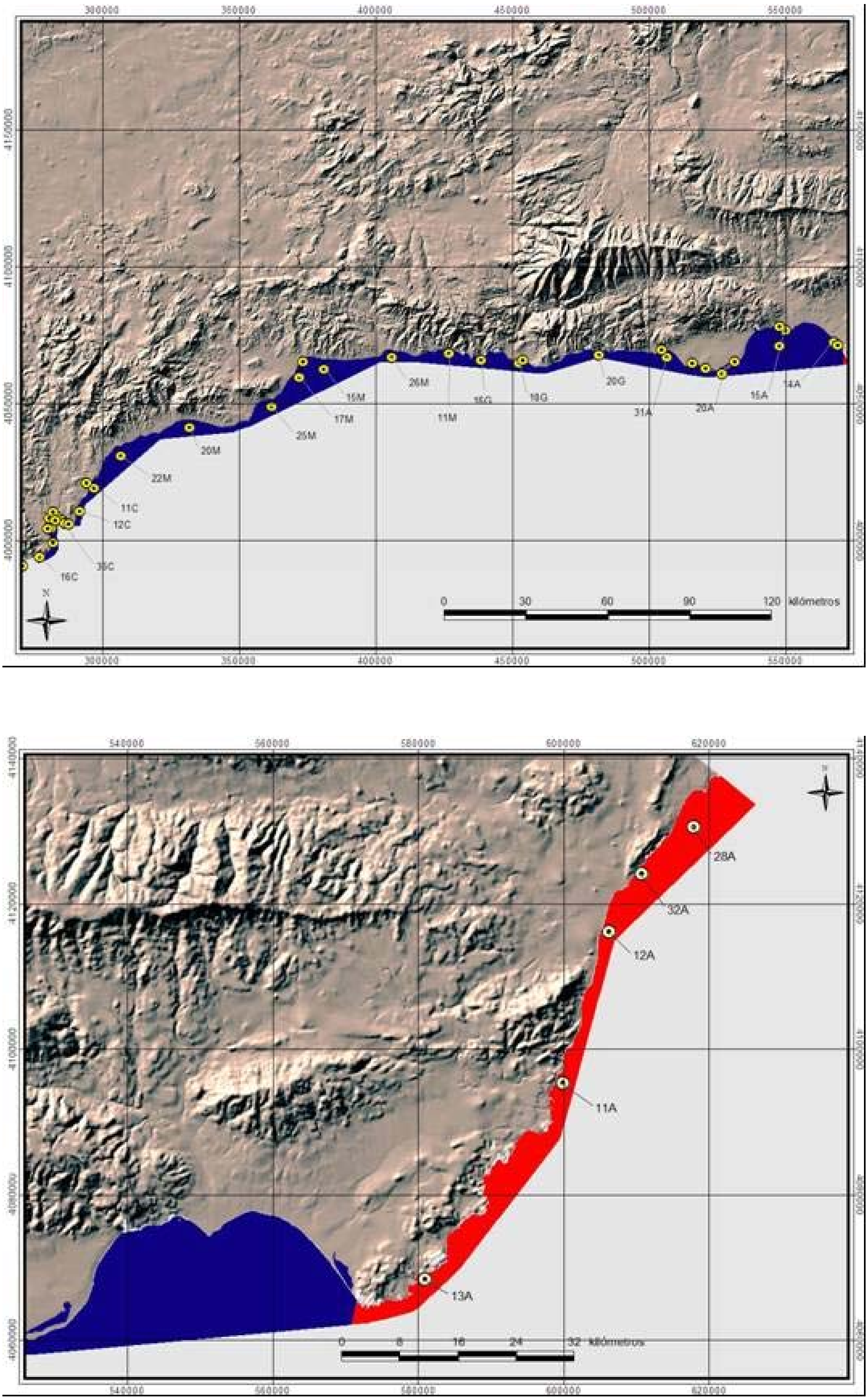



**Figure 11.** Monitoring sites along Andalusian coastal waters.

**Table 4.** Andalusian Monitoring sites: Location and type.

| STATION | CODE | X_UTM | Y_UTM | TYPE |
|---|---|---|---|---|
| Punta Acebuche | 16C | 277069 | 3992068 | Type II-B |
| Algeciras | 13C | 280715 | 4004488 | Type II-B |
| Palmones | 14C | 281846 | 4005813 | Type II-B |
| La Concha | 34C | 281889 | 4003418 | Type II-B |
| Getares | 33C | 281931 | 3997172 | Type II-B |
| Bahía Algeciras | 37C | 284455 | 4002059 | Type II-B |
| Campamento | 35C | 285204 | 4005418 | Type II-B |
| San Felipe | 36C | 286304 | 4004350 | Type II-B |
| La Línea-poniente | 15C | 287650 | 4004166 | Type II-B |
| La Línea-levante | 12C | 290124 | 4004882 | Type II-B |
| Guadiaro | 11C | 295781 | 4017652 | Type II-B |
| Manilva | 23M | 301089 | 4027347 | Type II-B |
| Estepona | 22M | 307330 | 4032016 | Type II-B |
| San Pedro de Alcántara | 21M | 322417 | 4038261 | Type II-B |
| Marbella | 20M | 332349 | 4041262 | Type II-B |
| Fuengirola | 19M | 355930 | 4046144 | Type II-B |
| Torremolinos | 18M | 365929 | 4052478 | Type II-B |
| Guadalhorce | 17M | 370916 | 4059488 | Type II-B |
| Puerto de Málaga | 16M | 372078 | 4062741 | Type II-B |
| El Palo | 15M | 379173 | 4064003 | Type II-B |
| Rincón de la Victoria | 14M | 384769 | 4063497 | Type II-B |
| Torre del Mar | 13M | 402399 | 4065833 | Type II-B |
| Nerja | 12M | 423373 | 4067376 | Type II-B |
| Maro | 11M | 427028 | 4067332 | Type II-B |
| La Herradura | 17G | 433572 | 4065463 | Type II-B |
| Almuñécar | 16G | 438466 | 4065464 | Type II-B |
| Salobreña | 15G | 447480 | 4064914 | Type II-B |
| Motril | 18G | 452267 | 4063759 | Type II-B |
| Motril | 14G | 453963 | 4064279 | Type II-B |
| Torrenueva | 13G | 456361 | 4061967 | Type II-B |
| Castell de Ferro | 12G | 468332 | 4064155 | Type II-B |
| La Rábita | 11G | 484567 | 4066896 | Type II-B |
| La Alcazaba | 22A | 494809 | 4066629 | Type II-B |
| Adra | 21A | 498585 | 4066408 | Type II-B |
| Punta Entinas | 20A | 526815 | 4059587 | Type II-B |
| Los Cerrillos | 17A | 531240 | 4061543 | Type II-B |
| Roquetas de Mar | 19A | 535514 | 4068493 | Type II-B |
| Aguadulce | 18A | 538586 | 4073717 | Type II-B |
| Bahía de Almería | 15A | 549942 | 4074950 | Type II-B |
| Costacabana | 16A | 555478 | 4076723 | Type II-B |
| Cabo Gata | 14A | 567499 | 4070257 | Type II-B |
| San José | 13A | 580178 | 4068888 | Type III |
| Carboneras | 11A | 599392 | 4095534 | Type III |
| Garrucha | 12A | 605597 | 4116251 | Type III |
| Villaricos | 23A | 610073 | 4124227 | Type III |

### 3.4. Balearic Islands

Coastal waters in Balearic Islands are mainly classified as type III, since no rivers or permanent freshwater discharges exist in these islands.

The Balearic phytoplankton monitoring programme is based on 63 stations (Fig. 12) located 500-700 m from the shoreline assigned to nearshore stations due to their physicochemical characteristics specially related to nutrient concentrations. Chlorophyll-a data are available for one annual cycle (2005-2006) with seasonal frequency sampling. The following parameters have been measured: transparency, temperature, salinity, dissolved oxygen, and inorganic dissolved nutrients. The inter annual mean of chlorophyll-a concentration in Balearic Islands is 0.16 µg/l, with a minimum value of 0.01 µg/l in summer and maximum value of 2.4 µg/l in winter season.

It is intended to define future and parallel monitoring cruises during the next two years, to confirm the values obtained introducing the in-shore field, and also verify the existence or not of type II coastal waters.

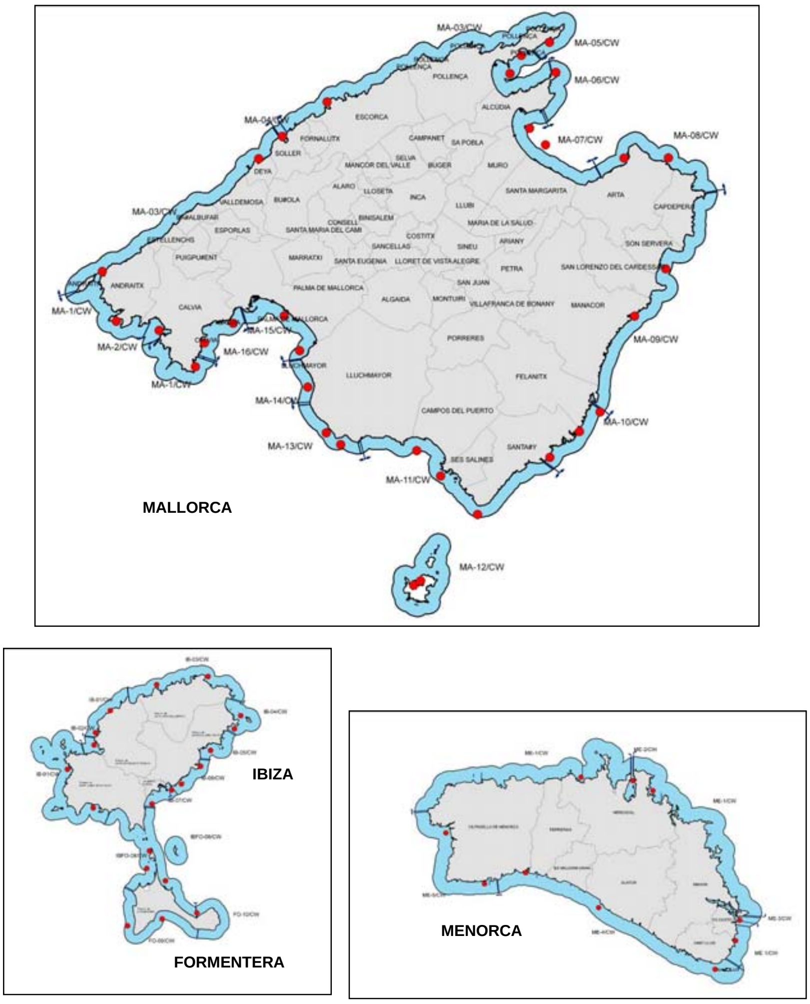



**Figure 12.** Monitoring sites along Balearic coastal waters.

# 4. METHODOLOGY

A methodology has been first developed for Catalan coastal waters and then adopted by other Mediterranean Spanish regions in order to reach, by common agreement, boundaries and RCs that represent all the Spanish Mediterranean coastal waters. The results for Catalan waters were obtained using the mean Chl-a concentration values. However, as a consensus between all Mediterranean Spanish Communities, and in order to homogenize with the results obtained by other GIGs, values were transformed into 90$^{th}$ percentile Chl-a, as described below.

