# Technical report coastal waters
# Mediterranean GIG

## PHYTOPLANKTON

Contributors:
**Croatia:** Robert Precali, Živana Ninčević
**France**: Catherine Belin, Rémi Buchet and Valérie Derolez
**Italy**: Franco Giovanardi, Emanuele Ponis, Cristina Mazziotti, Erika Magaletti, Andrea Tornambè, Federico Oteri
**Spain:** Eva Flo, Esther Garcés (Spanish coordinator), Emilio Garcia, Fernando Orozco, Immaculada Romero, Fuensanta Salas and Jordi Camp.
Justification method blooms: + Laura Arín, Albert Reñé, Magda Vila
**Slovenia:** Janja France, Patricija Mozetič
**Cyprus:** Marina Argyrou, Marilena Aplikioti
JRC: Wendy Bonne (conceptual and technical aspects)
Matus Maciak (technical calculation aspects benchmark standardization)

## 1 NATIONAL ASSESSMENT METHODS

### 1.1 THE LIST OF METHODS

| Member State | Method | Included in this IC exercise |
|---|---|---|
| Cyprus | Biomass - Chlorophyll a | Yes |
| Croatia | Biomass - Chlorophyll a | Yes |
| France | Biomass - Chlorophyll a | Yes |
| Greece | Biomass - Chlorophyll a | No (only 1st IC phase) |
| Italy | Biomass - Chlorophyll a | Yes |
| Spain | Biomass - Chlorophyll a | Yes |
| Slovenia | Biomass - Chlorophyll a | Yes |
| Malta | No method | No |

Increasing Chlorophyll-*a* concentrations above those considered natural are related to increasing nutrient enrichment which could be related to anthropogenic disturbances.

### 1.2 REQUIRED BQE PARAMETERS

| Member State | Full BQE method | Taxonomic composition | Abundance [a] | Diversity | Frequency and intensity of algal blooms | Biomass | Combination rule of metrics |
|---|---|---|---|---|---|---|---|
| Cyprus | No | No | No | | No | Chlorophyll a | No combination |
| Croatia | No | Work in progress | Work in progress | | Work in progress | Chlorophyll a | No combination |
| France | Yes | Work in progress | No | | Not presented in IC work | Chlorophyll a | No combination for IC |
| Greece | No | No | No | | No | Chlorophyll a | No combination |
| Italy | Yes | Work in progress | No | | Work in progress | Chlorophyll a | No combination |
| Spain | Yes | No | No | | No | Chlorophyll a | No combination |
| Slovenia | No | Work in progress | Work in progress | | Work in progress | Chlorophyll a | No combination |
| Malta | | | | | | | |

During the phytoplankton WG meeting on 17th and 18th January 2011 a common agreement was accepted regarding the use as biomass parameter (Chlorophyll a concentrations) as the only parameter for the BQE phytoplankton. The detailed explanation is provided below by the Member States.

Based on the Water Framework Directive (WFD) the phytoplankton biomass, taxonomic composition and abundance, together with the frequency and intensity of the bloom are the indicators to be assessed for the ecological quality element (BQE) phytoplankton.

In the first intercalibration phase, the Med-GiG considered only chlorophyll-*a* (Chl-*a*) as indicator of phytoplankton biomass (Technical Report. European Comission, 2009). However, for the second intercalibration phase, Member States were encouraged to incorporate other metrics in order to improve the existing methodology.

**FRANCE**

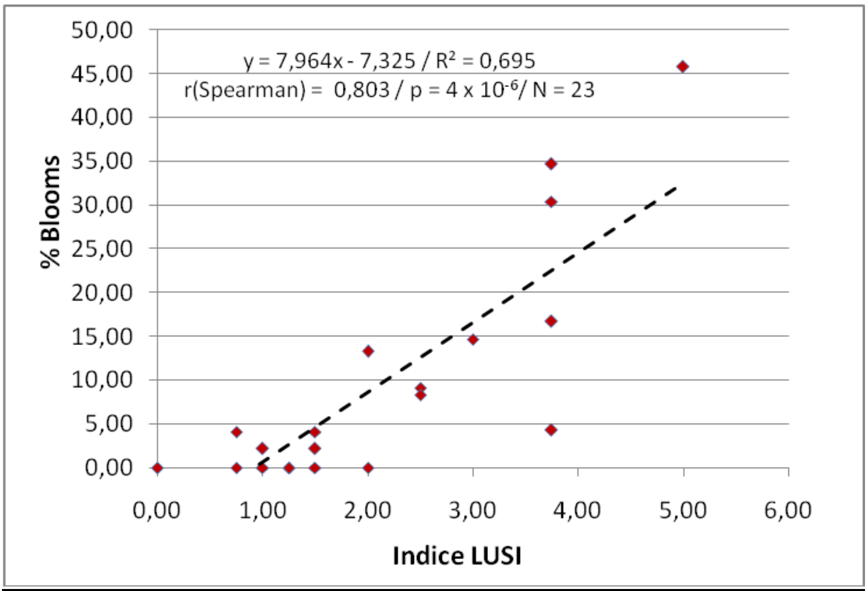
Abundance (frequency and intensity of algal blooms) is used as a complement of biomass index for the assessment of French Mediterranean coastal water bodies, but is not presented as a metric to intercalibrate in this exercise as agreed between MED GIG MS.
The extra metric used is the percentage (considering a 6 year period) of samples for which at least one taxa is:

- 100 000 cells/L (large sp.) or > 250 000 cells/L (small sp.) for all WB **except Corsica (type "Mediterranean island")**
- 25 000 cells/L for **Corsican type CW**

The reference values and class boundaries used for the assessment of Mediterranean CW bodies are the following:

| Reference value = 16.7 % (=2 blooms per year) | High | Good | Moderate | Poor | Bad |
|---|---|---|---|---|---|
| | | | | | |
| % blooms | < 20% | > 20 % | > 40 % | > 70 % | > 90 % |
| EQR | > 0.83 | > 0.42 | > 0.24 | > 0.19 | < 0.19 |

This metric shows a correlation with the Land Use Simplified Index (eutrophication) developed by Flo. *et al*. (2011) :




Also, there is still a work in progress on a phytoplankton composition index:

- Goffart, A., 2010. Mise au point de l'indice composition dans le cadre de l'indicateur phytoplancton. Les indices de composition phytoplanctonique en eaux côtières. Synthèse bibliographique. Convention Onema-Ifremer. 36pp.
- Soudant, D. & Belin, C., 2011. Note sur l'approche statistique de la diversité en écologie. Application à l'indice composition pour le phytoplancton.

- Goffart, A., 2011. Mise au point de l'indice composition dans le cadre de l'indicateur phytoplancton. Traitement des données pigmentaires des eaux côtières corses pour le développement d'un indice de composition phytoplanctonique. Convention Onema-Ifremer. 20pp.

**CROATIA and SLOVENIA**
**Justification on the use of Chl *a* concentration as the only parameter to assess the ecological quality of coastal waters**

During the 1st IC phase, only one phytoplankton parameter - biomass as chlorophyll a (Chl *a*) concentration - was calibrated between MS, with the commitment to evaluate the whole set of phytoplankton parameters (community composition and abundance, frequency and intensity of blooms) stated by the Water framework directive (WFD) in the 2nd phase. The idea was to improve the assessment of the response of phytoplankton to eutrophication in order to detect also alterations in the phytoplankton community not directly connected with the raise of phytoplankton biomass (e.g. shift in the community composition, blooms of small species).

During the first part of the 2nd IC phase, MS Croatia, Italy and Slovenia tested several methods to incorporate different phytoplankton parameters in the ecological status assessment. Some of these studies are explained below. However, during the phytoplankton group meeting at ISPRA, Rome, in January 2011, the participants agreed on the use of Chl *a* as the only parameter considered during the IC exercise.

*Examples of studies*

Testing and adapting two phytoplankton indices in the Slovenian coastal waters:
We focused on two phytoplankton indices developed by Devlin et al. (2007) for UK and Irish coastal waters, which are Index of seasonal succession of functional groups ($I_{SS}$) and Index of elevated phytoplankton counts ($I_E$). The first index assesses the shift of the seasonal succession of phytoplankton functional groups from the reference conditions and the second index represents the frequency of elevated phytoplankton counts.

Phytoplankton data for the testing were obtained during Slovenian national monitoring program at five coastal stations. The period in which all five stations were sampled was 2007-2008 with monthly sampling frequency. Reference conditions were tentatively determined on a data set of a site in the Gulf of Trieste with only slight anthropogenic influence.

The index of seasonal succession ($I_{SS}$) needed an initial construction of reference standardized seasonal occurrence curves for the four functional phytoplankton groups. Seasonality of these groups was very similar among stations (Table 1) and offered no possibility to distinguish between ecological statuses of the stations.

**Table 1.** Values of $I_{SS}$ (in %) for phytoplankton and its functional groups for the five Slovenian stations in the period 2007/08.

| group_station | 000F | 00MA | 00C4 | 0DB2 | 000K |
|---|---|---|---|---|---|
| nanoflagellates | 95.83 | 91.67 | 87.50 | 91.67 | 95.83 |
| diatoms | 95.83 | 91.67 | 87.50 | 91.67 | 95.83 |
| dinoflagellates | 100.00 | 87.50 | 95.83 | 91.67 | 95.83 |
| cocolithophorids | 95.83 | 87.50 | 83.33 | 83.33 | 83.33 |
| total | 96.88 | 89.58 | 88.54 | 89.58 | 92.71 |

Originally, the index of elevated phytoplankton counts is composed of 4 sub-metrics of which we used only three: 1. frequency of elevated Chl *a* records ($I_{Chl}$), 2. frequency of high phytoplankton counts of the whole community ($I_T$) and 3. frequency of high counts of any single taxa ($I_S$). The last attribute used by Devlin et al. (2007) concerns the number of blooms of *Phaeocystis*. We did not find any significant indicator species to substitute *Pheocystis* (which does not form blooms in the Northern Adriatic), so we left only 3 submetrics in the index.

$$I_E = \frac{I_T + I_S + I_{Chl}}{3} \times 100$$

Each sub-metric is calculated from the number of times that it exceeds the threshold as a proportion of the total number of sampling times. For the Chl *a* sub-metrics threshold was defined as the boundary between H/G for the phytoplankton biomass parameter. Thresholds for the total phytoplankton counts and for single taxa counts were defined after examining frequency distributions of 20 year data set of the reference station. For both, 90th percentile of all abundances was chosen as the threshold. To be able to differentiate between different ecological quality classes, we evaluated also the phytoplankton data of 2 stations off the Croatian Istrian coast (transect Rovinj-Po).

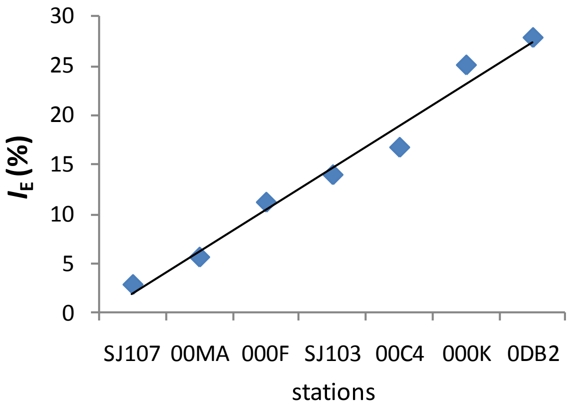



**Figure 1.** The distribution of index $I_E$ values along stations with different trophic status.

There is a clear gradient of index values among the 7 stations from the lowest values at the most oligotrophic station in the middle of the northern Adriatic to the highest values at the coastal stations in the inner part of Gulf of Trieste (Fig. 1). Similar gradient was observed with Chl *a* values and values of the TRIX index used as a pressure proxy. We found a very good agreement between TRIX index and index $I_E$ for all 7 stations.

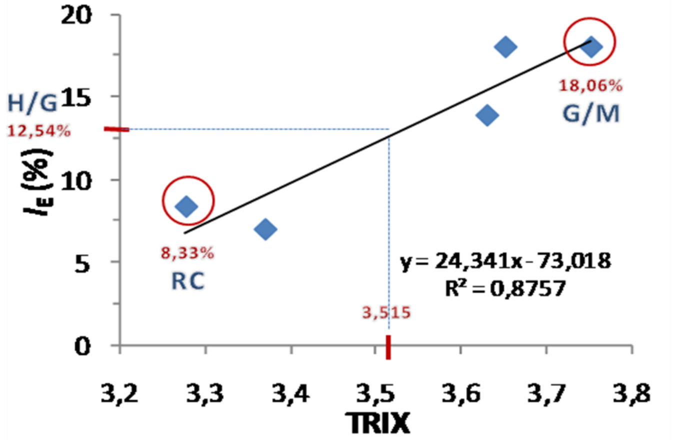



**Figure 2.** Relationship between TRIX and $I_E$ index values and tentative definition of H/G and G/M boundaries.

For Slovenian coastal stations only (all belonging to one water type), values of $I_E$ distributed along a gradient of increasing values TRIX index from the station 000F with only minor influence from the coast, characterised by the highest transparency and lowest nutrient concentrations, to the most anthropogenically influenced station 000K with the lowest transparency and highest nutrient concentrations. Establishment of tentative H/G boundary was therefore based on the relationship between TRIX and $I_E$ index (Fig. 2). Values for other class boundaries were defined by calculating EQRs for the remaining boundaries applying the equal distance between G/M, M/P and P/B EQRs. The corresponding values of $I_E$ were calculated from the equation of the regression curve (Table 2).