## 4.1. Inshore waters

### 4.1.1. Catalonia

In order to do an IC exercise within the Med-GIG group, and for waters not affected by freshwater inputs, High and Good IC sites (undisturbed or slightly disturbed coastal waters, respectively) have been selected by expert judgement. This kind of previous evaluation is not evident for freshwater influenced waters where the salinity gradient makes difficult the choice of representative High and Good IC sites. For this reason, two different approaches have been developed to set quality boundaries and RCs for non freshwater influenced waters (type III) and freshwater influenced ones (types I and II).

<u>*Class boundary setting*</u>

*Type III*

The H/G and G/M boundaries have been defined following the next steps (Fig. 13):

1) The mean Chl-a values of the High (Llarga PL48, Montjoi R10, Prat d'en Forés PL53, Cap Salou R06) and the Good (Cavaió PL24, El Callao PL25, Apartaments Blaumar, R25) IC sites were calculated (1.10 and 1.48 µg/l, respectively). High and Good stations correspond to undisturbed or slightly disturbed coastal waters, respectively (ACA, 2005).

2) In order to define the H/G boundary, half of the difference between these two values ((1.48 – 1.10)/2 = 0.19) was added to the mean Chl-a of the High IC sites. The same procedure has been applied to define the G/M boundary but in that case using the mean Chl-a of the Good IC sites.

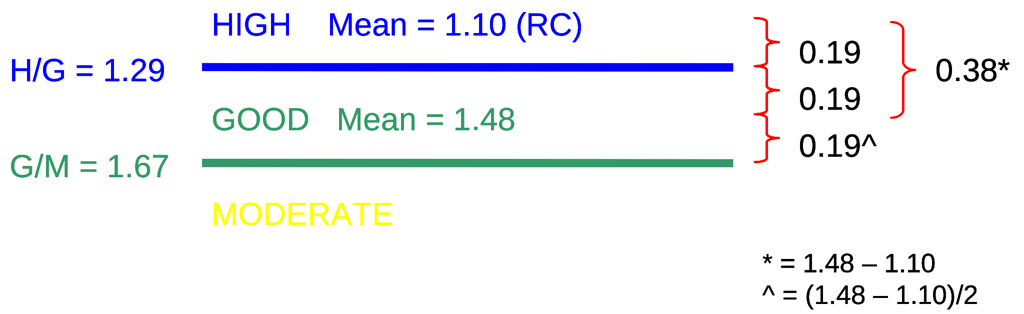



**Figure 13.** Steps followed to set RCs and quality boundaries for type III waters. Note: all values are Chl-a in µg/l.

The boundaries for type III are summarized in Table 5.

**Table 5.** Quality boundaries for type III.

| **Boundaries Mean Chl-a** | **Type III** |
|---|---|
| **H-G** | 1.29 |
| **G-M** | 1.67 |

*Types I and II-A*

Freshwater influenced waters have an additional natural input of nutrients associated with freshwater runoff from the continent which enhance phytoplankton growth. That means that reference conditions for these waters should be higher than that of the non freshwater influenced ones. Moreover, the reference conditions of such waters increase in parallel to the volume of the inflowing freshwater.

The two quality boundaries (H/G, G/M) have been estimated from the relationship observed between salinity and Chl-a (Fig. 14).

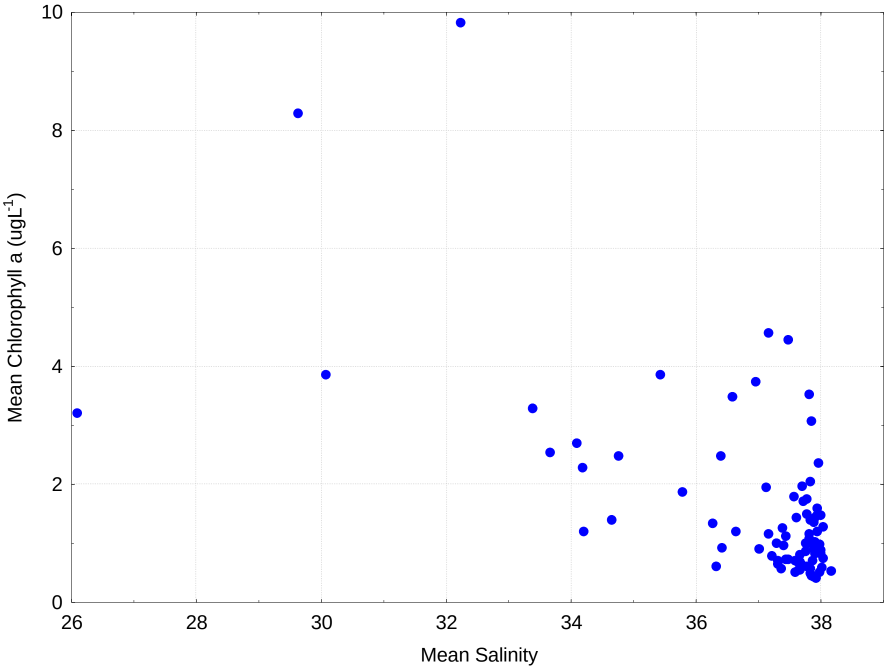



**Figure 14.** Scatterplot of Chl-a and salinity (mean station values) observed along the Catalan coastal waters.

Procedure for setting boundaries:

1) All the sampled stations along the Catalan waters (Fig. 14) have been classified as “good” or “bad” quality (Fig. 15) based on the multivariate physico-chemical analysis results which are related with human pressure (Flo *et al*, 2005). Physico-chemical multivariate analysis results are completely in agreement with expert judgement and with the results obtained from others Biological Quality Elements (ACA, 2005).

2) Within each data set (the “good” and the “bad”) a linear regression was fitted taking into account salinities higher than 34, where most of the values are located (Fig. 14). The linear regressions from the “good” and “bad” quality stations were then associated to H/G and P/B boundaries, respectively (Fig. 15).

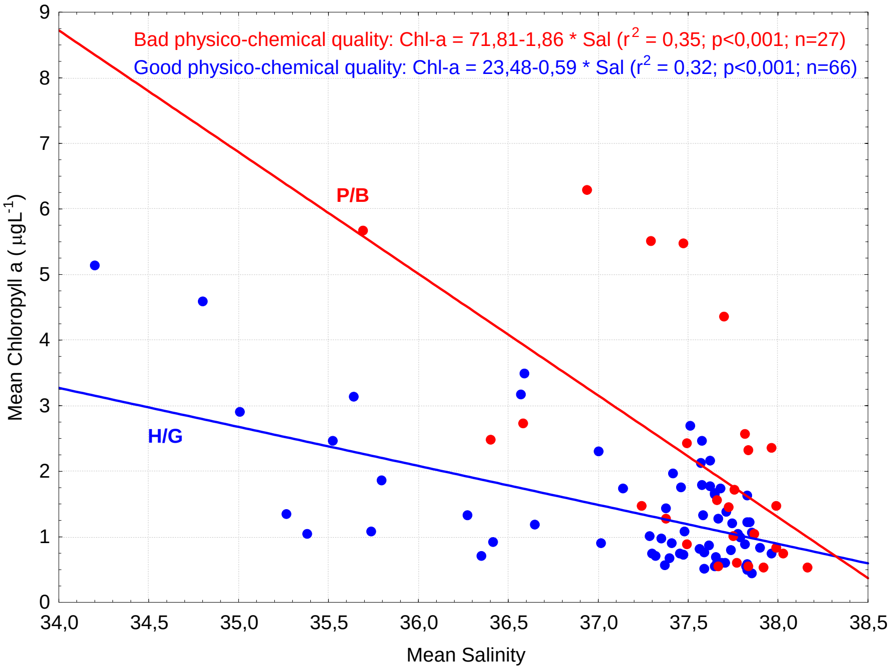



**Figure 15.** Scatterplot and linear relationship between Chl-a and salinity (mean station values) for the "good quality" stations (in blue) and for the "bad quality" stations (in red).

3) The G/M and M/P boundaries have been defined by the regression equations of two equidistant lines at Y-axis plotted between both, H/G and P/B regression lines (Fig. 16).

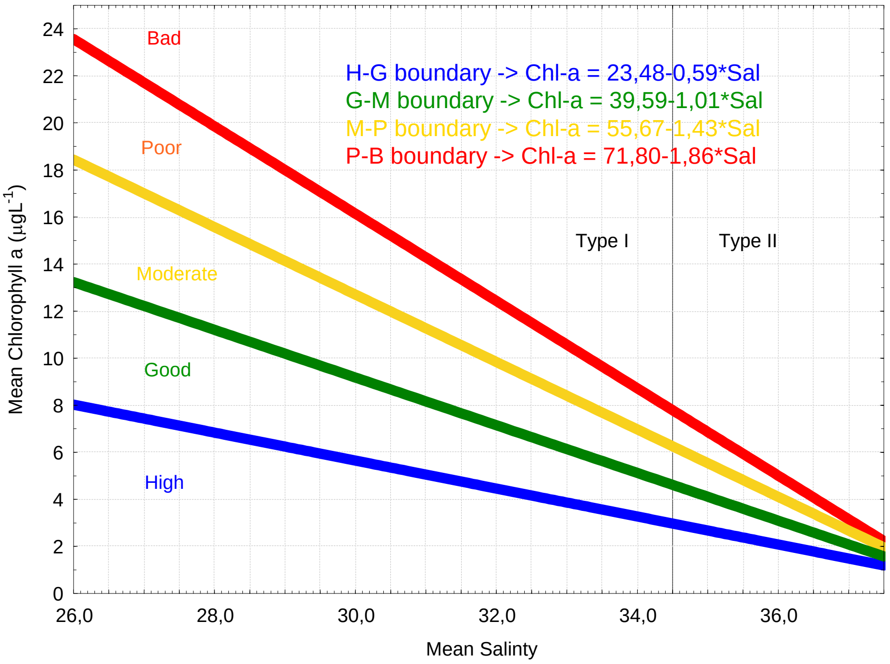



**Figure 16.** Regression lines used to define the H/G (in blue), G/M (in green), M/P (in yellow) and P/B (in red) boundaries.

4) The class boundaries (H/G, G/M, M/P, P/B) have been calculated using their respective regression equations (Fig. 16) at one fixed salinity value located in the middle of each type range (For Catalan waters => type I: salinity range = 26-34.5, salinity at the middle of the range = 30.25; type II-A: salinity range: 34.5-37.5, salinity at the middle of the range = 36). The results are summarized in Table 6.

**Table 6.** Quality boundaries for type I and II-A.

| **Boundaries**<br>**Mean Chl-a** | **Linear regression**<br>**Mean values** | **Type I**<br>**(Sal = 30.25)** | **Type II-A**<br>**(Sal = 36)** |
|---|---|---|---|
| **H-G** | Chl-a = 23.48-0.59*Sal | 5.50 | 2.08 |
| **G-M** | Chl-a = 39.59-1.01*Sal | 8.93 | 3.10 |
| **M-P** | Chl-a = 55.67-1.43*Sal | 12.35 | 4.11 |
| **P-B** | Chl-a = 71.80-1.86*Sal | 15.68 | 5.01 |

*Reference Conditions and EQRs*

Reference Condition for type III has been defined by the mean Chl-a of High IC sites (1.10 µg/l,Fig. 13).