**Table 2.** Tentative boundary values of $I_E$ and related EQRs for Slovenian WB.

| EQR | class | $I_E$ (%) |
|---|---|---|
| 1 | RC | 8.33 |
| 0.66 | High(H) | 12.54 |
| 0.46 | Good (G) | 18.06 |
| 0.31 | Moderate (M) | 26.92 |
| 0.15 | Poor (P) | 53.69 |

| | | |
|---|---|---|
| < 0.15 | Bad (B) | > 53.69 |

The index $I_{SS}$ was not considered as a suitable classification tool, mainly because seasonal succession can be hardly assessed solely on monthly basis due to fast generation times of phytoplankton (differences from year to year are substantial but natural). On the contrary, index $I_E$ seemed to be a quite robust index that reflected different trophic conditions: it indicates the intensity and the frequency of blooms and moreover, does not overlook the importance of low biomass blooms of nanoflagellates or conversely, low abundance blooms of dinoflagellates with elevated Chl *a* concentrations. Nevertheless, there are still many gaps in knowledge regarding the use of index of elevated counts as a tool to classify the ecological status of phytoplankton in relation to the eutrophication pressure. For example, defining a threshold value for bloom is a challenging task, since blooms can be governed by different forces, including physical processes not related to human pressures. A major problem relates also to biomass and structural changes observed in the last decades in the phytoplankton community (France, 2009), which makes the establishment of the reference conditions a difficult task.

Testing the differences between the species composition and abundance of Slovenian water bodies

Species composition and abundance was also studied in the Slovenian coastal waters. Slovenian water bodies resulted very similar in term of these parameters when examined by Principal Coordinates Analysis (PCoA). The factor that has the major influence to the discrimination of samples was the season. Similarly, we did not find any opportunistic phytoplankton species or substantial differences in the phytoplankton community structure where examining sampling stations located along a transect of growing distance from the river mouth (France, 2009).

*HABs as assessment tool:*

The blooms of harmful algae in the coastal waters of Slovenia and Croatia are mainly manifested as low biomass DSP events (France and Mozetič, 2006) and do not seem to be in relation to the eutrophication pressure. To the best of our knowledge, HAB events can not be related to the ecological status of the coastal waters.

*Conclusions*

As decided by all the members of the phytoplankton group during the meeting in January 2011 in Rome, Chl *a* metric will be the only one used for the assessment of phytoplankton ecological status. It is important to state that we agree with affirmations about advantages of using Chl *a* concentrations stated by Spain and France group, and we will not repeat those statements. An exception is done with Type III waters (see below), for which Chl *a* is not a suitable assessment tool (see explanation below).

We also state here that we absolutely agree on the need of future/continuous studies on phytoplankton community. Better knowledge of species composition, abundance and distribution would allow us to properly address the true state parameters of phytoplankton, not only the estimate of biomass.

**SPAIN**

During the intercalibration exercises, Spain has been working with the phytoplankton community at different levels: harmful species and functional or taxonomic groups, as well as bloom frequency. However, we believe that those metrics, in absolute terms, are not synonymous of environmental quality, and do not add more information than Chl-*a*. Those indices depend on many factors, and their use cannot be considered for management purposes.

This document shows the results obtained in relation to phytoplankton community (those which are more comparable with the rest of MS) in order to justify that these new metrics do not provide any relevant information.

**Phytoplankton community**

The stations used for this study are grouped into: Undisturbed stations (US - sites with minor or low levels of disturbance from human activities), risk stations (sites where the risk of Harmful Algal Blooms - HAB - is high) and reference stations (sites with no, or very minor, disturbance from human activities). Those stations were sampled for the assessment of the ecological status by the different calculated metrics (Chl-*a*, HAB index, diatom:dinoflagellates ratio and bloom frequency). All the stations were located very near the coastal line, where the water column is around 1 m depth (inshore stations).

*Harmful species index*

Harmful or noxious phytoplankton species were recorded in 6 US and 11 risk stations during two years (2005-2006) with a monthly frequency (see Reñé *et al.* (2007)). Phytoplankton species were grouped in 6 categories:

- Tox1: *Dinophysis* spp., *Protoceratium reticulatum, Lingulodinium polyedrum*
- Tox2: *Alexandrium minutum, A. catenella, Prorocentrum rhathymum, P. minimum, Akashiwo sanguinea*
- Epiphytes: *Coolia monotis, Ostreopsis* spp., *Prorocentrum lima*
- Dino: bloom-forming dinoflagellates
- Diat: bloom-forming diatoms
- Nano: bloom-forming nanoflagellates

A HAB has been defined when cell concentration (cells/L) exceeds a certain threshold (different for each category) giving a value of 1 or 2 depending on its intensity, as is show in Table 1. The sum of HAB values can range between 0 and 12.

Table 1. Threshold (cells/L) for the 6 phytoplankton categories (see text) and the corresponding HAB value (1 or 2, depending on the HAB intensity).

| HAB value | Tox1 | Tox2 | Epiphytes | Dino. | Diat. | Nano. |
|---|---|---|---|---|---|---|
| 1 | >200 | >1000 | >5000 | $>10^5$ | $>10^5$ | $>10^6$ |
| 2 | >1000 | $>10^4$ | $>10^4$ | $>10^6$ | $>10^6$ | $>10^7$ |

Finally, a HAB index was calculated as the percentage of toxic or harmful blooms according to the following formula:

$$\text{HAB index} = (\text{Sum of HAB values} / (\text{N}^{\text{o}} \text{ samples} * F)) * 100$$

where F = “12”, which is the maximum sum of the HAB values that can be obtained for each sample.

The Table 2 shows the HAB index results together with the quality of the WB obtained by the Chl-*a* methodology.

Table 2 - Comparison between quality of the WB by the Chl-*a* methodology (by means Chl-*a* values) and the HAB index.

| **Station** | **Type** | **Chl-*a*** | **Chl-*a* quality** | **HAB index** |
|---|---|---|---|---|
| L'Eucaliptus (EUC) | I | 2,65 | High | 2,78 |
| Desemb. Muga (MUG) | I | 10,07 | Moderate | 8,33 |
| Parc del Litoral (LIT) | II | 0,90 | High | 1,39 |
| L'Alguer (ALG) | II | 0,96 | High | 0,69 |
| L'Estartit 3 (EST) | II | 2,64 | Good | 2,78 |
| Parc de Garbí (GAR) | II | 3,67 | Moderate | 3,82 |
| Ses Illetes (ILL) | III | 0,54 | High | 1,39 |
| Canyet (CAT) | III | 0,56 | High | 0,69 |
| Canyelles (CAP) | III | 0,65 | High | 0,35 |
| La Fosca (FOS) | III | 0,86 | High | 1,74 |
| Cavaió d’Arenys (CAV) | III | 1,27 | High | 1,39 |
| Tossa (TOS) | III | 1,41 | Good | 0,00 |
| L'Arenal (ARE) | III | 1,44 | Good | 0,00 |
| Llevant (LLE) | III | 1,59 | Good | 1,39 |
| Sitges (SIT) | III | 1,62 | Good | 2,08 |
| Llavaneres (LLAV) | III | 2,48 | Bad | 4,17 |
| Castelldefels (CSF) | III | 3,08 | Bad | 1,74 |

At low salinity waters (Type I and II) lower Chl-*a* concentrations were related with lower HAB index. This was not seen in Type III waters, where in several cases, the water quality by the Chl-*a* methodology was good compared to a bad evaluation for harmful phytoplankton (high HAB index) (Fig. 1). We concluded that considering only a part of the phytoplankton community (the toxic or harmful species) is not a good option to evaluate the water quality because it has no direct relation with eutrophication. Similar conclusions were obtained by Revilla *et al.* (2009) who removed the cell counts of harmful species from the calculation of the quality index, as it did not provide any relevant information in their study area (Basque coast – NEA region). Also, it is in agreement with Collos *et al.* (2009), who conclude that "HABs are not related to eutrophication of the Mediterranean zone" (in CIESM, 2010).

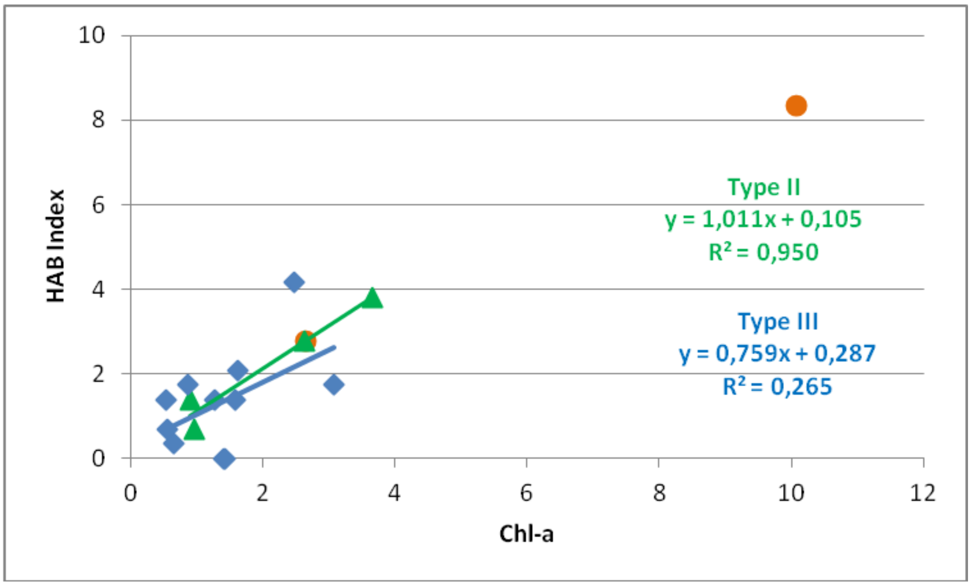



Figure 1 – HAB index and Chl-*a* relationship for the 17 stations listed in Table 2 (two from Type I, 4 from Type II and 11 from Type III).

*Diatom:dinoflagellate ratio*

Diatoms and dinoflagellates were counted from two years (2007-2008) samples (with a monthly frequency) from 2 reference stations, 6 US stations and 6 risk stations (Table 3).

Table 3 – Sampling stations (2 reference, 6 US and 6 risk stations) with their corresponding typology (type I, II or III).

| Codi | Water Body (WB) | Station | Type | |
|---|---|---|---|---|
| C05 | Cap Norfeu | Montjoi | III | Ref |
| C06 | Canyelles | Canyelles Petites | III | US |
| C07 | Roses-Castelló d'Empúries | La Muga | II | Risk |
| C11 | Torroella de Montgrí-El Ter | L'Estartit (3) | II | Risk |
| C14 | Begur-Blanes | La Fosca | III | Risk |
| | | Canyet | III | US |
| | | Ses Illetes | III | US |
| C16 | Pineda de Mar-Mataró | Cavaió | III | US |
| | | Apartaments Blaumar | III | Risk |
| C22 | El Prat de Llobregat-Castelldefels | Castelldefels | III | Risk |
| C26 | Tarragona Nord | Llarga | III | Ref |
| C31 | Vandellós i L'Hospitalet de l'Infant | L'Arenal (Vandellós) | III | US |
| C32 | L'Ametlla de Mar | L'Alguer | III | US |
| C34 | Delta Sud | Desembocadura de la platjola | I | Risk |

The aim was to test if the diatoms:dinoflagellates ratio allowed us to differentiate US and risk stations during the annual cycle. We expected to find a lower diatom:dinoflagellate ratio in the risk (problematic) than in the US (non-problematic) stations. As it has been proposed, long term changes in the diatom:dinoflagellate ratio can be linked to changes in eutrophication (Marasović *et al.* 2005).

The results showed that while there is a clear difference applying the Chl-*a* methodology between non-problematic (US) and problematic (risk) stations (Fig. 2), the diatoms:dinoflagellates ratio did not show differences between both types of stations (Fig. 3). Although on summer it was observed a slight predominance of dinoflagellates on diatoms, basically, what the data indicate is the seasonal pattern of phytoplankton which is usually observed in the NW Mediterranean (Margalef and Catellví 1967, Vila and Masó 2005).

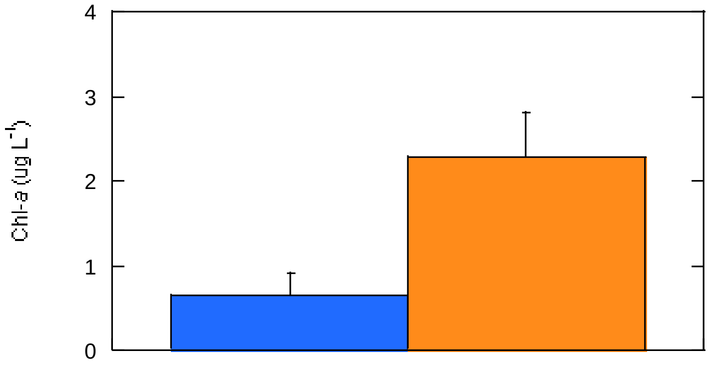



Figure 2 - Mean Chl-*a* concentration at the US (blue) and risk (orange) stations.