No RCs for types I and II-A exist; therefore they have been calculated assuming the same proportion between RC and H/G boundary as found in type III (i.e. 1.10*100/1.29= 85%).

Then, the EQR has been calculated as the ratio between the RC value and the boundary value.

The RCs values and the EQR boundaries for each water typology and category are summarized in Table 7.

**Table 7.** Reference conditions values and EQR boundaries for types I, II-A and III inshore waters.

| | | **Type I** | **Type II-A** | **Type III** |
|---|---|---|---|---|
| **RC*** | | 4.69 | 1.77 | 1.10 |
| **EQR** | **H-G** | 0.85 | 0.85 | 0.85 |
| | **G-M** | 0.53 | 0.57 | 0.66 |

*Mean Chl-a (type III) or calculated Chl-a (type I and II). Values are given in µg/l.

### 4.1.2. Valencia

In the Valencian Community there are only types II-A and III.

For type III waters data related to other BQE indicators such as posidonia and macroinvertebrates (sampling cruises developed for the WFD in 2005-2006) have been used to identify reference sites (Fig. 17) and set quality boundaries.

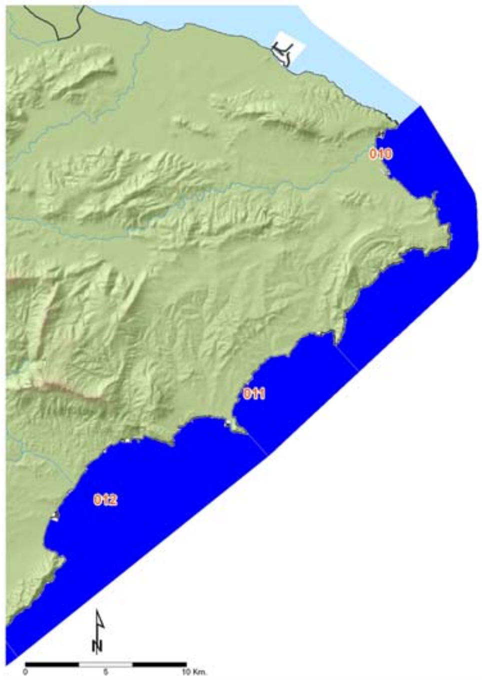



**Figure 17.** Type III Reference sites, water bodies 010,011,012.

The H/G and G/M boundaries have been defined following the next steps:

1) Based on the posidonia and macroinvertebrates results (both biological quality indicator parameters), the representative high (sites 10, 11 and 12), good (site 13) and moderate (site 15) IC sites are chosen.
2) From the representative selected zones for High, Good and Moderate IC sites, the mean chlorophyll-a concentration is selected (0.91, 1.02 and 2.06 µg/l, respectively). The mean chlorophyll-a of selected site as High is used as a reference condition
3) In order to define the H/G boundary, the half of the difference between these two values [(1.02 – 0.91)/2 =0.06] is added to the mean Chl-a of the High IC sites (0.91+0.06=0.97).
4) The same procedure has been applied to define the G/M boundary, the half of the difference between these two values [(2.06 – 1.02)/2 =0.52] is added to the mean Chl-a of the Good IC sites (1.02+0.52=1.54).

**Table 8.** Reference conditions values and EQR boundaries for type III waters.

| Status | Zone | Mean Chl-a |
|---|---|---|
| **High** | 10,11, 12 | 0.91 |
| **Good** | 13 | 1.02 |
| **Moderate** | 15 | 2.06 |

| Boundary | Mean Chl-a | EQR |
|---|---|---|
| **RC** | 0.91 | |
| **H/G** | 0.97 | 0.93 |
| **G/M** | 1.54 | 0.59 |

For type II-A waters (influenced by freshwater inputs), Valencian Community has also posidonia and macroinvertebrates data (data that has been obtained in sampling cruises developed for the WFD in 2005-2006). The methodology developed to set quality boundaries, and the establishment of reference sites (Fig. 18) is based in the data from these BQE.

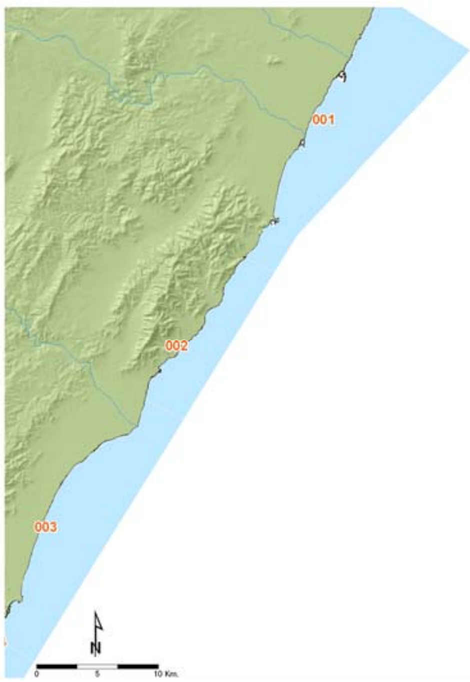



**Figure 18.** Type II-A reference sites, water bodies 001,002,003.

The H/G and G/M boundaries have been defined following the next steps:

1) Based on the posidonia and macroinvertebrates results (both biological quality indicator parameters), the representative high (sites 1, 2 and 3 zones), good (sites 5 and 9) and moderate (site 8) IC sites are chosen.
2) From the representative selected sites for High, Good and the Moderate IC sites, the mean chlorophyll-*a* concentration is selected (1.32, 2.04 and 3.55 µg/l, respectively). The mean chlorophyll-a of selected site as High is used as a reference condition.
3) In order to define the H/G boundary, the half of the difference between these two values [(2.04 – 1.32)/2 = 0.36] is added to the mean Chl-a of the High IC sites (1.32+0.36=1.68).
4) The same procedure has been applied to define the G/M boundary, the half of the difference between these two values [(3.55 – 2.00)/2 = 0.78] is added to the mean Chl-a of the Good IC sites (2.00+0.78=2.78).

**Table 9.** Reference conditions values and EQR boundaries for type II A waters.

| **Status** | **Site** | **Mean Chl-a** |
|---|---|---|
| **High** | 1, 2, 3 | 1.32 |
| **Good** | 5, 9 | 2.04 |
| **Moderate** | 8 | 3.55 |

| **Boundary/RC** | **Mean Chl-a** | **EQR** |
|---|---|---|
| **RC** | 1.32 | |
| **H/G** | 1.68 | 0.79 |
| **G/M** | 2.80 | 0.47 |

## 4.2. Nearshore waters

### 4.2.1. Catalonia

At nearshore waters only type II-A and III are found, whereas for inshore waters the three types are present.

Some stations for both typologies were classified as High by expert judgement (For type III: M11 Blanes, M03 Cadaqués, M10 Tossa and for type II-A: M29 Ametlla, M32 Trabucador, M05 Badia Roses Sud, M35 Les Cases Alcanar). Reference conditions for each typology were defined by the mean Chl-a value from their respective High stations (0.98 µg/l for type II-A and 0.46 µg/l for type III).

The boundaries for both types were calculated using the same proportion between RC and the 2 quality boundaries (H/G, G/M) for inshore waters (i.e. RC of inshore type III = 1.10 µg/l and G/M boundary of inshore type III = 1.67 µg/l => Proportion = 1.67/1.10 = 1.52; RC of nearshore type III = 0.46 µg/l => G/M of inshore type III = 0.46*1.52 = 0.70 µg/l).

The reference and quality boundary values and EQRs obtained for the nearshore waters are shown in Table 10.

**Table 10.** Reference conditions, quality boundaries and EQR boundaries for type II-A and III nearshore waters.

| | Type II-A | | Type III | |
|---|---|---|---|---|
| | **Mean Chl-a** | **EQR** | **Mean Chl-a** | **EQR** |
| **RC** | 0.98 | | 0.46 | |
| **H/G** | 1.15 | 0.85 | 0.54 | 0.85 |
| **G/M** | 1.72 | 0.57 | 0.70 | 0.66 |

RCs and boundaries for Nearshore waters corresponded to the 55% and the 42% of those defined for Inshore waters for type II-A and III, respectively.

### 4.2.2. Andalusia and Balearic Islands

*Type III*

Reference conditions, quality boundaries and EQR based on the methodology applied by Catalonia are also applicable for type III coastal waters in Andalusia and Balearic Islands. Chlorophyll-a data from Balearic nearshore stations are showed in Fig. 19. These data come from four campaigns (summer 2005, winter, spring and summer 2006) encompassing a yearly cycle. Not general monitoring data are available before these cruises around all Balearic Islands.

The inter annual mean of chlorophyll-a in Balearic Islands results in 0.16 µg/l, with a minimum value of 0.01 µg/l in summer and maximum value of 2.4 µg/l in winter season.

These stations can be preliminary classified as high status based on data coming from parameters related to other BQE.

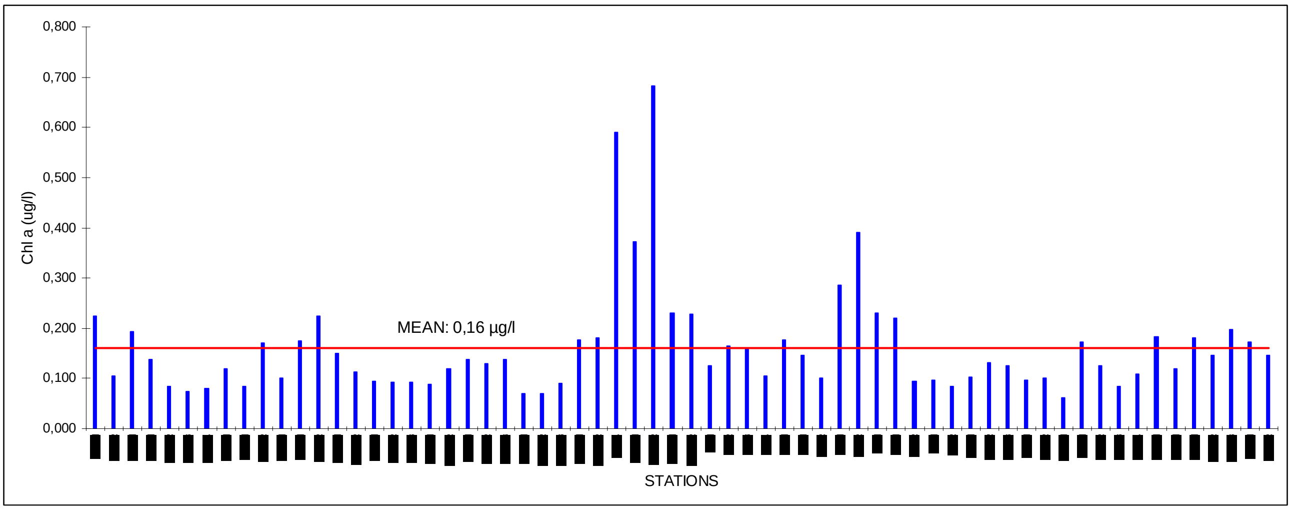



**Figure 19.** Inter-annual mean of Chl-a for Balearic monitoring sites

Andalusian stations, located 1500 m from the shoreline provide chlorophyll-a data (Fig. 20, stations in red colour) resulting in mean values around 0,5 µg/l and 90$^{th}$ percentile values around 0.8 µg/l These stations, not affected by significant human pressure, can be classified as high status (23 A) or even as reference sites (11A, 12A, 13 A), *Type II-B*

The Andalusian region agreed in the application of the same methodology for Atlantic and Mediterranean coastal waters, based on the calculation of the -90th percentile for the surface water chlorophyll-a data , from the monitoring eutrophication programme .

These stations are located at a distance of 1500m from the shore line and they have been sampled in a discontinuous way with a monthly or seasonal frequency, from the year 2000.

The station 16C, strongly influenced by the Atlantic waters, was selected as reference site for the type II-B, with a surface chlorophyll-a value of 1,99 µg/l.

After analyzing studying the results from the eutrophication monitoring programme , performed from the year 2000, it can be defined 3 µg/l as the boundary between high and good status (H/G) and 6 µg/l as the boundary between good and moderate status (G/M).

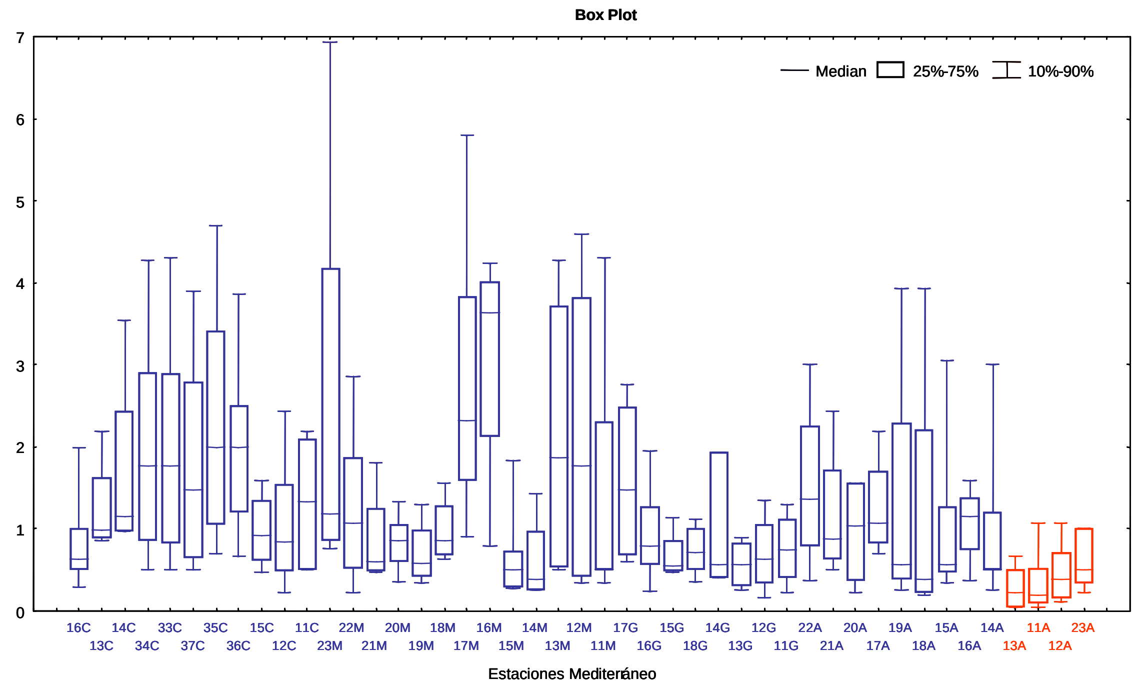



**Figure 20.** Box whisker plots of chlorophyll-a concentration from Andalusian Monitoring sites. The end whisker corresponds to 90th percentile value

On the other hand, it must be taken into account the continuity with the atlantic water masses, that should be reflected in the establishment of similar boundaries.

Therefore, it is proposed to consider 3 µg/l and 6 µg/l as the boundaries for the chlorophyll-a H/G and G/M boundaries, respectively. These boundary values are in consonance with the transition zone between the Atlantic values, type NEA1/26a, (5-10 µg/l) and the values proposed for the Mediterranean type-III (1,3-1,8 µg/l) which are included in Section 5.

**Table 11.** Reference conditions, quality boundaries and EQR boundaries for type II-B waters.

| | **Type II-B** | |
|---|---|---|
| | **90th%ile** | **EQR** |
| **RC** | 2.0 | |
| **H/G** | 3.0 | 0,67 |
| **G/M** | 6.0 | 0,33 |

a. The reference and quality boundary values have been obtained from the chlorophyll-a values derived from the eutrophication monitoring programme as indicated in paragraph b. Levels adopted for the Mediterranean type III waters have been also considered. As well, the Atlantic waters levels were compared, as the type II-B waters represent an intermediate state between the Andalusian Atlantic waters and the Andalusian Mediterranean waters not affected by the Atlantic waters. For the EQR values, it has been used the following equation: EQR = CR / Real value. So for a H/G level of 3, the EQR is calculated as EQR = 2 / 3 = 0,67. The same procedure is used for the calculation of the other EQR´s. These EQRs are identical to those defined for type NEA1/26a (see Technical Report, Section 3.4.5)

b. Procedure for setting boundaries:
All the stations along the Andalusian waters have been used to represent a box whisker plot, for the chlorophyll-a values. Considering those stations with less anthropogenic impacts, it has been defined the reference value and the boundary levels.

## 4.3. Reference and boundary values expressed in terms of 90$^{th}$ percentile

### Catalonia

Within the Med-GIG IC exercises, there was an agreement to use the 90$^{th}$ percentile Chl-a as the metric to set quality boundaries. For this reason, the values obtained for Catalan waters by the mean Chl-a were transformed into 90$^{th}$ percentile Chl-a applying equations obtained by linear regressions between both statistics.

For the transformation of the inshore values, type specific linear equations obtained from the relationship between means and 90$^{th}$ percentiles of each station has been used (Fig. 21). However for the nearshore values, the transformation has been done using a unique linear equation obtained from all nearshore stations (Fig. 22).

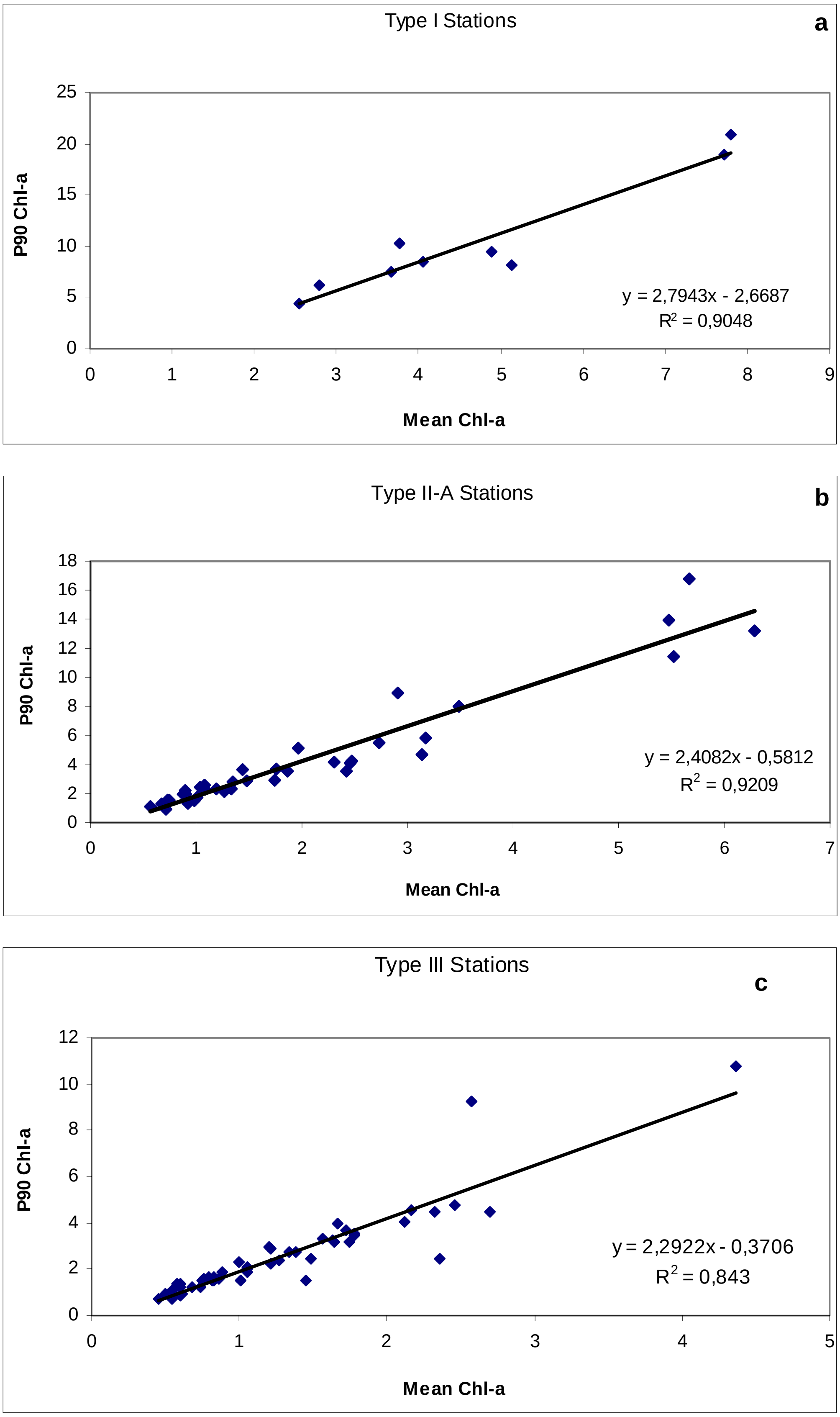



**Figure 21.** Type specific linear regressions between mean and 90th percentile Chl-a at inshore stations; a) type I stations, b) type II-A stations and c) type III stations.