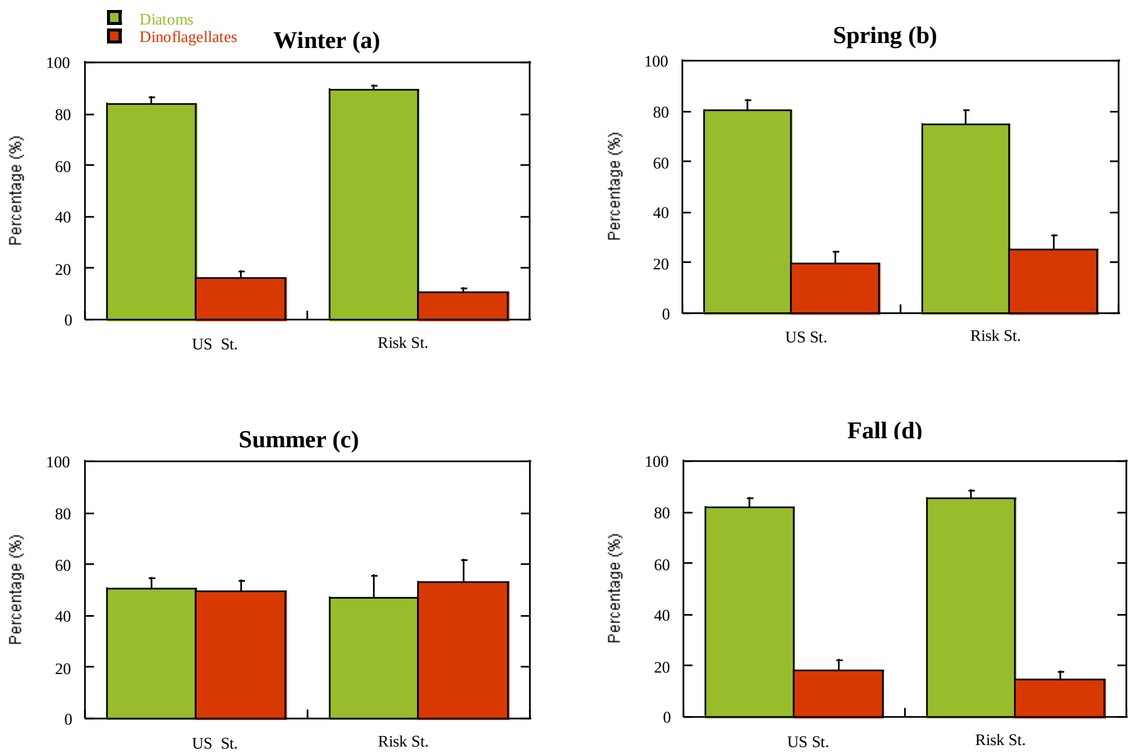



Figure 3 - Diatoms and dinoflagellates percentage (%) at the US and risk stations for the 4 seasons: a) winter, b) spring c) summer and d) fall.

*Bloom frequency*

Data of bloom frequency was obtained from five years (2005-2009) in 14 stations sampled with a monthly frequency. Stations samples were: 2 reference, 6 US and 6 risk stations.

The thresholds for bloom determination were defined as:

- $>10^5$ cells/L – for any diatom and dinoflagellate taxa
- $>10^6$ cells/L – for any coccolithophorid taxa
- $>10^6$ cells/L – for nanoflagellates

Then, the percentage of bloom in relation to the total number of samples was calculated.
The quality categories due to bloom percentage were assigned according to the type specific thresholds (Table 4).

Table 4 – Thresholds for the different quality categories (H/G, G/M, M/P, P/B) and reference condition (Ref) due to bloom percentage.

| Categories | Bloom percentage |
|---|---|
| Ref | 5 |
| H/G | 6 |
| G/M | 12 |
| M/P | 24 |
| P/B | 48 |

Table 5 shows the water quality of the WB obtained by the Chl-*a* and the Bloom frequency methodologies.

Table 5 – Water quality of the WB obtained by the Chl-*a* and the Bloom frequency methodologies at the 11 studied WB.

| Code | Water Body (WB) | Station | Type | | Chl-*a* Quality | Quality-Bloom Frequency |
|---|---|---|---|---|---|---|
| C05 | Cap Norfeu | Montjoi | III | Ref | High | High |
| C06 | Canyelles | Canyelles Petites | III | US | High | High |
| C07 | Roses-Castelló d'Empúries | La Muga | II | Risk | Bad | Poor |
| C11 | Torroella de Montgrí-El Ter | L'Estartit (3) | II | Risk | High | Poor |
| C14 | Begur-Blanes | La Fosca | III | Risk | High | Good |
| | | Canyet | III | US | | |
| | | Ses Illetes | III | US | | |
| C16 | Pineda de Mar-Mataró | Cavaió | III | US | Good | Good |
| | | Apartaments Blaumar | III | Risk | | |
| C22 | El Prat de Llobregat-Castelldefels | Castelldefels | III | Risk | Bad | Good |
| C26 | Tarragona Nord | Llarga | III | Ref | High | High |
| C31 | Vandellós i L'Hospitalet de l'Infant | L'Arenal (Vandellós) | III | US | Good | High |
| C32 | L'Ametlla de Mar | L'Alguer | III | US | High | High |
| C34 | Delta Sud | Desembocadura de la platjola | I | Risk | Good | Moderate |

In most cases, the assessment of WB is similar with both methodologies (high or good, poor or bad). Only in three cases (C11, C22 and C34) the WB quality is different. However, the relationship between the two metrics is good ($r^2 = 0.63$) as it is show in the Fig. 4.

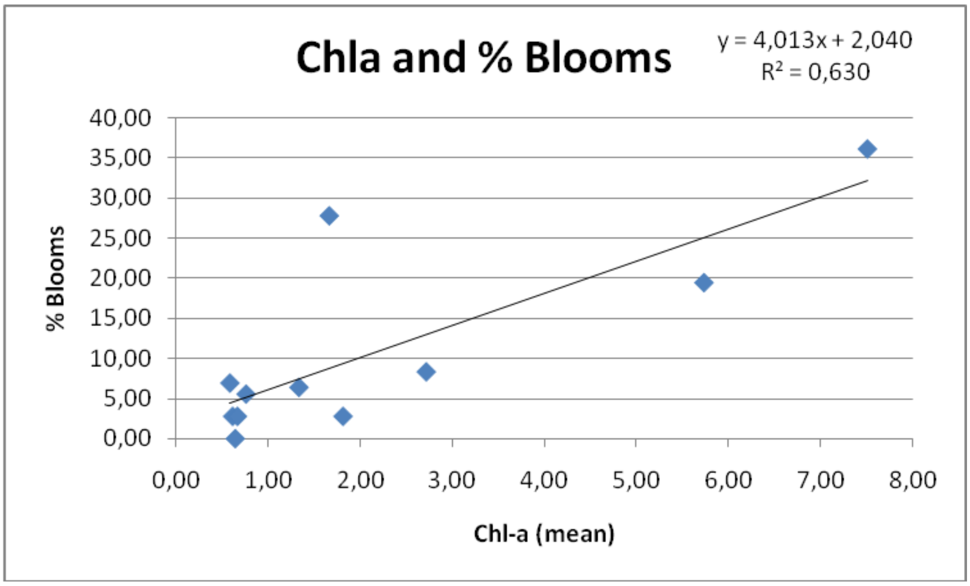



Fig. 4 – Relationship between Chl-*a* and Bloom frequency.

**Validation of Chl-*a* and Bloom frequency methods against pressure**

Figure 5 shows the relationship between LUSI (**Land Uses Simplified Index -** See Annex II ) and inshore Chl-*a* for the 36 WB (34 coastal + 2 transitional waters) existing in Catalonia for a three years data set (2007-2009). However, the bloom frequency has been only analysed at 11 WB. At those WB, a significant relationship was observed between LUSI and Chl-*a* (Fig. 6a) and between LUSI and Bloom frequency (Fig. 6b).

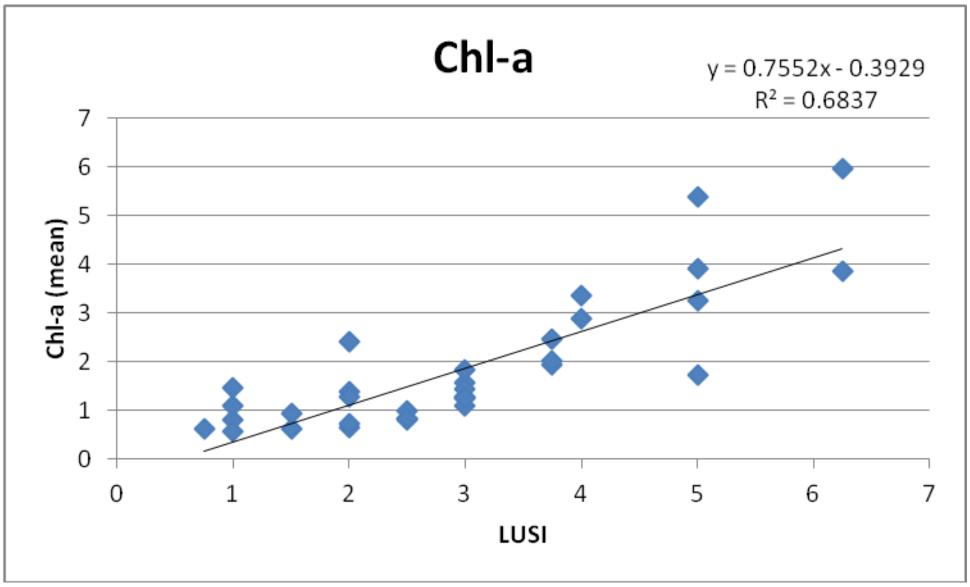
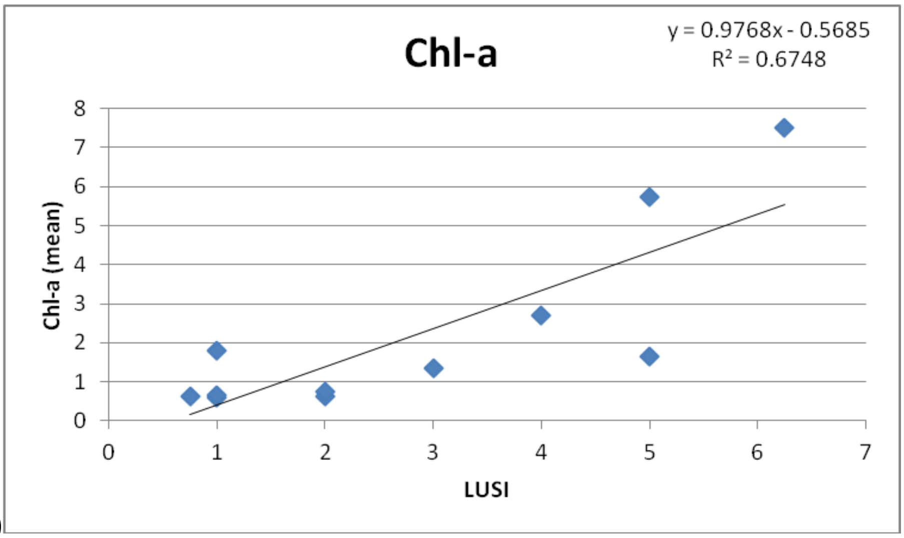
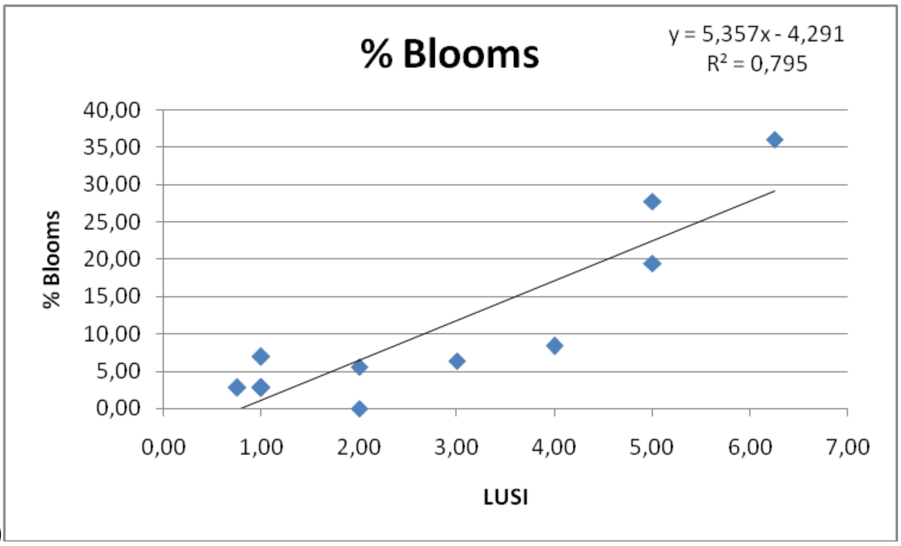



Figure 5 – Relationship between inshore Chl-*a* and LUSI for the 36 Catalan WB.




**(a)**




**(b)**

Fig. 6 – (a) Relationship between Chl-*a* and LUSI and (b) Relationship between Bloom frequency and LUSI for a selected 11 WB.

In summary, bloom frequency presented a very similar relationship as the Chl-*a* with LUSI. Thus, bloom frequency did not provide more information in the study area.

**Why to use Chlorophyll-*a* methodology instead the others methodologies?**

Since few years ago, at the Mediterranean Spanish region we have been exploring the possibility of using the phytoplankton marine community as an indicator of the quality of marine coastal waters. Despite the effort of sampling, counting and subsequent interpretation of data, until now we have not been able to find any indicator that improves the information provided by chlorophyll in relation to eutrophication.

There is general agreement about the link between mean value of nutrients and mean values of chlorophyll in the coastal waters, to the extent that satellite chlorophyll maps are usually used as maps of marine eutrophication (Technical Report. European Environment Agency, 2002). This is not the case of other descriptors proposed for the BQE phytoplankton (phytoplankton composition and abundance and phytoplankton bloom frequency and intensity). As concluded in the “ICES sponsored meeting” held in Tisvildeleje, Denmark (September 2006) from large phytoplankton temporal series (ranging from about one to four decades), no clear signals are detectable in phytoplankton composition and dynamics in coastal waters disturbed or not disturbed by human activities. In addition, they argued, that “reference conditions are difficult (impossible?) to establish”.

Many studies used Chl-*a* as indicator of eutrophy or water quality (Harding 1994 in Harding and Perry 1997, Boyer *et al.* 2009) due to its very simple and integrative analysis. For example in Chesapeake

Bay, Chl-*a* was used as biomass indicator of N and P enrichment (Harding 1994 in Harding and Perry 1997).

In addition, as pointed out Boyer *et al.* (2009), "the Chl-*a* indicator has three specific components, bloom magnitude (incidence of Chl-*a* concentrations that exceed the baseline value per zone per month), bloom frequency (number of month per year when Chl-*a* concentrations in each zone exceed the specific threshold value for that zone), and
bloom spatial extent (area-weighted Chl-*a* concentrations within a region per month exceeding the threshold concentration for the region)".