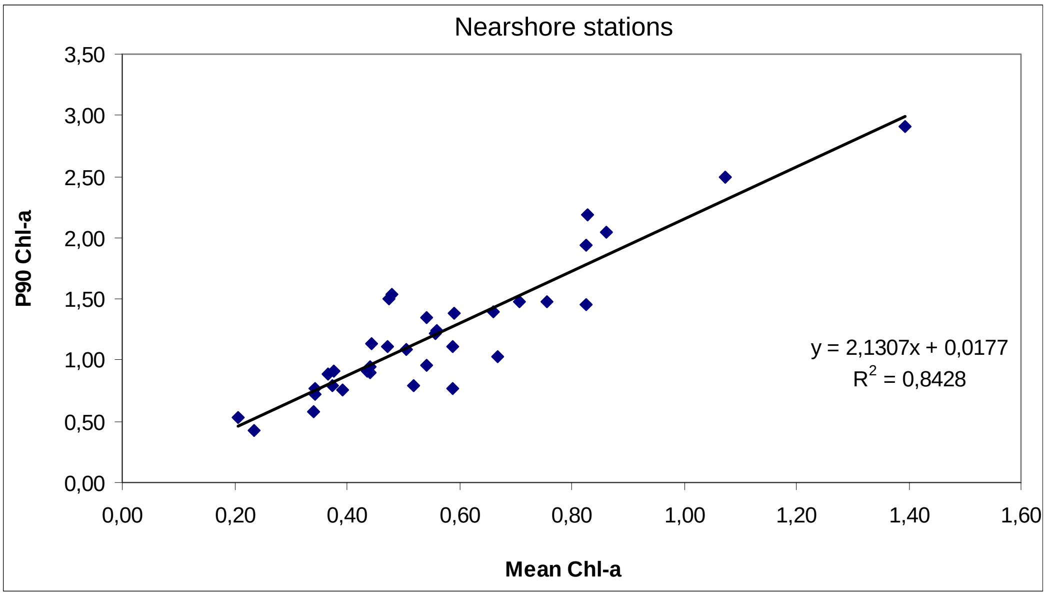



**Figure 22.** Linear regression between mean and 90th percentile Chl-a at nearshore stations.

**Table 12.** Reference conditions, quality boundaries and EQR boundaries of type I, II-A and III at inshore waters in terms of 90th percentile Chl-a.

| | Type I | | Type II-A | | Type III | |
|---|---|---|---|---|---|---|
| | **90th%ile** | **EQR** | **90th%ile** | **EQR** | **90th%ile** | **EQR** |
| **RC** | 10.44 | | 3.68 | | 2.15 | |
| **H/G** | 12.70 | 0.82 | 4.43 | 0.83 | 2.59 | 0.83 |
| **G/M** | 22.28 | 0.47 | 6.88 | 0.53 | 3.46 | 0.62 |

**Table 13.** Reference conditions, quality and EQR boundaries for type II-A and III nearshore waters in terms of 90th percentile.

| | Type II-A | | Type III | |
|---|---|---|---|---|
| | **90th%ile** | **EQR** | **90th%ile** | **EQR** |
| **RC** | 2.11 | | 1.00 | |
| **H/G** | 2.47 | 0.85 | 1.17 | 0.85 |
| **G/M** | 3.68 | 0.57 | 1.51 | 0.66 |

The values obtained from the transformation of the mean Chl-a values into 90th percentile by means the regression lines are practicaly the same than those reached using directly the 90th percentile (from raw data).

**Valencia**

*Type II-A*

For the transformation of the inshore mean values in 90th percentile values, type specific linear equations, obtained from the relationship between means and 90th percentiles of each zone, have been used in type II-A waters.

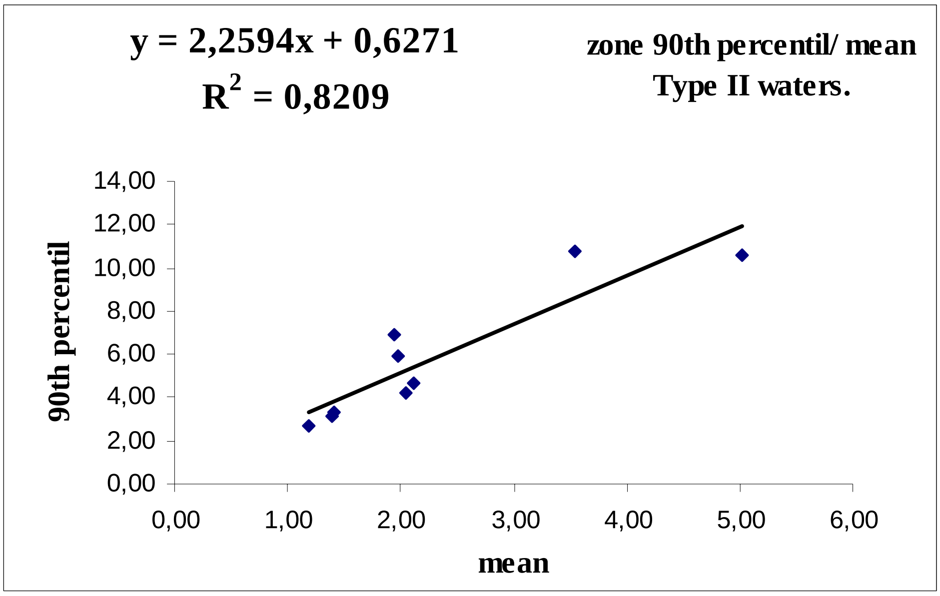



**Figure 23.** Specific linear regression between mean and 90th percentile Chl-a for inshore stations in type II-A waters.

| Type II-A | Mean Chl-a | 90th percentile |
|---|---|---|
| **RC** | 1.32 | 3,61 |
| **H/G** | 1,68 | 4,42 |
| **G/M** | 2.80 | 6,95 |

*Type III*

For the transformation of the inshore mean values in 90th percentile, type specific linear equations, obtained from the relationship between means and 90th percentiles of each zone, have been used in type III waters.

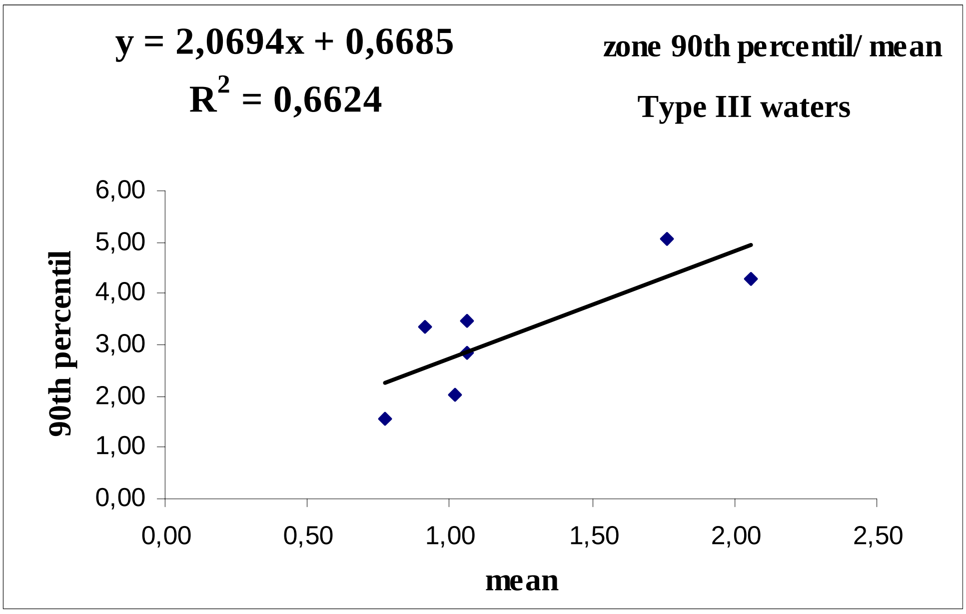



**Figure 24.** Specific linear regression between mean and 90th percentile Chl-a for inshore stations in type III waters.

| Type III | Mean Chl-a | 90th percentile |
|---|---|---|
| RC | 0,91 | 2,55 |
| H/G | 0,97 | 2,68 |
| G/M | 1,54 | 3,86 |

## 5. RESULTS OF THE HARMONISATION – BOUNDARY EQR VALUES

As a common agreement among the Mediterranean Spanish Regions, and in order to contribute to the intercalibration exercise (IC), slightly modified Catalan boundaries (in $90^{th}$ percentile) for nearshore waters were adopted as representative boundaries for Spanish Mediterranean coastal waters (based on data from other regions). The common nearshore agreed boundaries (Table 14) correspond to around 50% of those for Catalan Inshore waters (Table 15). This percentage is similar that the obtained comparing nearshore and inshore values for Catalan waters (see section 4.3).

**Table 14.** Reference conditions, quality and EQR boundaries agreed for type II-A, type II-B (Andalusia) and type III nearshore waters in terms of $90^{th}$ percentile for Spanish Mediterranean coastal waters.

| | Type II – A<br>Sal: 34.5 – 37.5<br>Influenced by freshwater inputs | | Type II - B<br>Sal: 34.5 – 37.5<br>Influenced by Atlantic waters | | Type III<br>Sal > 37.5<br>Not influenced by freshwater inputs | |
|---|---|---|---|---|---|---|
| | $90^{th}$%ile | EQR | $90^{th}$%ile | EQR | $90^{th}$%ile | EQR |
| RC | 1.9 | | 2.0 | | 1.1 | |
| H/G | 2.3 | 0.83 | 3.0 | 0.67 | 1.3 | 0.85 |
| G/M | 3.5 | 0.54 | 6.0 | 0.33 | 1.8 | 0.61 |

The following table (Table 15) shows the values agreed by the Mediterranean Spanish Regions for inshore waters.

**Table 15.** Reference conditions, quality and EQR boundaries agreed for type I, type II-A, type II-B and type III inshore waters in terms of $90^{th}$ percentile for Spanish Mediterranean coastal waters.

| | Type I<br>Sal < 34.5<br>Highly influenced by freshwater inputs | | Type II – A<br>Sal: 34.5 – 37.5<br>Influenced by freshwater inputs | | Type II – B<br>Sal: 34.5 – 37.5<br>Influenced by Atlantic waters | | Type III<br>Sal > 37.5<br>Not influenced by freshwater inputs | |
|---|---|---|---|---|---|---|---|---|
| | $90^{th}$%ile | EQR | $90^{th}$%ile | EQR | $90^{th}$%ile | EQR | $90^{th}$%ile | EQR |
| RC | 10.44 | | 3.8 | | 4.0 | | 2.2 | |
| H/G | 12.70 | 0.82 | 4.6 | 0.83 | 6.0 | 0.67 | 2.6 | 0.85 |
| G/M | 22.28 | 0.47 | 7.0 | 0.54 | 12.0 | 0.33 | 3.6 | 0.61 |

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# ANNEX 1: NATIONAL METHODS INCLUDED IN THE INTERCALIBRATION

## FRANCE : National Methods for Phytoplankton

### 1. National Method included in the intercalibration : biomass, chlorophyll-a

**Metric** : 90$^{th}$ percentile of Chl-a concentrations (µg/L) on six-year data.
Sampling period : all year for coastal water bodies (from June to August for lagoons).
Sampling frequency : once a month.
Concern : coastal and transitional water bodies.
Boundaries :

| Type | Reference | H / G | G / M | M / P | P / B |
|---|---|---|---|---|---|
| **1**<br>(water bodies highly affected by Rhône river) | ? | under development | | | |
| **2 A**<br>French Western Mediterranean water bodies, except Banyuls | < 2 | 2 – 2.4 | 3.6 – 4 | under development | |
| **3 WM**<br>French Eastern Mediterranean water bodies, Banyuls water body, and Corsica water bodies | < 1 | 1 – 1.1 | 1.8 – 2 | under development | |

The boundaries are presented as ranges, since the consequences of the compromise made for intercalibration has to be further discussed, and the boundaries clearly precised. It is also for this reason that the boundaries between Moderate and Poor, and between Poor and Bad, are not yet precised in the table.