Other advantages of using Chl-*a* as a metric:

1) Chl-*a* integers all the phytoplankton community.

"Phytoplankton biomass is a direct measurement of phytoplankton abundances. Chlorophyll concentration represents a very simple and integrative measure of phytoplankton community response to nutrient enrichment" (in: Devlin *et al.* 2007)

2) Chl-*a* is an indicator of eutrophication, which means that could be managed.

"There is generally a good agreement between planktonic primary production and algal biomass, and algal biomass is an excellent trophic state indicator. Furthermore, algal biomass is associated with the visible symptoms of eutrophication, and it is usually the cause of the practical problems resulting from eutrophication" (in: Boyer *et al.* 2009).

*3)* 3) Chl-*a* is easily to sample and analyze.

"Chl-*a* is relatively easy to measure compared to algal biomass" (in: Boyer *et al.* 2009). It is not necessary a specialization for Chl-*a* analysis. It is easily comparable between different laboratories. It is cheap and affordable for a great number of samples (even replicates, if necessary).

Weaknesses of frequency of blooms and community composition respect to chlorophyll-a:

1) There is a lack of knowledge about the mechanisms of pressure-impact in the BQE "phytoplankton bloom frequency and phytoplankton community composition indices". Thus, nowadays it could not be managed.

2) Phytoplankton community has a rapid response to changes in environmental conditions; however it does not integrate in time. Thus, the link between certain environmental conditions and some phytoplankton community structures can only be interpreted statistically by a sufficient number of samples in a suitable space-time framework. This requires large numbers of samples that greatly increase the monitoring effort. In the test, we did analyzed between 14 and 20 stations (depending on the indicator tested). To do this kind of analysis for the 76 samples taken for Chl-*a* is not affordable for us.

3) It is required a high specialization of scientists. The effort invested per sample of phytoplankton increases significantly respect to the effort invested per samples of Chl-*a*. Moreover, the level of phytoplankton identification between different scientists is different; some labs have more experience with diatoms, others with dinoflagellates, etc.

In the Med-GIG meeting held in Rome in 2011, Mediterranean member states agreed that:

- Chl-*a* index will be an effective and relevant BQE for coastal ecosystems and it is universally accepted
- nowadays, we do not know the mechanism about the link between eutrophication and phytoplankton bloom frequency and phytoplankton community composition indices
- phytoplankton bloom frequency and community composition indices do not add more information for management than the Chl-*a* as BQE, at least, in the Mediterranean Sea. At management level it is impractical.

We concluded that the phytoplankton community should be explored as a potential indicator of water quality in a middle future, but at the level of knowledge we currently have, we discourage their use for management.

## 1.3 SAMPLING AND DATA PROCESSING

Any details that could have been provided in the WISER project assessment method questionnaires are not copied here yet.

| Information provided in the online WISER project assessment method questionnaires | |
|---|---|
| - Question B.08: How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? | |
| - Question B.09: Sampling/survey months | |
| - Question B.10: Which method is used to select the sampling /survey site or area? | |
| - Question B.11: How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? | |
| - Question B.12: Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based | |
| - Question B.13-B.14-B.15: Short description of field sampling/survey procedure and processing (sub-sampling) | |

France:

Sampling by Niskin or Hydrobios bottle, in sub-surface (0-1m), monthly.
Chlorophyll-a analysis is done by spectrophotometry (monochromatic – Lorenzen) or fluorimetry (Holm-Hansen), both methods described in Aminot & Kerouel (2004)[1].

Spain (by regions):

Catalonia: Analytical methodology followed Yentsh, C.S., Menzel, D.W., 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep Sea research 10, 221-23.
Valencia: The Chlorophyll-*a* content was determined using trichromatic method (APHA, 1998) based on visible spectroscopy and using Jeffrey and Humprey's (1975) equations to obtain the concentration.
Murcia: Chlorophyll-*a* was analysed with the spectrophotometric methods reported by Parson et al (1984) Parsons, T. R., Y. Maita & C. M. Lalli, 1984. A Manual of Chemical and Biological Methods for Sea-Water Analysis. Pergamon Press, Oxford: 173 pp.
Andalucia: Chlorophyll-*a* was analysed with the spectrophotometric methods reported. Standard Methods. Methods for Examination of Water and Wastewater, ed. 17.
Balearic Islands: Method EPA 445.0 "In vitro determination of Chlorophyll-*a* and Pheophytin a in marine and freshwater algae by fluorescence" revision 1.2, 1997.

Niskin bottle were usually used for sampling within Spanish waters. Spanish sampling is done within inshore (within Valencia and Catalonia regions) and nearshore (all regions except Valencia) waters in surface (Inshore <500 m; Nearshore = from inshore limit to the limit of the WB, in Spain our nearshore is approximately at 1000 m). When inshore data are used and required in the common data set, inshore data are converted to nearshore data according to the relationship demonstrated in the document 1st IC MED-GIG Technical Report, Section 3 Annex I Spain. Therefore, Spanish data are nearshore in order to compare the data with France. The frequency sampling was monthly or 4 times a year depending on the region, distance to the shore and previous knowledge of the water bodies.

[1] Aminot, A., Kerouel, R., 2004. Hydrologie des écosystemes marins. Paramètres et analyses. Editions IFREMER, Plouzané (France), 336 p.

## 1.4 NATIONAL REFERENCE CONDITIONS SETTING

| Member State | Type and period of reference conditions | Number of reference sites | Location of reference sites | Reference criteria used for selection |
|---|---|---|---|---|
| Cyprus | Existing near-natural reference sites (pristine areas-Natura 2000 sites), expert knowledge, historical data covering a 4-year-period | 2 sites for Type III-E | Type III-E: South-East Cyprus: WB name: Cape Greco (Code CY_25-C3-S1, and South-West Cyprus, WB name Akamas (Code CY_5-C1) | Pressure: LUSI ≤ 2<br>Type III-E 90th percentile Chl *a* (µg/L) <0.1<br>(Remark: Levantine is naturally nutrient-deficient and highly oligotrophic , In general Chl *a* concentrations rarely exceed 0.1 µg/L) |
| Greece | | | | |
| Croatia<br>Italy<br>Slovenia | Period: 2000-2010<br>Sites: Among the same sites already used for defining typologies (Tyrrhenian and Adriatic sites) | All data used for defining one common reference condition. | Threshold values used, defined from common database | Pressure: dilution factor as the primary indicator of pressure from land. |
| France<br>Spain | Existing undisturbed sites or sites with only very minor disturbance.<br><br>Please check Annex II-France and Spain working document for the type and period of reference conditions | Island-W: 39 WB selected by pressure (LUSI) criteria and 4 by expert judgement.<br><br>Type II-A: 8 WB selected by pressure (LUSI) criteria and 2 by expert judgement.<br><br>Type III-W: 26 WB selected by pressure (LUSI) criteria and 9 by expert judgement. | Please check Annex II-France and Spain working document for the locations of reference sites. | Pressures (LUSI) and expert judgement were taken into account when selecting reference sites:<br>1) a water body was chosen as reference if it was an undisturbed site or a site with only very minor disturbance, which are associated with LUSI values that not exceed 2 for Type II-W and Type Islands and with LUSI values that not exceed 3 for Type II-A, as this typology is naturally affected by freshwater inputs.<br>2) a water body was chosen as reference by expert judgement based on IMPRESS documents, high ecological status of others BQEs, high physicochemical status, no risk of breach the WFD environmental objectives, anthropogenic pressures, territory and population analysis, protected natural areas, historical data, etc.<br>More detailed information could be found in Annex II-France and Spain working document. |

The coordinates of the reference are provided below:

**France and Spain**
See the section on reference conditions setting.

**Croatia, Italy and Slovenia**
No sites have been identified.

**Cyprus**

| Type | Station Code | Name | Lon_WGS84 | Lat_WGS84 |
|---|---|---|---|---|
| III-E | CY_5-C1_S1/LR2 | Akamas/Lara-20m | 32.303133° | 34.963517° |
| III-E | CY_5-C1_S1/LR3 | Akamas/Lara-30m | 32.300083° | 34.966267° |
| III-E | CY_5-C1_S1/LT3 | Akamas/Latsi-30m | 32.402167° | 35.061500° |
| III-E | CY_5-C1_S1/LT4 | Akamas/Latsi-40m | 32.408000° | 35.066000° |

| | | | | |
|---|---|---|---|---|
| III-E | CY_5-C1_S1/B2 | Akamas | 32.294950° | 35.018700° |
| III-E | CY_25-C3_S1/B2 | Cape Greco | 34.083933° | 34.970267° |

## 1.5 NATIONAL BOUNDARY SETTING

| Member State | Type of boundary setting: Expert judgment – statistical – ecological discontinuity – or mixed for different boundaries? | Specific approach for H/G boundary | Specific approach for G/M boundary | Boundary setting procedure: method tested against pressure |
|---|---|---|---|---|
| France and Spain | France and Spain followed the guidance document Nº14 on the intercalibration process (ANNEX IV: The development of a boundary setting protocol for the purposes of the inter-calibration exercise).<br>Briefly, there were not discontinuities in the relationship between the metric and the gradient of impact represented by the data set (Step 4). France and Spain were not able to use paired metrics to assess class centres or class boundaries (Step 6). Afterwards (Step 8), France and Spain divided the continuum of impact below the high-good boundary into four equal width classes but the values of the metric of the quality element represented at the good and moderate status class boundaries did not agree with the normative definitions. Finally, France and Spain revised the boundaries by expert judgement until values represented in the good and moderate status classes were consistent with the normative definitions.<br>Boundary values obtained are the result of a combination of several things: historical data analysis compared to expert judgment. | Derived from metric variability at high and good status by expert judgement according with normative definitions. | Derived from expert judgement. | Yes, LUSI |
| Croatia Italy Slovenia | Joint boundary setting. No boundary setting is possible with Croatia and Slovenian data only; stations are in a narrow trophic window.<br>Data were merged with Italian and a common database was built with Type I and Type IIA data.<br>A combination of expert judgement and statistical approach was used. | Derived by expert judgement in combination with statistical analysis of the common database. | Derived by expert judgement in combination with statistical analysis of the common database. | Yes, Total phosphorus |
| Cyprus | Boundary values resulted mainly from modification of the Greek eutrophication scale in line with expert judgement and consensus from the 1st phase of IC exercise | Derived from boundary between oligotrophic and lower mesotrophic class in line with expert judgement | Derived from an equidistant split of the lower mesotrophic class, where the median is taken as the G/M boundary | Yes, LUSI |

## 1.6 RESULTS WFD COMPLIANCE CHECKING

| Compliance criteria | Compliance checking conclusions |
|---|---|
| 1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad). | |
| 2. High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)<br>See info from WISER Questionnaires: | |
| - Question A.12: Scope of detected pressures | |
| - Question A.13: Has the pressure-impact relationship of the assessment method been tested? | |
| - Question C.14: Setting of ecological status boundaries: methodology and reasoning to derive and set boundaries | |
| - Question C.15: Boundary setting procedure in relation to the pressure:<br>Which amount of data/pressure indicators have been related to the method and what was the outcome of the relation? | |
| - Question C.11 + C.16: Reference and Good status community description:<br>Is a description of the communities of reference/high – good – moderate status provided? Not only a formula or an EQR value, but the range of values for the different parameters included in the method that result in high – good – moderate status | |
| 3. **All relevant parameters** indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole<br>See info from WISER Questionnaires: | |
| - Question C.01: Complete list of biological metric(s) used in assessment | |
| - Question C.02: Data basis for metric calculation | |
| - Question C.04: Combination rule for multimetrics | |
| 4. Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT<br>See info from WISER Questionnaires: | |
| - Question A.14: Is the assessment method applied to water bodies in the whole country? | |
| - Question A.15: Specify common intercalibration types | |
| - Question C.03: Does the selection of metrics differ between types of water bodies? | |
| 5. The water body is assessed against **type-specific near-natural reference conditions**<br>See info from WISER Questionnaires: | |
| - Question C.05: Scope of reference conditions | |

| | |
|---|---|
| - Question C.06: Key source(s) to derive reference conditions | |
| - Question C.07-C.08-C.09: Number of sites, location and geographical coverage of sites used to derive reference conditions | |
| - Question C.10: Time period (months+years) of data of sites used to derive reference conditions | |
| - Question C.12: Reference site characterisation: criteria to select them | |
| - Is a true reference used for the definition of High status or an alternative benchmark estimation? | |
| 6. Assessment results are expressed as **EQRs:**<br>- Question C.13: Are the assessment results expressed as Ecological Quality Ratios (EQR)? | |
| 7. Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**<br>See info from WISER Questionnaires: | |
| - Question C.17: Has the uncertainty of the method been quantified and is it regarded in the assessment ? | |
| - Question C.18: Specify how the uncertainty has been quantified and regarded | |

| Compliance criteria | Compliance checking conclusions |
|---|---|
| 8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**<br>See info from WISER Questionnaires: | Italy, France, Spain-all regions, Croatia: yes |
| 9. Selected taxonomic level achieves adequate **confidence and precision** in classification<br>See info from WISER Questionnaires: | No taxonomy is needed for this methods |
| - Question B.02-B.03: Sampling/survey device | Catalan: transect, quadrates |
| - Question B.04: Minimum size of organisms sampled and processed | Catalan: shoot |
| - Question B.16-B.17: Record of biological data: level of taxonomical identification – what groups to which level | Catalan: not necessary |

General conclusion of the compliance checking:

There are no gaps in the French and Spanish method.

There a no gaps in the Croatian, Italian and Slovenian methods.

There are no gaps in the Cypriot method.

# 2 IC FEASIBILITY CHECKING

## 2.1 TYPOLOGY

Description of the common intercalibration water body types and list the MS sharing each type

| Common IC type | Type characteristics | Mean salinity | MS sharing IC common type |
|---|---|---|---|
| Type I | Influenced by freshwater inputs. | | France, Italy |
| Type II - A | Moderately influenced by freshwater inputs. | 34,5 ≤ SAL <37,5 | France, Spain |
| Type II - A Tyrrhenian (see Annex I) | Moderately influenced by freshwater inputs. Tyrrhenian. Tyrrhenian considered separately from the rest of the Mediterranean Sea type II - A. See Fig. 4. | | Italy |
| Type II – A Adriatic (see Annex I) | Moderately influenced by freshwater inputs. Adriatic considered separately from the rest of the Mediterranean Sea type II - A. | | Croatia, Italy, Slovenia |
| Type III - W | Continental coast. Not affected by freshwater inputs. Western basin. | SAL ≥ 37,5 | Croatia, France, Italy, Spain |
| Type III – E | Not affected by freshwater input. Eastern Basin. | | Cyprus, Greece |
| Type Island-W | Mediterranean island, not affected by freshwater inputs. Western basin. | All range | France, Spain<br>Italy has not defined any islands as belonging to this type. |

Note: for some WB, salinity dataset available was not consistent enough to derive an annual mean of salinity. In these cases, **expert judgment** was used to address the relevant typology to the WB.
Island-W Typology was introduced, since Chlorophyll-*a* reference values use to be lower in these coastal areas, and pressure-impact relationship shows different pattern.
National typology corresponds to common typology for all the Mediterranean countries.

Results obtained for Type I in IC exercise done by Slovenia, Croatia and Italia may not be relevant for the only French CW body of type 1 (facing Rhône river). Indeed, boundary setting done by IT, Sl and CR was strictly done on an Adriatic dataset".

Type II-B is not included in the intercalibration exercise for Spain (only present in one Member State Spain: Andalucía region).

The criterion adopted to identify different typologies of coastal water bodies (see the Decision of the Commission EU - 2008/915/CE) is currently based on seawater density, as Sigma_t annual mean values: Type I: Sigma_t < 25. Type II: 27> Sigma_t >25. Type III: Sigma_t >27.

**Separation of the NW Adriatic Sea from type II A defined in the 1st Intercalibration phase.**

The whole NW Adriatic Sea area, affected by the Po River inputs (i.e. the Emilia Romagna coast), belongs to Type I. The remaining part of the Adriatic coast (to say: the Veneto and Friuli-Venezia Giulia coasts, the Gulf of Trieste with the Slovenian coast), influenced by other major rivers that flow into the N Adriatic Sea, belongs mainly to Type IIA.

The examination of the common nearshore data base, prepared among the Mediterranean MS, in the framework of the IC exercise, confirms that no other part of the Mediterranean coasts is classified as Type I, with rare exceptions, to be however referred to as transitional water bodies. Similarly, the coastal stretches of Italy belonging to Type IIA, are mainly located in the Adriatic Sea and some coastal stretches also in the Tyrrhenian sea. In this case however, as well documented in the following, the response of the Tyrrhenian coastal systems are quite different in trophodynamic terms (Fig. 4). For this reason we have made a distinction between Type IIA (Adriatic Sea) and Type IIA (Tyrrhenian Sea) and named the Adriatic Sea type as Type IIA Adriatic.

The following figure illustrates the different response of chlorophyll a to TP of the Adriatic Sea (Type I and Type IIA) in relation to the rest of the Type IIA of the Mediterranean Sea (Tyrrhenian Sea data included here).

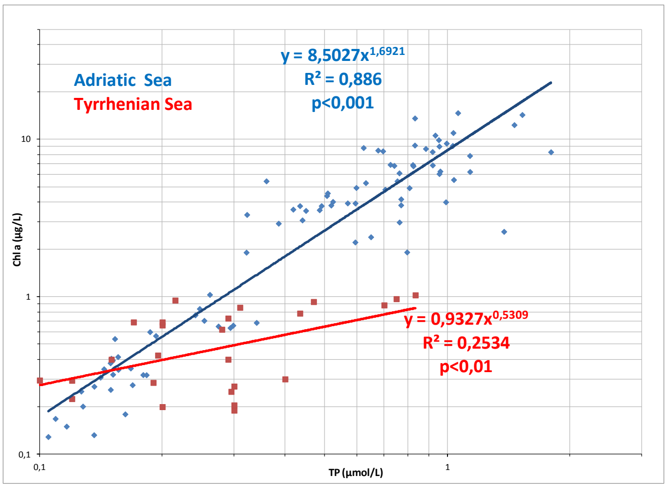



**Figure 4.** Geomean of Chlorophyll *a* (Chl *a*) vs. Total Phosphorous (TP) concentration in Adriatic and Tyrrhenian Sea

What is the outcome of the feasibility evaluation in terms of typology? Were all assessment methods appropriate for the common intercalibration water body types, or subtypes?

| Method | Appropriate for IC types / subtypes | Participating Member States |
|---|---|---|
| Biomass - Chlorophyll a | Type II A, Type III W, Type Island-W | France and Spain (Croatia and Italy declared that it was not possible to develop an assessment system for Type IIIW, see explanation below) |
| Biomass - Chlorophyll a | Type I, Type II A Adriatic | Joint IC for Croatia, Italy and Slovenia (France and Italy did not compare Type I) |
| Biomass - Chlorophyll a | Type II A Tyrrhenian | Italy |
| Biomass - Chlorophyll a | Type III - E | Cyprus and Greece |
| **Conclusion**<br>Is the Intercalibration feasible in terms of typology **? Yes** | | |

**Type III - W: no assessment for Croatia and Italy**

When designing a classification scheme, with the aim of comparing different trophic levels, the question that arises is how many samples are needed to obtain a reliable estimate of the difference between two contiguous Chl *a* means. Obviously this Discrimination Limit (i.e., the resolution power of a test on the differences), depends on the sample size.

In general, it is possible to evaluate a priori the minimum level of resolution requested. With small samples (N <50) randomly extracted from the same normal population, the following condition is worth:

$$dM = s_p \cdot t_{(\alpha/2;N_1+N_2-2)} \cdot \sqrt{(1/N_1 + 1/N_2)} \neq 0$$

,

where *dM* represents the Discriminant limit expressed as absolute value. In the particular case N1 = N2 = N, the degrees of freedom for the variable *t* become (2N-2) and the term under root becomes (2/N).

In the case of two Chl *a* sample distributions, as already discussed above, the normality conditions are achieved by means of a 10 Log transformation. In such a way, the variances of the log data become stable with a St. Dev. around 0.30-0.40. Assuming therefore a pooled St. Dev. for the logarithms of Chl *a*: $s_p$ = 0.3, at an opportune significance level α/2 = 0.025 (with P = 95%), the following results are obtained:

| | | | | |
|---|---|---|---|---|
| 1) With: | *N = 12* | *t = 2.074* | *√(2/12) = 0.408* | *dM > \|0.25\|* |
| 2) | *N = 52* | *t= 1.983* | *√(2/52) = 0.196* | *dM > \|0.12\|* |
| 3) | *N = 100* | *t= 1.972* | *√(2/100) = 0.141* | *dM > \|0.08\|* |

The Type III Chl *a* data available are mainly related to the oligotrophic Tyrrhenian coastal waters of Sardinia, Calabria, etc. Here, the annual geometric means of Chl *a* do not exceed concentration values of 0.2 µg/L, with maximum seasonal peaks that are unlikely to exceed 1 µg/L. Trying to build up a classification criterion based on the Chl *a* in these conditions, it means setting a range from 0 to 1 µg/L with 4 intermediate boundaries.

Suppose to adopt the "alternative benchmarking" rule, to say the rule of the equidistant range applied to log-transformed Chl *a* data. For Type III we would have the following table, with the related boundaries assigned for the chlorophyll concentrations (µg/L), as annual geomeans:

| Ref. Values | H/G | G/M | M/P | P/B |
|---|---|---|---|---|
| 0.11 | 0.19 | 0.33 | 0.58 | 1 |

So, converting the H/G and G/M boundaries in 10Log, we will have:

Log(0.19)= -0.72.    Log(0.33)= -0.48

Therefore, with an yearly monitoring programme and a monthly sampling frequency (case 1), we would reach a discrimination level between two log(Chl) averages equal to |0.25|, not indeed favourable for a status classification, when the range between the two boundaries is = 0.24.

With a weekly sampling frequency (case 2), the limit descends to 0.12, a value that surely does not help to provide an acceptable level of uncertainty.

In conclusion, we think that for this type of waters, the Chl *a* is not a suitable indicator, but as requested by the directive, the EQB Phytoplankton must be tested in the future against the biodiversity decay. We have to take into account that these coastal environments are particularly vulnerable and sensitive to the trophic levels increase and in general to the human-induced pressures, which may result in a considerable reduction of the phytoplankton diversity.

## 2.2 PRESSURES ADDRESSED

Is the Intercalibration feasible in terms of **pressures** addressed by the methods?

Description of the pressures addressed by the MS assessment methods

| Member State | Metrics tested | Pressure | Pressure indicators | Amount of data | Strenght of relationship |
|---|---|---|---|---|---|
| Croatia<br>Italy<br>(Adriatic)<br>Slovenia | Geometric mean of Chl *a* [µg/L] | Anthropogenic and natural pressures from land | Total phosphorus | Type I and IIA Adriatic:<br>89 | Type I and IIA Adriatic combined<br>Chl *a* = 8.5027 $TP^{1.6921}$<br>$r^2$ = 0.886, $p<0.001$ |
| France and Spain | $90^{th}$ percentile of Chl *a* [µg/L] | Land uses and continental pressures | LUSI | 116 Water bodies (42 water bodies of Type Island-W; 51 water bodies of Type III-W; and 23 water bodies of Type II-A). | y = 0.08 + 0.49*x<br>$r^2$ = 0.45 (Spearman)<br>p = 0.00<br>n = 116<br>(All types combined) |
| Italy (Tyrrhe-nian) | Geometric mean of Chl a [µg/l] | Anthropogenic and natural pressures from land | Total phosphorus | Type IIA Tyrrhenian:<br>29 | Chl a = 0.9327 $TP^{0.5309}$<br>$r^2$ = 0.253, $p<0.01$ |
| Cyprus | $90^{th}$ percentile of Chl *a* [µg/L] | Land uses | LUSI | Type III - E : 17 | y = 0.013x + 0.070<br>$r^2$ = 0.332<br>p=<0.05 |

Note: Please check Annex for further explanations of LUSI and pressure - impact relationships.

**Conclusion**
Is the Intercalibration feasible in terms of **pressures** addressed by the methods? Yes.

**Croatia, Italy and Slovenia:** Type I and IIA Adriatic

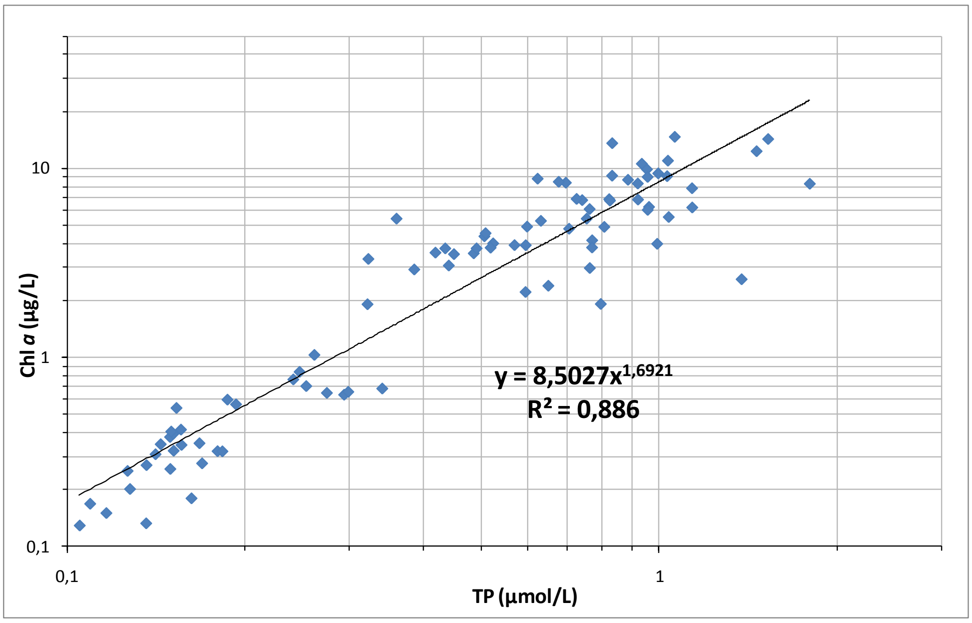



**Figure 2**: Relationship between pressure (TP) and impact (Chl *a*) in Type I and IIA Adriatic combined. Use of TP for pressure is explained in the common boundary setting procedure for Type IIA-Adriatic.