Concerning the reference conditions, as no reference site has been designated for the types 1 and 2A in France, it is only possible to precise that the reference conditions are below the boundary High / Good for these types. For type 3WM, the data on the two reference sites (Banyuls and Villefranche) do not allow to assign a unique reference value, so the reference value will be probably the value which has been retained for Intercalibration (0.9).

### 2. National Methods not included in the intercalibration

#### *1.1 2.1. ABUNDANCE : BLOOMS OF ALL SPECIES*

**Metric** : percentage of samples on six-year data, where a single taxa count is above the exceeding threshold.
Definition of the exceeding threshold : 100 000 cells per liter for large cells, and 250 000 cells per liter for small cells.
Sampling period : all the year
Sampling frequency : once a month.
Concern : coastal and transitional water bodies.
Boundaries :

| Type | Reference | H / G | G / M | M / P | P / B |
|---|---|---|---|---|---|
| **all types** | < 20 | 20 | 40 | 70 | 90 |

## 1.2 2.2. COMPOSITION : BLOOMS OF HARMFUL SPECIES

**Metric** : percentage of samples on six-year data, where a single harmful taxa count is above the exceeding threshold.
Harmful species are defined as : toxic or harmful species to marine fauna, and eutrophication indicator species. The list of these harmful species has yet to be precised.
Definition of the exceeding threshold : 1 000 000 cells per liter.
Sampling period : all the year
Sampling frequency : once a month.
Concern : coastal and transitional water bodies.
Boundaries : under development.

# NUTRIENTS AND THEIR RATIOS IN RELATION TO EUTROPHICATION AND HAB OCCURENCE. THE CASE OF EASTERN MEDITERRANEAN COASTAL WATERS

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## INTRODUCTION

The problem of eutrophication in the Mediterranean appears to be limited largely to specific coastal and adjacent offshore areas. However, several (severe sometimes) eutrophication events occur. Especially in enclosed coastal areas, which receive anthropogenically-enhanced nutrient loads from rivers and the direct discharges of untreated domestic and industrial waste-water. Uncontrolled expansion of aquaculture may also cause local environmental problems in the Eastern Mediterranean. According to Izzo & Pagou (1999), eutrophication problems are likely to increase in the future due to rapid population expansion, applied production technologies and inadequate environmental policies and enforcement. These local and regional eutrophication problems can negatively affect marine life and have a severe socio-economic and potential adverse impact on tourism, aquaculture, fisheries and other water uses.

It is not feasible to assess the Mediterranean eutrophication phenomena based only on nutrient concentrations. However, the establishment of nutrient thresholds for each Mediterranean sub-basin are needed as tools for communication between scientists, policy makers and the public. Furthermore, according to Moncheva *et al*. (2001) subsequent comparative studies between different basins could be a step toward to highlight common patterns and modes of ecosystem response to anthropogenic eutrophication and to suggest common indices to scale eutrophication impact.

The Eastern Mediterranean is one of the world's poorest seas Azov (1991). The Aegean Sea is one of the Eastern Mediterranean basins displaying a complicated hydrographic and ecological structure due to its geographical position between the Black Sea and the Ionian and Levantine Seas (Siokou-Frangou *et al*., 2002; Zervakis *et al*., 2000). A number of recent studies in Aegean Sea confirmed its oligotrophic status (Gotsis-Skretas *et al*., 1999; Siokou-Frangou *et al*., 2002; Ignatiades *et al*., in press). Data collected during the MTP-II-MATER (MAST-III) multidisciplinary EU project in Aegean Sea (Ignatiades *et al*., in press) showed that all nutrients exhibited very low concentrations not differing significantly between the N. and the S. Aegean Sea. The average levels in the entire area ranged for phosphate from 0.013 to 0.049μM, for ammonium from 0.067 to 0.397 μM, for nitrite+nitrate from 0.287 to 0.881 μM and for silicate from 1.177 to 2.395 μM. The overall average level of chl α ranged from 0.119 to 0.371 mg $m^{-3}$, whereas daily primary production rates in the N. Aegean (81.36 mgC $m^{-2}$ $d^{-1}$) is higher than that in the S. Aegean (38.88 mgC $m^{-2}$ $d^{-1}$), but both areas are oligotrophic as having productivity values lower than 270 mgC $m^{-2}$ $d^{-1}$ (Nixon, 1995).

Despite the above, eutrophication problems have been recognized in a number of Aegean coastal areas, affected by urban and industrial waste-waters and/or nutrient inputs from rivers and agricultural activities (Pagou, 1990; Pagou & Ignatiades, 1988, 1990; Gotsis-Skretas, 1995).

The most disturbed Greek coastal areas, regarding anthropogenic nutrient enrichment, are the Saronikos and Thermaikos Gulfs situated in Central and Northern Aegean Sea, respectively (FIG. 1).

Saronikos Gulf (mean depth ~100m) was impacted by untreated sewage for more than 30 years from the metropolitan Athens (up to 3 million inhabitants during that period) discharged in the surface of its northern part, by a surface outfall (Keratsini Bay, FIG.1). The industrial effluents from the Elefsis Bay (a shallow semi-enclosed area to the north of Saronikos gulf) contributed also to the pollution of Saronikos. Elefsis Bay was the most industrialized area of Greece, where anoxic conditions near the bottom were recorded every summer. From summer 1994 Saronikos Gulf receives the primary treated effluents (domestic and industrial) from the Psittalia Treatment Plant, through an outfall positioned at 60m depth.

The Inner Thermaikos Gulf (mean depth ~30m) on the other hand and especially its northern part (Bay and Gulf of Thessaloniki) are the marine receptors of urban and industrial wastes from the city of Thessaloniki, along with agricultural effluents from the relevant practices taking place in the adjacent land, which includes drainage basins of rivers, streams and channels discharging in

Thermaikos Gulf. At the same time in Inner Thermaikos Gulf several fishing practices and aquacultures (the most important aquaculture ground of Greece) exist together with recreational and touristic activities concentrated along its coastline. The western coast of the Inner Thermaikos Gulf is influenced by the three major river estuaries (Axios, Loudias, Aliakmon). The inflow of large amounts of freshwater enriched in nutrients (from agriculture run-off) and matter through these rivers determines significantly the trophic condition in the ecosystem. In fact this inflow is the second most serious (after sewage) impact for the coastal environment of the Inner Thermaikos Gulf. However, from June 2000 a treatment plant with an outfall discharging at 25m depth started operating in the northern part of Thermaikos including biological treatment of the influents. Load removal efficiency of the treatment plant has reached 90% for nitrogen and 50% for phosphorus.

The purpose of the paper is an attempt to produce estimates on nutrient thresholds for Eastern Mediterranean coastal marine ecosystems. Some methodological procedures are presented and used in order to define eutrophication scales, assign critical values and, consequently evaluate water quality. The two Greek gulfs were selected as case studies.

## METHODOLOGY: DEFINING NUTRIENT THRESHOLDS AND EUTROPHICATION ASSESMENT CRITERIA

1. Eutrophication scale based on nutrient and phytoplankton data:

Definition of concentration ranges from nutrient and phytoplankton data sets, specific for this region of the Eastern Mediterranean and characteristic of the different trophic levels in the Greek seas have been conducted by local authorities in the two gulfs. A eutrophication scale was developed by Ignatiades *et al*. (1992) and Karydis (1999), based on nutrient data from several Greek marine areas coastal and offshore waters influenced or not by industrial and/or domestic effluents). Later the original eutrophication scale was modified in order to include also phytoplankton parameters (Siokou & Pagou, 2000; Pagou, 2000), which were undergone the same statistical treatment. The derived eutrophication scale is given in Table I.

**Table I**: Trophic classification ranges based on nutrients (phosphates, nitrates, ammonium), chlorophyll α and total number of phytoplankton cells. Ranges are given for the oligotrophic, lower mesotrophic, higher mesotrophic and eutrophic system. Nutrients concentrations are given in μM, phytoplankton cells number in cells $l^{-1}$ and chlorophyll in μg $l^{-1}$.

| Parameter | Oligotrophic | Lower mesotrophic | Higher mesotrophic | Eutrophic |
|---|---|---|---|---|
| Phosphates ($PO_4$) | <0.07 | 0.07-0.14 | 0.14-0.68 | >0.68 |
| Nitrates ($NO_3$) | <0.62 | 0.62-0.65 | 0.65-1.19 | >1.19 |
| Ammonium ($NH_4$) | <0.55 | 0.55-1.05 | 1.05-2.2 | >2.2 |
| Phytoplankton | $<6x10^3$ | $6x10^3$-$1.5x10^5$ | $1.5x10^5$-$9.6x10^5$ | $>9.6x10^5$ |
| Chlorophyll α | <0.1 | 0.1-0.6 | 0.6-2.21 | >2.21 |

Four levels of eutrophication are defined in this scale: eutrophic, higher mesotrophic, lower mesotrophic and oligotrophic. These levels could be considered as corresponding to the five categories of environmental status as defined by the WFD: eutrophic for bad, higher mesotrophic for poor, lower mesotrophic for both moderate and good and oligotrophic for high. Therefore, nutrient concentrations above the limit defining eutrophic conditions could be considered as estimates of threshold values for marine coastal areas of Eastern Mediterranean. In addition, nutrient concentrations which define higher mesotrophic conditions indicate “sensitive” ecosystems (can be eutrophic in the future, if an increasing trend in eutrophication parameters is detected). Thus, concentrations characteristics of higher mesotrophic conditions can serve as “red flags” for ecosystems potentially threatened by future human impacts.

The data sets from Saronikos (1998-1999) and Thermaikos Gulfs (1997-1998), used as examples to evaluate this tool, were mean annual values based on mean integrated values over the water column of the above mentioned parameters. The mean values (of the log-transformed data) were calculated using the method “Box-and-Whisker” plot (Ott 1988). For the exclusion of outliers, the statistical package STATGRAPHICS PLUS, version 3.1 was used.

2. Thematic maps:

a) The methodological procedure for the generation of thematic maps regarding the spatial distribution of each nutrient and chlorophyll was based on the application of the spatial interpolation Kriging method (Lancaster & Salkauskas, 1986). The method was applied in a spatial resolution of 100x100m using Geographic Information System (ARC/INFO version 7.1.2). The values of each generated continuous surface were categorised based on nutrient and chlorophyll concentration scaling given in Table I. Therefore again four levels of eutrophication were defined on the thematic maps: eutrophic, higher mesotrophic, lower mesotrophic and oligotrophic.

b) For the synthesis of all measured parameters to create the final eutrophication map the below described procedure was followed:

Values assigned to the limits of each trophic level of Table 1, were standardised according to the equation (Sneath & Sokal, 1973):

$$\mathbf{Z=X-X\mu/sd}$$

where, **Z** is the standardized value for the data **X** of the parameter, **Xμ** is the mean value of the parameter and **sd** is the standard deviation of the parameter. Standardisation is necessary in order to compare and combine parameters-criteria measured in different scales, as are nutrients and chlrophyll. Thus, new ranges were produced for each trophic level, which were pure numbers (scores) and were added to produce a new eutrophication scale based on the combination of all examined parameters. The same standardisation procedure was followed for the data sets used as case studies and the results were compared to those of the newly formed eutrophication scale.

Afterwards in order to produce the final synthetic eutrophication map, the same procedure as before (2a) was followed, but for the categorisation of the area was based to the new synthetic limits produced for each trophic level.

Thus, two series of maps were produced: a) categorical maps for the distribution of each nutrient and chlorophyll α, separately (FIG. 2), and b) synthetic categorical maps of the eutrophication level) (FIG.3). The data sets used for the evaluation of this tool were from Inner Saronikos Gulf (1998-1999, for surface and for the mean integrated value of the water column) and before the application of the above described procedure exclusion of the outliers was carried out.

3. <u>Trend analysis</u>

The investigation of the existence of trends in the evolution of the trophic condition parameters is very important, both for the assessment of the eutrophication levels and also in order to test the effectiveness of management plans applied in coastal marine areas. Nutrients and chlorophyll α data from 1987 to 1999 for the Saronikos Gulf and from 1993 to 2000 for the Thermaikos Gulf were tested in order to assess any trend existence.

In all time series of the elaborated parameters (after their log transformation) the outliers were excluded using the “Box-and-Whisker” plot (Ott, 1988) and a simple regression analysis was applied in order to test if the overall trend was statistically significant. The statistical package STATGRAPHICS PLUS, version 3.1 was used.

4. <u>Nutrient ratios:</u>

Nutrient ratios (ΣN/P, Si/ΣN, Si/P, where ΣN is the sum of nitrate+nitrite+ammonium) were calculated for the same data sets as before. In addition, the available time series of nutrient ratios were analyzed for trends and the more important results are presented.

CASE STUDIES

Data from the Saronikos and Thermaikos Gulfs were applied as case studies to assess the effectiveness of the above described tools.

<u>Eutrophication scale</u>: The application of this criterion showed that overall the Inner Saronikos Gulf (the sewage receptor water body) revealed a high mesotrophic character regarding nutrients and phytoplanktonic cells and low mesotrophic regarding chlorophyll α. A small area of its southern part is oligotrophic, whereas the northern (Keratsini bay) is high mesotrophic (Table II).

On the other hand, the northern part (port and sewage outfall area) of the Inner Thermaikos gulf has a higher mesotrophic to eutrophic character (depending on the parameter), whereas estuaries can be characterized as mesotrophic (towards the upper levels). The central and southern part of the Inner Thermaikos Gulf has a variable character from oligotrophic to higher mesotrophic, depending on the parameter. It must be mentioned again that this is not a deficiency of the examined tool but different parameters have different spatial behavior (Table III).

**Table II.** Mean annual values based on mean integrated values (May 1998 - May 1999) of the studied parameters in the water column. Symbols: E=Eutrophic, HM=Higher Mesotrophic, LM=Lower Mesotrophic, O=Oligotrophic.

| **Area** | **$PO_4$** (μgat/l) | **$NO_3$** (μgat/l) | **$NH_4$** (μgat/l) | **Chl-α** (μg/l) | **Phytoplankton** (cells/l) |
|---|---|---|---|---|---|
| Keratsini bay (former outfall) | 0,44 **HM** | 1,15 **HM** | 0,92 **LM** | 0.92 **HM** | |
| Psitallia (new outfall) | 0,23 **HM** | 0,70 **HM** | 1,17 **HM** | 0,30 **LM** | $3{,}25X10^5$ **HM** |
| Inner Gulf (8.5km to the SE from new outfall | 0,09 **LM** | 0,98 **HM** | 0,509 **O** | 0,29 **LM** | $4{,}11X10^5$ **HM** |
| Inner Gulf (15km to the SW from new outfall) | 0,13 **LM** | 1,262 **E** | 0,25 **O** | 0,29 **LM** | |
| Southern Inner Gulf (18km to the SE from new outfall) | 0,06 **O** | 0,609 **O** | 0,24 **O** | 0,23 **LM** | |

**Table III.** Mean annual values based on mean integrated values of the studied parameters for the period May 1997-May 1998 in the water column. Symbols: E=Eutrophic, HM=Higher Mesotrophic, LM=Lower Mesotrophic, O=Oligotrophic.

| **Area** | **$PO_4$** (μgat/l) | **$NO_3$** (μgat/l) | **$NH_4$** (μgat/l) | **Chl-α** (μg/l) |
|---|---|---|---|---|
| Thessaloniki Bay - port | 0,45 **HM** | 1,04 **HM** | 1,63 **HM** | 3,79 **E** |
| Thessaloniki Gulf - outfall | 0,34 **HM** | 1,08 **HM** | 1,64 **HM** | 2,37 **E** |
| Estuaries | 0,33 **HM** | 1,59 **E** | 0,77 **LM** | 1,67 **HM** |
| Inner Thermaikos Gulf | 0,10 **LM** | 0,57 **O** | 0,93 **LM** | 1,16 **HM** |

Thematic maps: The classification of the sub-areas of the Inner Saronikos Gulf derived by recent nutrient and chlorophyll data sets (FIG. 2) showed that almost all Inner Saronikos Gulf is high mesotrophic regarding phosphates and only the southeastern part is oligotrophic. Regarding ammonium, the area up to a distance of 4km from the outfall is characterised as high mesotrophic, up to 10km from the outfall as low mesotrophic, whereas the south Inner Saronikos is oligotrophic. Based on nitrates measurements, almost the entire Inner Saronikos is high mesotrophic with few delimited eutrophic areas, whereas based on chlorophyll α the whole area is low mesotrophic.

From these results it is obvious that the different horizontal distribution patterns of the various eutrophication parameters renders the characterization of water quality at a spatial scale very difficult, when producing categorical maps for each examined variable. Nutrient loads tend to homogenize environmental gradients (Giovanardi & Tromellini, 1992), whereas phytoplankton parameters show spatial heterogeneity (Li & Reynolds, 1994). Furthermore, according to Lipiatou (2002) nutrient threshold is referring to the sum of organic and inorganic nitrogen and phosphorus and Carbon and Si.

Thus, a synthetic trophic condition presentation was produced based on all available environmental parameters (FIG. 3). Taking into account the whole water column and the annual mean values, the Inner Saronikos is characterized as high mesotrophic, but the southeastern part is low mesotrophic to oligotrophic. Keratsini Bay (the northern part of Inner Saronikos) has been revealed as eutrophic. The figure (3A) based on the surface and stratification period data is similar to that for the water column (FIG. 3B). This figure (FIG. 3A, stratification period) compared to that given by Kitsiou & Karydis (1998; data from 1980-1982) reveals the change of the trophic conditions in the area, where the former sewage outfall was discharging, from eutrophic to high mesotrophic and of the southern Inner Saronikos from high mesotrophic to low mesotrophic. These changes can be attributed to the operation of the new treatment plant.

Trend analysis: For the Saronikos Gulf eutrophication trends had not a consistent character, since a different trend was detected for nutrients (eutrophication causative) when compared to chlorophyll (eutrophication result). Among nutrients only nitrates revealed a clear increasing trend, whereas chlorophyll revealed a more or less decreasing trend. The observed increasing trends of nutrients in the sewage outfall area can be explained by the increase of the discharge volume. Especially for nitrates, the increase observed at all sampling sites could be related to the discharges increase and diffusion due to circulation patterns and probably the entrance from the atmosphere should also be considered (Carpenter *et al*., 1998). However the decreasing trends of chlorophyll α concentrations suggest that nutrients (and mainly nitrates) are not exclusively used by the autotrophic organisms (heterotrophic bacteria also use nutrients). On the other hand not favorable relative concentration ratios in the sewage area (N/P, Si/N, Si/P), or even light limitation, since sewage is discharged in a greater depth than before 1994, should also be encountered. Before the operation of the Psittalia treatment plant (1994) the highest chlorophyll values were recorded in the surface layer, whereas surface and column integrated values are very low after 1994 (NCMR, 1999). The observed trends suggest an ecosystem being in evolution, since only nitrates among nutrients (eutrophication cause) revealed a clear increasing trend, whereas chlorophyll (eutrophication result) revealed a more or less decreasing trend.

Trend analyses of nutrients and chlorophyll time series from the Thermaikos Gulf revealed also some important features. Nutrients and chlorophyll α concentrations showed a general trend to decrease the last years, according to the trend analysis performed in time series data from 1993 to 2000. These decreasing trends were often statistically significant. However, an exception was recorded. In the reference area, nitrate and ammonium showed an increasing trend, which respectively were not statistically significant and statistically significant. The reference area was near the southern boundaries of Inner Thermaikos with the Outer Gulf where it is assumed minimum impact from the sewage. Therefore, is suggested the existence of a local inflow of nutrients, which can possibly be of a local nature. This increase needs further investigation. In conclusion nutrients and chlorophyll concentrations show a decreasing trend in the most affected areas of the Inner Thermaikos Gulf during the last years.

Nutrient ratios: It is known that anthropogenic nutrient enrichment is confined to N and P mainly, whereas Si supply remains constant or decreases as a consequence of increased diatom production and subsequent increased deposition and retention of Si in the sediments. Furthermore, this shift in relative nutrient concentrations increases the occurrences of Si limitation to phytoplankton growth. Si limitation could diminish the importance of diatoms in the phytoplankton population and replace it by noxious and toxic forms such as dinoflagellates (Parker, 1987; Dortch & Whitledge, 1992; Justic *et al*., 1995). Therefore, marine ecosystems impacted by such nutrient enrichment show changes in resource supply ratios that can cause changes in microplankton population.

Studies on nutrient uptake kinetics have pointed out that ambient ratios of dissolved $N/P<10$ and $Si/N>1$ indicate stoichiometric N limitation, $Si/N<1$ and $Si/P<3$ indicate Si limitation and $N/P>20$-$30$ suggests P limitation (Dortch & Whitledge, 1992; Justic *et al.,* 1995). In Eastern Mediterranean open waters productivity was shown to be P limited (Krom *et al*., 1991).

At this point it is very interesting to compare data on nutrient ratios and phytoplankton blooms in the two Aegean Gulfs chosen as case studies, before and after 1995.

Moncheva *et al*. (2001) examined differences and similarities in phytoplankton response to anthropogenic nutrient enrichment in coastal sites of the Western Black Sea and the Eastern Mediterranean (Aegean Sea). From the Aegean Sea the Saronikos and the Thermaikos Gulf were selected for this study and historical data on physicochemical parameters and phytoplankton populations from the 80ties up to 1995 were studied. The most important conclusions regarding the two Greek gulfs were:

- Diatoms were less common blooming species in Aegean Sea coastal areas with the exception of Thermaikos Gulf. In Thermaikos diatoms dominated even during summer, whereas spring blooms were due to diatom assemblages. On the other hand in Saronikos gulf, dinoflagellates, chrysophyceae and chlorophyceae species frequently outcompeted diatoms.
- The numerical displacement of diatoms with other species in Saronikos in relation to Thermaikos was attributed to differences in the nature of nutrient loads and nutrient ratios. Higher dominance of the Si demanding diatoms in Thermaikos were more efficient in utilizing high nutrient levels (especially of Si) as mainly supplied by the rivers run-off. On the contrary, the sewage outfalls, rich in dissolved organic matter (a major source of eutrophication in Saronikos Gulf) stimulate the growth of mixotrophic microalgae.