**France and Spain:** Type II A, Type III W, Type Island-W

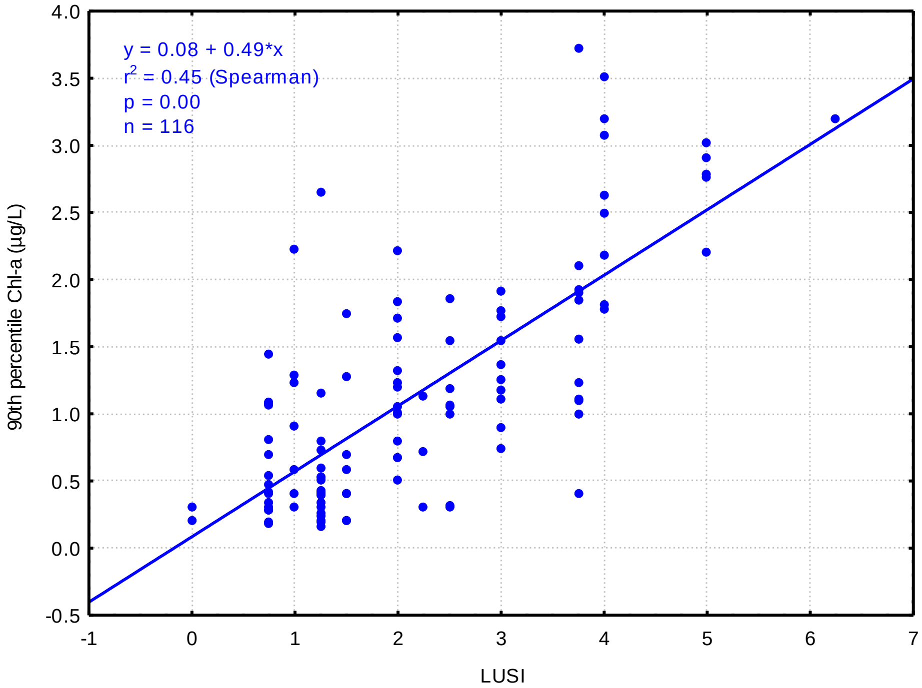



**Figure 1**: Relationship between pressure index and impact of French and Spanish data.

**Italy:** Type II-A Tyrrhenian

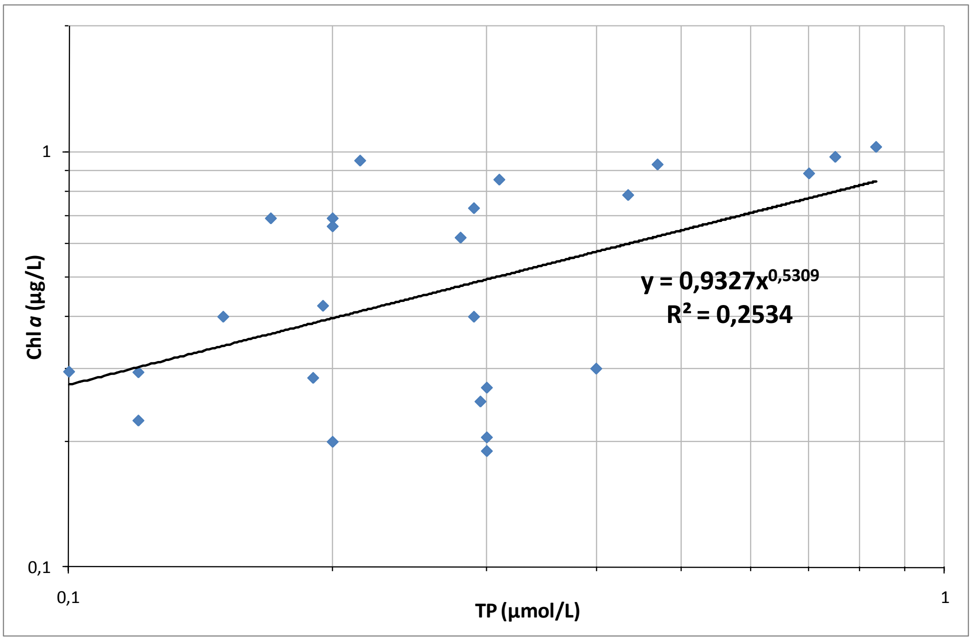



**Figure 3**: Relationship between pressure (TP) and impact (Chl a) in Type IIA Tyrrhenian.

**Cyprus:** Type III - E

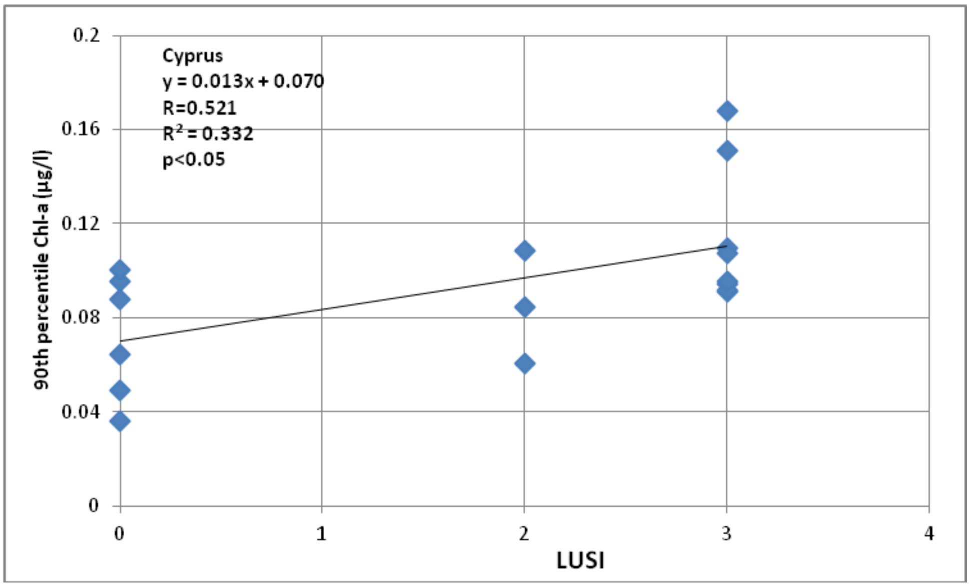



**Figure 4**: Relationship between pressure index LUSI and impact of Cyprus data.

## 2.3 ASSESSMENT CONCEPT

| Method | Assessment concept | Remarks |
|---|---|---|
| Biomass - Chlorophyll *a* (France and Spain) | Increasing Chlorophyll *a* concentrations above those considered natural are related to increasing nutrient enrichment which could be related to anthropogenic disturbances. LUSI are based on land uses and continental pressures potentially sources of nutrients inputs. | Further explanations of LUSI in ANNEX II |
| Biomass - Chlorophyll *a* (Croatia, Italy and Slovenia) | Increasing Chlorophyll *a* concentrations above those considered natural are related to increasing nutrient enrichment which can be related to anthropogenic disturbances. Total phosphorus best represent the pressures (combined anthropogenic and natural pressures) in the area of the three countries. | Total phosphorus as pressure indicator. |
| Biomass - Chlorophyll *a* (Cyprus) | Increasing Chlorophyll *a* concentrations above those considered natural are related to increasing nutrient enrichment which could be related to anthropogenic disturbances. LUSI is based on land uses and continental pressures. | Further explanations of LUSI in ANNEX II |
| **Conclusion**<br>Is the Intercalibration feasible in terms of **assessment concepts? Yes** | | |

# 3 IC DATASET COLLECTED AND BENCHMARKING

## 3.1 DATASET DESCRIPTION

Description of the data collection within the GIG.

| | |
|---|---|
| Size of common dataset: total number of sites | |
| Number of Member States | 6 (excluding Greece and Malta) |
| Repackage/disaggregation of samples/WB results? | No, use of water body integrated values for Type IIA, Type Island W and Type III W<br>Yes, disaggregation at sample level to analyse chlorophyll a measurements for Type IIA-Adriatic |
| Gradient of ecological quality | Truncated, including upper ecological gradient of |

| Coverage per ecological quality class | |
|---|---|

### 3.1.1 IC common types **France and Spain**

**The France and Spain common data set include water bodies of Type II-A, Type III-W and Type Island-W. The total number of water bodies per Member State are given below.**

| Member State | Number of sites or samples or data values | | |
|---|---|---|---|
| | Biological data | Physico - chemical data | Pressure data |
| France | 24 WB | 24 WB | 24 WB |
| Spain | 92 WB | 92 WB | 92 WB |
| Total | 116 WB | 116 WB | 116 WB |

**Table 3:** Number of water bodies within the common data set by typology and country.

| Number of water bodies | | Typology | | | |
|---|---|---|---|---|---|
| | | Island-W | Type III-W | Type II-A | Total |
| Country | France | 12 | 8 | 4 | 24 |
| | Spain | 30 | 43 | 19 | 92 |
| Total | | 42 | 51 | 23 | 116 |

There is sufficient covering of all relevant quality classes per typology.

As Spain used inshore and nearshore data, all inshore data were transformed to nearshore data according to 1st IC MED-GIG Technical Report, Section 3 Annex I Spain. A more detailed characterisation of inshore and nearshore data could be found in Flo *et al*. 2011[2]
The common data set includes information on water bodies, on anthropogenic pressure estimated at water body level (LUSI) and impacts (Chlorophyll-*a*), and on salinity and density (in order to specify typology following the criteria agreed in IC phase 1).

Chlorophyll-*a* data are expressed in P90th percentile values in µg/l. For France P90th percentile was calculated on a 6 year period and for Spain, depending on the region, this value was calculated on a 2 (Balearic Islands), 3 (Murcia), 4 (Catalonia) or 5 (Valencia) year period.

**The intercalibration is feasible in terms of common data for 3 Mediterranean CW common typologies.**

### 3.1.2 IC common type **Croatia, Italy and Slovenia Type II Adriatic + Italy Type II A Tyrrhenian**

| Member State | Number of sites or samples or data values | | |
|---|---|---|---|
| | Biological data | Physico - chemical data | Pressure data |
| Croatia | 598 data values | 598 data values | 598 data values |
| Italy | 432 data values | 432 data values | 432 data values |
| Slovenia | 180 data values | 180 data values | 180 data values |

### 3.1.3 IC common type **Greece and Cyprus**

| Member State | Number of sites or samples or data values | | |
|---|---|---|---|
| | Biological data | Physico - chemical data | Pressure data |
| Cyprus | 10 WB | 10 WB | 10 WB |
| Greece | No data in 2nd phase | No data in 2nd phase | No data in 2nd phase |

[2] Flo, E.; Garcés, E.; Manzanera, M. & Camp, J. 2011. Coastal Inshore Waters In The NW Mediterranean: Physicochemical And Biological Characterization And Management Implications. Estuarine, Coastal and Shelf Science, 93 (4), 279-289.

## 3.2 DATA ACCEPTANCE CRITERIA

List of data acceptance criteria used for the data quality control and describe the data acceptance checking process and results

| Data acceptance criteria | Data acceptance checking |
|---|---|
| Data requirements (obligatory and optional) | Salinity values needed for type definition were provided by all countries.<br>Sampling procedure should be representative in space and time, avoiding seasonality bias. |
| The sampling and analytical methodology | **Croatia**: Chlorophyll a – Niskin bottle sampling (surface layer, 0,5 m), monthly frequency; fluorimetric analysis<br>**Cyprus**: Chlorophyll a – Niskin bottle sampling (3 depth intervals: surface layer- 10m-20m below surface), monthly frequency; fluorimetric analysis<br>**France**:<br>Sampling : by Niskin or Hydrobios bottle, at 1m under the surface<br>Chlorophyll a analysis :<br>Spectrophotometry (monochromatic – Lorenzen) or fluorimetry (Holm-Hansen), both methods described in Aminot & Kerouel (2004)<br>Italy: Chlorophyll a – Niskin bottle sampling (surface layer, 0,5 m), monthly frequency; fluorimetric analysis<br>Slovenia: Chlorophyll a – Niskin bottle sampling (surface layer, 0,5 m), monthly frequency; fluorimetric analysis<br>**Spain** (by regions):<br>Catalonia: Chlorophyll a. Standard methods were used for sampling (Niskin bottle) and analysing of Yentsh, C.S., Menzel, D.W., 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep Sea research 10, 221-23.<br>Valencia: The chlorophyll a content was determined using trichromatic method (APHA, 1998) based on visible spectroscopy and using Jeffrey and Humprey's (1975) equations to obtain the concentration.<br>Murcia: Chlorophyll a was analysed with the spectrophotometric methods reported by Parson et al (1984) Parsons, T. R., Y. Maita & C. M. Lalli, 1984. A Manual of Chemical and Biological Methods for Sea-Water Analysis. Pergamon Press, Oxford: 173 pp.<br>Andalucia: Chlorophyll a was analysed with the spectrophotometric methods reported. Standard Methods. Methods for Examination of Water and Wastewater, ed. 17.<br>Baleares: Método EPA 445.0 “In vitro determination of Chlorophylly a and Pheopytin a in marine and freshwater algae by fluorescence” revision 1.2, 1997. |
| Level of taxonomic precision required and taxalists with codes | / |
| The minimum number of sites / samples per intercalibration type | All intercalibration types had a sufficient number of sampling sites. |
| Sufficient covering of all relevant quality classes per type | France and Spain: Yes<br>Croatia, Italy and Slovenia: Yes<br>Italy: Yes<br>Greece and Cyprus: Yes |

## TECHNICAL REPORT CONTINUATION PART I

## TYPE I AND TYPE IIA TYRRHENIAN (ITALY), AND TYPE IIA ADRIATIC (CROATIA, ITALY AND SLOVENIA)

### 3.3 COMMON BENCHMARK: IC REFERENCE CONDITIONS OR ALTERNATIVE BENCHMARK

The group has defined reference conditions.

**Reference conditions**

- Common approach for setting reference conditions (true reference sites or indicative partial reference sites, see Annex III of the IC guidance):

**Croatia, Italy and Slovenia** used a modelling approach to define RC. For the 2 IC types the minimal threshold value was defined on the base of the common dataset as reference one.

- Detailed description of **reference criteria** for screening of sites in near-natural conditions (abiotic characterisation, pressure indicators):

**Croatia, Italy and Slovenia** have chosen reference condition on the base of the dilution factor coupled with the minimum value of Chl *a* as the best measure of no or minimal pressure at the type scale. In details explained below.

- Identify the **reference sites** for each Member State in each common IC type. Is their number sufficient to make a statistically reliable estimate?

**Croatia, Italy and Slovenia / Italy:** Reference conditions are not linked to definite reference sites but rather represent a statistically/modelling estimated conditions.

- Explain how you have screened the biological data for impacts caused by pressures not regarded in the reference criteria to make sure that true reference sites are selected:

**Croatia, Italy and Slovenia / Italy:** not considered because Chl *a* values are related mainly to the eutrophication pressure.

- Give detailed description of **setting reference condition**s (summary statistics used)

*Annual geometric mean of Chl a as a metric*

Due to the particular nature of Chlorophyll *a* data, functionally related to phenomena of exponential type like biomass growth and nutrient uptake and release, decimal log transformation of all the above parameter point values has been adopted, by considering this preliminary transformation of the original data, proper and sufficient to normalize each statistical distribution. (Note: About this topic, a rich literature is available, since 1965, as demonstrated by the citation reported below. Margalef, R., 1965. See also Giovanardi and Vollenweider, 2004, Giovanardi et al. 2006).

> In the working of correlations and regressions, the rough data frequently do not approach a normal distribution. In such cases a transformation is required before further statistical analysis. A logarithmic transformation often proves appropiate for parameters referring to populations (chlorophyll content, production, number of cells) and to environmental factors strongly influenced by organisms (nutrient concentrations). Multiplication and diffusion in a non-uniform environment lead commonly to a type of distribution in which density of populations decreases exponentially with increasing distance from a center of maximum density. If samples are taken with a regular spacing or a regular periodicity, chances are that in any series of samples, not the actual densities, but the logarithms of the densities approach normal distribution. Other variables (temperature, salinity) frequently do not require transformation.

Consequently, in order to characterize each sampling station, we have adopted the annual geometrical means (to say the arithmetic mean of the logarithms, re-converted into numbers), as the main metrics actually accounting for the trophic levels of the areas under consideration.

*Defining reference conditions for Croatia, Italy and Slovenia*

In order to fix a reference value for Chl *a*, we used the values of the dilution factor for WBs of the whole phytoplankton group dataset (Croatia, France, Italy, Slovenia and Spain). Values of the dilution factor were plotted against geometric means of Chl *a* (see Fig. 3).

*Dilution factor*

The Dilution factor defines the freshwater content of the sea (Yentsch 1975). It is calculated as:

$$F = \frac{S - s}{S}$$

with S = open sea salinity; s = measured salinity. In the following, the Dilution factor, abbreviated as F_dil, will be represented as a percent value.

On the basis of all the available data of Croatia, France, Italy, Slovenia and Spain, plotted in a Chl *a* vs. F_dil (%) diagram (Fig. 3), a realistic lower demarcation for the scattering area is provided by the curve that separates the data points from the empty area below, assuming that the line represents the natural conditions attainable depending on the freshwater inputs.

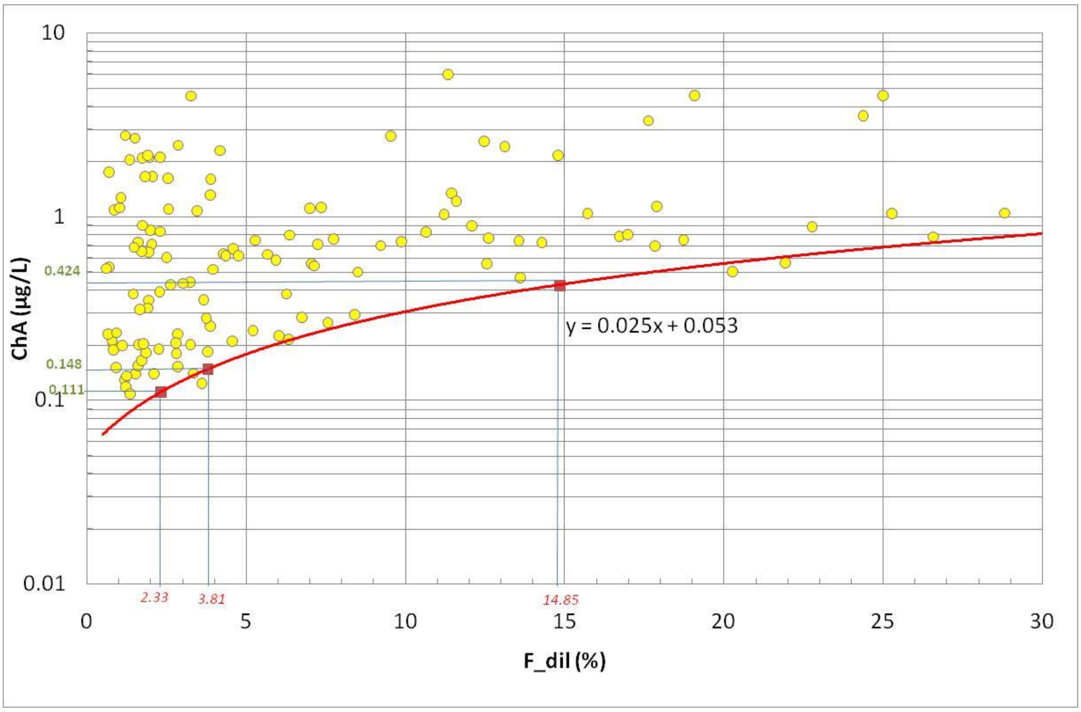



**Figure 3.** A scatterplot of annual geomeans of Chl *a* of sampling stations in the common database plotted against the dilution factor, showing the red line as a lower limit of Chl *a* values as a function of the dilution factor.

This line is meant as the minimum threshold for the Chl *a* concentration, to be assigned to the geometric means of sampling station in a water type, as a function of the dilution factor.

The table 3 shows the reference values for Chl *a*, for each of the three typologies (Type III included). The g-means (geometric mean), the minimum and maximum values are reported, taking into consideration that the 10Log-transformation of the original data approximates the Chl *a* distribution to the normality, with a Standard Deviation of the 10Logs nearly constant, between 0.30 and 0.40.

**Table 3.** Reference values minimum and maximum values for Chl *a*, for each of the three typologies

| | Type I | | | Type IIA | | | Type III | | |
|---|---|---|---|---|---|---|---|---|---|
| Ref. value | G_Mean | Min | Max | G_Mean | Min | Max | G_Mean | Min | Max |
| Chl *a* [µg/L] | 0.80 | 0.09 | 1.94 | 0.15 | 0.03 | 0.68 | 0.11 | 0.02 | 0.51 |

## 3.4 BENCHMARK STANDARDIZATION

Regional differences have been taken into account by separating the Adriatic Sea from the rest of the Mediterranean Sea. The different Member States within the Adriatic are considered to respond similarly dependent from the dilution factor. One reference has been set for the entire type IIA Adriatic, although there is also variation within this subtype dependent on the dilution factor, but this is not further taken into account.

# 4 COMPARISON OF METHODS AND BOUNDARIES

## 4.1 IC OPTION AND COMMON METRICS

- IC option: Option 1 with common national boundary setting.

- Explanation for the choice of the IC option:

**Croatia, Italy and Slovenia** developed a common assessment methodology for use at the basin scale (Adriatic). It is based on the common dataset built for which identical data acquisition and numerical evaluation was applied. A JOINT BOUNDARY SETTING was considered.

## 4.2 DESCRIPTION OF BOUNDARY SETTING PROCEDURE SET FOR THE COMMON IC TYPE

Summarize how boundaries were set following the framework of the BSP:

– Provide a description how you applied the full procedure (use of discontinuities, paired metrics, equidistant division of continuum)

**Croatia, Italy and Slovenia:** The procedure of boundary setting was the same for Type I and Type IIA Adriatic.

During the process of intercalibration we realized that no boundary setting was possible with Croatian and Slovenian data only, because stations do not cover the whole trophic scale. Therefore, data was merged with Italian and a common database was built with Type I and Type IIA data. A combination of expert judgement and statistical approach was used.

– Provide pressure-response relationships (describe how the biological quality element changes as the impact of the pressure or pressures on supporting elements increases)

**Croatia, Italy and Slovenia:** To assess pressure Croatia, Italy and Slovenia have used Total phosphorous values.

We have tested the sensitivity of the Chl *a* variability against the nutrient concentrations, the Oxygen % saturation (expressed as aD_O, absolute % deviation from the saturation), the Dilution factor F%, etc.

From the common data set, in the first approach Multiple linear regression analysis (Linear models) was applied to Type I and Type IIA sampling stations average data, in order to test the variability of the Chl *a* depending on different pressure indicators. (Note: for Type IIIW sampling stations this procedure could not be applied, due to a poor and not significant sample size).

**Type I**

The considered sampling stations belonging to Type I were 26.
Among all the possible combinations, Stepwise regression technique provided the following linear model:

lm(formula = ChA ~ f_dil + aD_O + TP + DIN, data = Type_I)

The numerical output of the multiple regression analysis is the following:

| | Estimate | Std. Error | t value | Pr(>\|t\|) | |
|---|---|---|---|---|---|
| **(Intercept)** | -2.45363 | 0.52147 | -4.705 | 0.000338 | *** |
| **f_dil** | 0.15981 | 0.03720 | 4.296 | 0.000739 | *** |
| **aD_O** | 0.32117 | 0.06128 | 5.241 | 0.000125 | *** |
| **TP** | 3.65302 | 0.45542 | 8.021 | 1.33e-06 | *** |
| **DIN** | -0.11004 | 0.01949 | -5.646 | 6.04e-05 | *** |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Multiple R-squared: 0.8886, Adjusted R-squared: 0.8568

F-statistic: 27.93 on 4 and 14 DF, p-value: 1.533e-06

The results show that Total Phosphorus accounts for the maximum weight in determining the variability of Chl *a*; the other regressors, although highly significant, have lowest effects. The fitted LM explains at least the 86% of the total Chl *a* variability and the tests performed on the residuals insure us that the remaining Chl *a* variability is not affected by other independent variables, not considered in the adopted linear model.

### Type IIA

The considered sampling stations belonging to Type IIA were 30.
The linear model adopted by the Stepwise Regression Technique was the following:

lm(formula = ChA ~ f_dil + TP, data = Type_IIA)

The multiple regression analysis provided the following results:

| | Estimate | Std. Error | t value | Pr(>\|t\|) | |
|---|---|---|---|---|---|
| **(Intercept)** | -0.00971 | -0.05824 | -0.167 | 0.869170 | n.s. |
| **f_dil** | 0.04135 | 0.01244 | 3.323 | 0.003231 | ** |
| **TP** | 1.62190 | 0.39665 | 4.089 | 0.000525 | *** |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Multiple R-squared: 0.7758, Adjusted R-squared: 0.7544
F-statistic: 36.33 on 2 and 21 DF, p-value: 1.521e-07

For Type IIA situation, the resulting Linear Model is very simple, only two regressors have been chosen, with a prevailing weight of TP. The Multiple R_squared obtained shows that the variability of Chl *a* explained by the model is cca. 77%. And that also in this case Total Phosphorus is the limiting factor.

After understanding that most of the Chl *a* changes in the ecosystem can be explained by TP changes and that Phosphorous is accounting for far the most share in the eutrophication pressure, the relationship curves were built. We also realized that no boundary setting was possible with Croatian and Slovenian data only, because stations do not cover the whole trophic scale. Therefore, data was merged and relationship curves were built with Type I and Type IIA data. However, the response of the Tyrrhenian coastal system compared to the Adriatic one was quite different in trophodynamic terms (Fig. 5). With the equal raise in TP concentrations much more biomass is built up in the Adriatic Sea than in Tyrrhenian Sea, where factors other than phosphorus limit the phytoplankton growth. The regression curves statistics are presented in the next Table:

| | relationship | equation | $r^2$ | p |
|---|---|---|---|---|
| **Type I + Type IIA Adriatic** | Log -log | Chl $a$=8.5027 $TP^{1.6921}$ | 0.886 | <0.001 |
| **Type IIA in Tyrrhenian** | Log -log | Chl $a$=0.9327 $TP^{0.5309}$ | 0.253 | <0.01 |

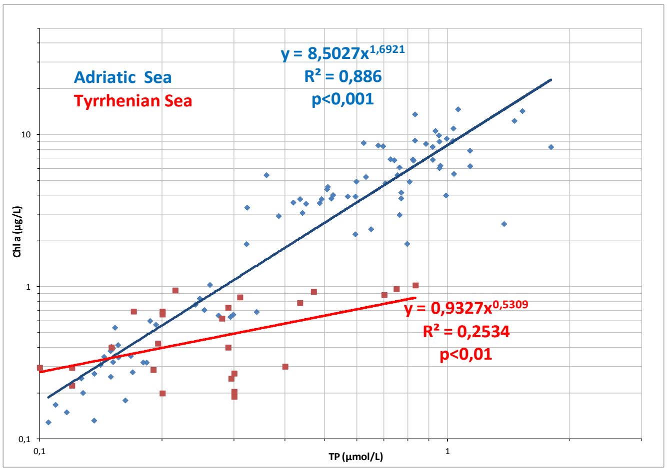



**Figure 5.** Geomean of Chlorophyll a (Chl *a*) vs. Total Phosphorous (TP) concentration in Adriatic and Tyrrhenian Sea

*Test of LUSI index for Croatia, Italy and Slovenia:*

Spanish experts have developed their own approach for identifying the pressure through the LUSI Index, just because they want to address the effects of human-induced pressures mainly on this type of waters (CIW), rather than on the whole CW water body. We believe that Spain is legitimate to do that and test the effects of LUSI on the Chl *a* and on these restricted coastal environments.

We presume that the use of LUSI is not fully adequate to synthesize the anthropogenic pressures on the trophic levels, using Chl *a* concentration as indicator. It can be surely used at a local scale, like the Spanish data on CIW seem to demonstrate. Applying LUSI to data Type I and II and also to Type III (Adriatic and Tyrrhenian seas sampling stations), we found no significant correlation; mainly because our data are referred to different distances from the shore; with the aim of characterizing the entire coastal water body (Fig. 6).