After 1995 dramatic changes occurred in both areas. In Saronikos no significant phytoplankton bloom have been reported and this can attributed to the lowering of the trophic status of the most eutrophic areas (from eutrophic to high mesotrophic) due to the operation of the new waste treatment plants.

However minimum values (annual means) of N/P ratios (5-9) were found in areas near the sewage outfall in Saronikos Gulf after 1995, according to recent data (1998-1999). The ratio increases with distance from the outfall (14-18) and remains close to the theoretical value 16 (Redfield ratio), due to the decrease of phosphates, which are domestic sewage indicator. Time series analysis of N/P ratio (1987-1999) showed a significant increasing trend in all areas except the one where the outfall discharges. But even in this area N/P ratio remains stable or it is showing a no statistically significant increase. It can be concluded that in the Saronikos Gulf N/P ratio trend is to reach values close to those found in undisturbed marine waters of E. Mediterranean. Thus, the ecosystem from N limited due to the anthropogenic impact is changing to P limited. At this point it is worth mentioning again that no exceptional phytoplanktonic blooms were detected in the Inner Saronikos Gulf during the last 10 years. Even in the most eutrophic areas, blooms are scarce in contrast to what happened in the 80ties, when red tides were observed quite often (Pagou, 1990).

A more interesting situation has been revealed in Thermaikos Gulf. The ΣN/P ratio values were lower than the Redfield ratio in all the examined areas, being lowest in the Bay and the northern part of the Gulf of Thessaloniki (most eutrophic areas due to sewage) and comparativelly higher in the central area of the Inner Thermaikos Gulf near to the boundaries with the Aegean Sea. In fact in the northern part of the Inner Thermaikos Gulf the N/P ratios are exhibiting extremely lower values, than the theoretical and range between 0.6 (spring) to 4.9 (winter) (1997-1998 data) indicating that a strong nitrate deficit occurs during all seasons, in the area. It is also interesting that during 2000 only once (September) the N/P ratio (mean spatial value) exceeded the theoretical Redfield value.

These extremely low values of N/P can be attributed not only to sewage enrichment but also to additional enrichment through the river freshwater inflow. Orthophosphate input in Thermaikos Gulf from the rivers is at the same range and higher from that by the Rhone river in the Gulf of Lions, confirming thus the statement at the European Environment Agency report (EEA, 1999), that phosphate concentrations have increased dramatically in Greece. Furthermore, when calculating budgets of non conservative materials for Thermaikos Gulf during winter and spring, *ΔDIP* was found to be positive, indicating that there is a net release of DIP probably related to organic matter regeneration processes (Pagou *et al*., 2001).

On the other hand in the Thermaikos Gulf the frequent diatom blooms observed before 1996 changed to even noxious dinoflagellate blooms, that can be related to the very low N/P ratios recorded at the northern part of Inner Thermaikos gulf, the site where usually the exceptional blooms have started. Trend analysis of N/P ratios in Inner Thermaikos Gulf from 1993 to 2000 showed that in Thessaloniki bay where the port is situated, there was not a trend and N/P ratio values were very low with seasonal means that did not exceeded 12. South to the port, in the area where the new sewage outfall is discharging and nearby is an area with many musselcultures, N/P is showing a decreasing trend with maximum seasonal means <17. Finally, in the estuaries area N/P ratio showed an increasing trend until 2000. Variability in all areas was irregular and abrupt. However, during 2000 very low values were recorded again (<5). These trends should be related to changes to river discharges and rainfall among years.

As it was mentioned above, dinoflagellates were the blooming species from 1996 and among them the toxic species *Dinophysis acuminata*, a DSP causative, with substantial socio-economic impact in the area (economic losses of ~3 million Euros every year). The first confirmed "bloom" of *Dinophysis acuminata* was recorded from January to May 2000. This one was the most severe up to mow with cell abundances >50000cells $l^{-1}$ and okadaic acid concentrations up to 1600ng $g^{-1}$ of mussel tissue (8 times higher than limits). The *Dinophysis acuminata* bloom was repeated the two following years (January-April 2001, February-May 2002).

It is interesting to study the ratios Si/P and Si/ΣN during the 3 seasons that *D. acuminata* was blooming. N/P ratios were very low during all 3 periods: mean spatial value <13 for January - May 2000 (and the preceding December 1999), whereas in the sites where the most dense *D. acuminata* populations were recorded was ~3-5. Si/ΣN ranged from 0.01 to 9.77 (May), with mean values per month ranging from 0.90-2.80. Spatial distribution of low values (<1) was the same as for N/P. Si/P<3 was recorded at the same sites except during December 1999 (>4.6). These data indicate Si limitation, in addition to the N limitation (from N/P ratios). N/P ratios were very low again during 2001 (spatial mean <5, except in February = 10.3), Si/N ratios presented values <1 (February 2001), whereas the other months of the bloom minimum values were ~1. During 2002 the same patterns and low values were recorded again. Menesquen (1999) reported the increase of dinoflagellate species blooms including some toxic species (*Dinophysis sp*.) due to constant lowering of Si/N ratios of marine waters in late spring. In Thermaikos the toxic bloom of *Dinophysis* is taking place from late winter to spring, probably due to higher temperatures than in other European Seas. Furthermore, vertical haline stratification in front of the river mouth due to the freshwater inflow during this period favors dinoflagellate production also.

The so far presented data on nutrient ratios indicate that a more detailed study on their time series trend analysis and spatial distributions must be performed, as well as an effort to elucidate their possible relationship with HABs occurrence in Thermaikos.

From the above it is obvious that though both Saronikos and Thermaikos gulf present nutrient concentrations characteristic of high mesotrophic and eutrophic conditions according to the scale produced for this region, negative effects in general were recorded in Thermaikos (like HABs). Therefore, establishing nutrient thresholds (including nutrient ratios) is not enough, but other changes like those in community structure, or occurrence of potential toxic species, trends of eutrophication parameters, or even oxygen deficiency, have to be considered in order to evaluate these tools and assess the concept of “threshold”.

Finally, regarding coastal marine ecosystems of E. Mediterranean, is worth mentioning that in general, the above nutrient values characterized as thresholds (Table I) are within the lower ranges of values found in other European seas (ETC/MCE/EEA, 2000). This confirms the very oligotrophic character of the E. Mediterranean Sea even for gulfs, whose trophic conditions have been changed due to the input of anthropogenic effluents or rivers. Therefore, it becomes clear that in such areas a eutrophication scale based on the E. Mediterranean characteristics should be applied, in order to reveal the results of the trophic conditions variability.

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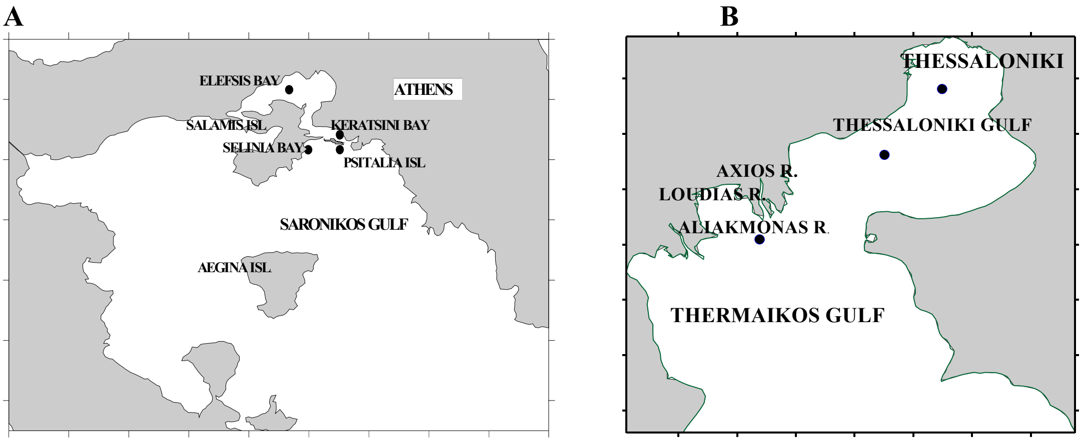
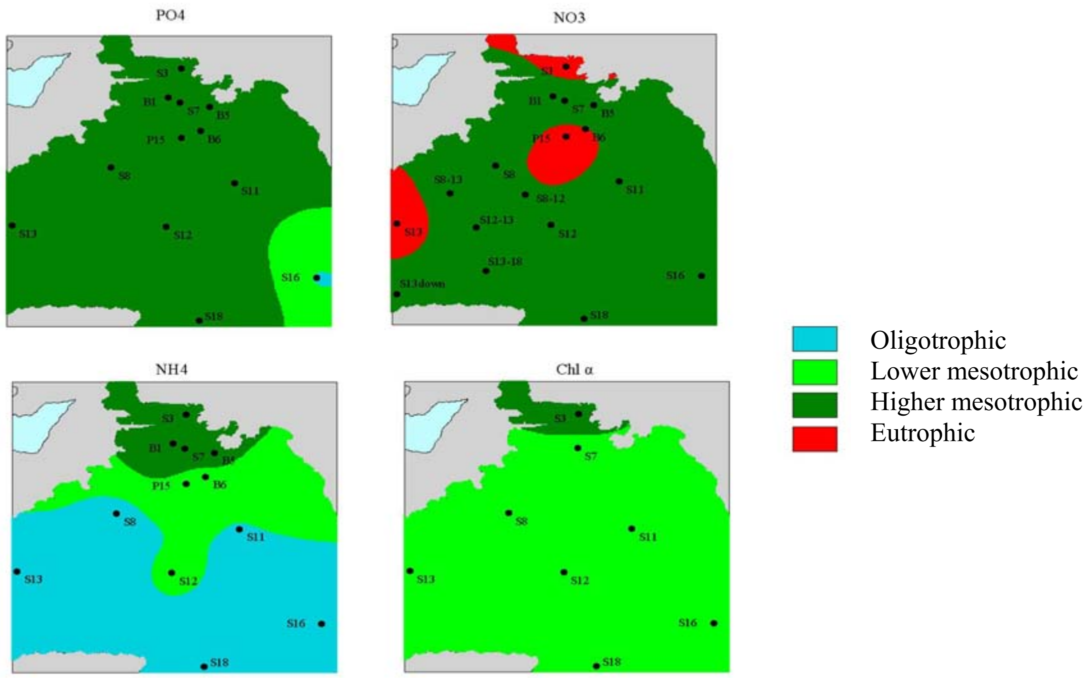



FIG. 1. Maps of case study areas. A: Saronikos Gulf, B: Thermaikos Gulf.




**A.**

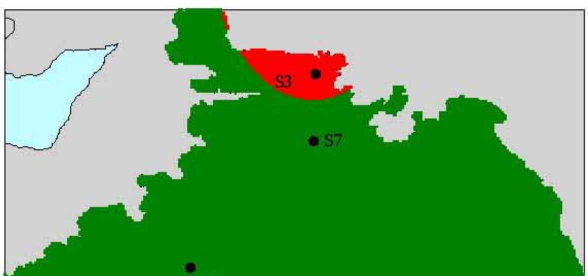


FIG.3. Thematic maps of eutrophication levels in Saronikos Gulf according to the synthesis of all examined parameters. A. Surface during stratification period. B. Water column.