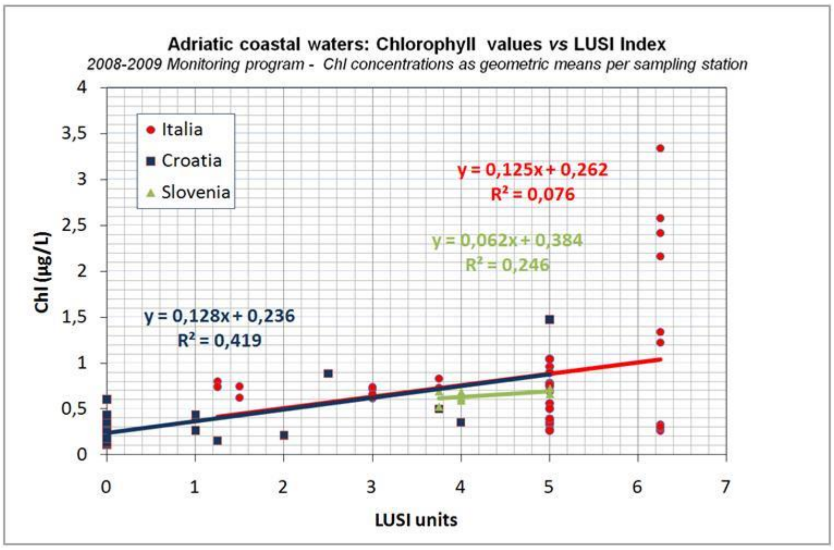



**Figure 6.** Chlorophyll *a* concentration annual geometric mean per station vs. LUSI.

– Provide a comparison with WFD Annex V, normative definitions for each QE/ metrics and type

For all IC types, definitions of the metric used (representing Chl *a* biomass) at high, good and moderate status are according the normative definitions.

For management purposes, TRIX index as such is more functional and useful to represent the ecological status of the BQE Phytoplankton, rather than a single indicator such as Chl *a*. Depending on the formulation of TRIX, this index encompass the main characteristics of the planktonic community, but in addition it contains also the nutrients as pressure indicators, that allow to fix objectives and to adopt strategies and policies for correct sanitation plans. TRIX is explained in ANNEX I.

We decided therefore to use TRIX as common metric to evaluate the corresponding values of Chl *a* (on which the classification criterion for BQE Phytoplankton is built up) and the related TP concentration, as pressure indicator.

The use of TRIX as common metric for Chl *a* and Total Phosphorus, as mentioned above, reflects a "Management Approach" more than an "Eco-system approach". In the case of BQE Phytoplankton, it is believed that the ecosystem approach promoted by the Directive, is still premature at the current state of knowledge. Preliminary studies about the effects of trophic level increase on the biodiversity expressed by phytoplankton, have shown promising results. There have been in fact identified ranges of variation of the main indexes in use (Shannon-Weaver Index, Margalef Index, etc...) and these ranges are in good agreement with the values provided by the literature for coastal waters more or less impacted by the human activities. Nevertheless much remains to understand about the strategies and the dynamics of phytoplankton algal growth. E.g., in recent years blooms of phytoplankton species characterized by small size (<3 µm) are becoming more and more frequent, with a large number of cells/L. Nevertheless, here we encounter difficulties not only of taxonomic kind, but also in the understanding the causes of these blooms, which apparently occur in a totally random way and lead however to a rapid decay in diversity.

By the way, these studies were made possible by the fact that the Adriatic countries have multi-year series of data on species composition and abundance of phytoplankton. These determinations are also included in the national monitoring programs, as expressly required by the Directive.

The relationship between TRIX and Chl *a* and TP is presented in Figure 7.

The boundaries were set applying a combination of expert judgement and statistical approach. First the G/M boundary was set, readapting the boundaries reported by Rinaldi and Giovanardi (2011) by expert judgment taking into account the typology difference. Than an equidistant scale of TRIX were built for every type considering the maximal expected values of TRIX to be found. The boundaries are than calculated from the relationship curves for TRIX/Chl *a* and TRIX/TP (Fig. 7).

The boundaries for all the types are reported in the Table 4.

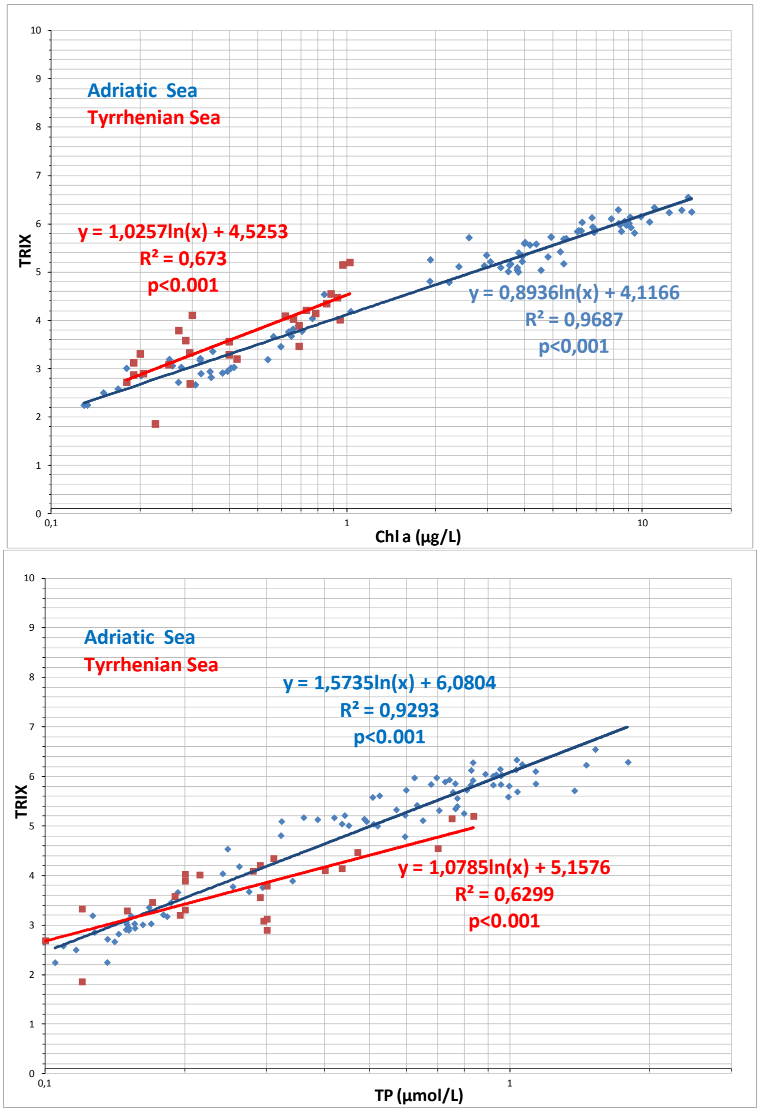



**Figure 7.** TRIX vs Chl *a* and TP for the various types with relationship curves.

**Table 4.** Boundaries for TRIX, Chl *a* (g. Mean and 90th per.), Total Phosphorous (TP) and EQR (real and normalized) by Type.

**Type I**

| Boundaries | TRIX | Chl *a* annual g.means (µg/L) | Chl *a* 90th percentile (µg/L) | EQRs real | EQRs normalized | TP annual g.means (µM/L) |
|---|---|---|---|---|---|---|
| Ref. Values | - | 0.8 | 2.3 | 1 | 1 | 0.24 |
| H/G | 5.0 | 2.5 | 7.0 | 0.32 | 0.78 | 0.4 |
| G/M | 5.7 | 6.2 | 17.3 | 0.13 | 0.59 | 0.6 |
| M/P | 6.4 | 15.1 | 42.5 | 0.05 | 0.39 | 0.9 |
| P/B | 7.1 | 37.1 | 104.4 | 0.02 | 0.20 | 1.6 |

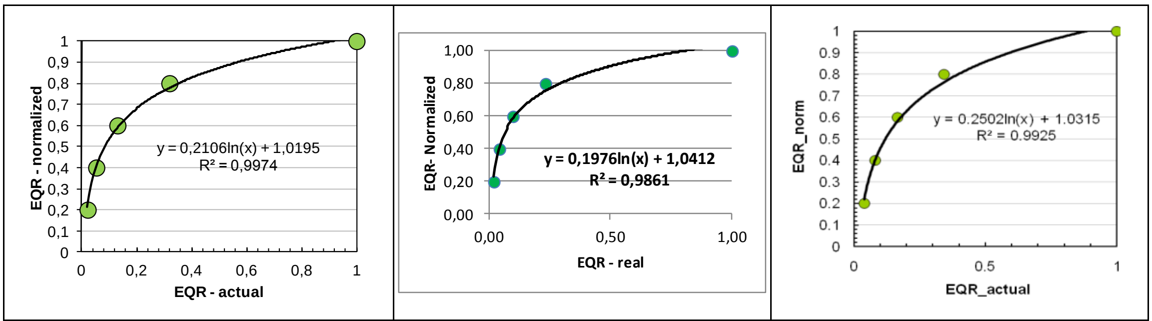
**Type IIA- Adriatic Sea**

| Boundaries | TRIX | Chl *a* annual g.means (µg/L) | Chl *a* 90th percentile (µg/L) | EQRs real | EQRs normalized | TP annual g.means (µM/L) |
|---|---|---|---|---|---|---|
| Ref. Values | - | 0.15 | 0.36 | 1 | 1 | - |
| H/G | 3.7 | 0.65 | 1.58 | 0.230 | 0.75 | 0.23 |
| G/M | 4.5 | 1.57 | 3.81 | 0.095 | 0.58 | 0.37 |
| M/P | 5.3 | 3.79 | 9.20 | 0.040 | 0.41 | 0.61 |
| P/B | 6.1 | 9.14 | 22.17 | 0.016 | 0.22 | 1.01 |

**Type IIA- Tyrrhenian Sea**

| Boundaries | TRIX | Chl a annual g.means (µg/L) | Chl a 90th percentile (µg/L) | EQRs real | EQRs normalized | TP annual g.means (µM/L) |
|---|---|---|---|---|---|---|
| Ref. Values | - | 0.15 | 0.36 | 1 | 1 | - |
| H/G | 3.7 | 0.4 | 1.06 | 0.34 | 0.76 | 0.26 |
| G/M | 4.5 | 0.9 | 2.19 | 0.17 | 0.59 | 0.54 |
| M/P | 5.3 | 1.9 | 4.51 | 0.08 | 0.40 | 1.14 |
| P/B | 6.1 | 3.8 | 9.30 | 0.04 | 0.23 | 2.40 |

The real obtained EQRs were normalized applying a conversion function obtained fitting a logarithmic function between real and equidistantly distributed EQRs.




| Type I | Type IIA Adriatic | Type IIA Tyrrhenian |
|---|---|---|

**Figure 8.** Conversion function for EQRs by type.

The pressure (TP) – response (EQR) relationship calculated for combined Type I and Type IIA Adriatic data is presented on Figure 9.

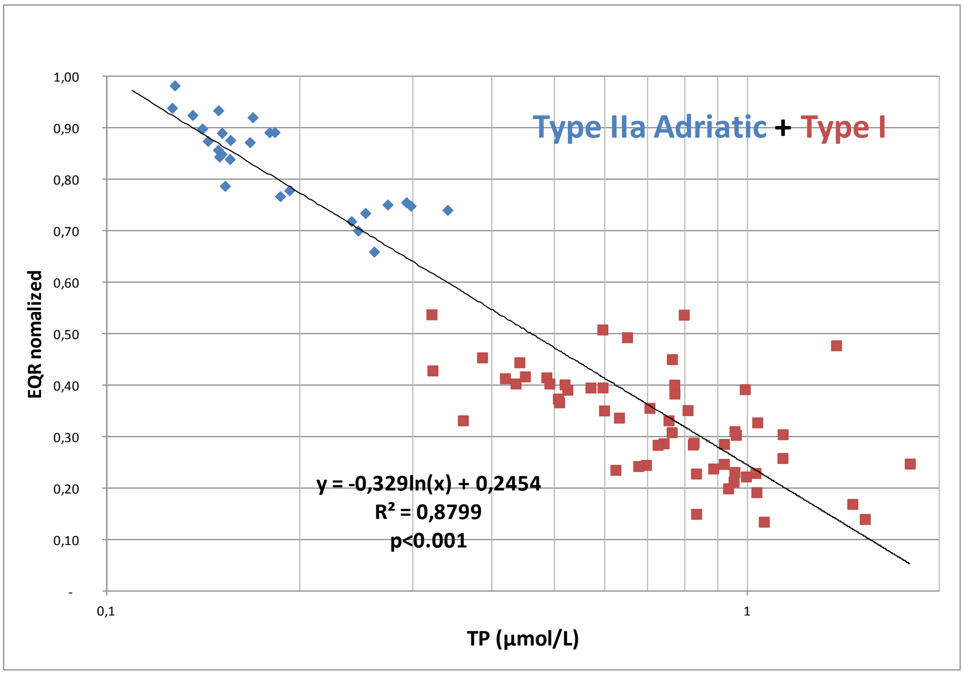



**Figure 9.** Response (EQR) vs. pressure (TP) relationship for combined Type I and Type IIA Adriatic.

## TECHNICAL REPORT CONTINUATION PART II

## TYPE IIA + TYPE IIIW + TYPE ISLAND

### FRANCE AND SPAIN INTERCALIBRATING

The group has defined reference conditions.

**Reference conditions**

- Common approach for setting reference conditions (true reference sites or indicative partial reference sites, see Annex III of the IC guidance):

**France and Spain** have selected true reference sites separately but using the same criteria: LUSI values and expert judgement. Afterwards, by using this common data base, ranges for reference conditions for each typology were set. Finally, reference conditions were set within these ranges (see below for further explanations). So, expert judgement approach was used to set French and Spanish reference conditions.

- Detailed description of **reference criteria** for screening of sites in near-natural conditions (abiotic characterisation, pressure indicators):

**France and Spain**: chosen reference criteria were according to low values of LUSI (LUSI are based on land uses and continental pressures) and according with several expert judgment criteria. These criteria were indicative of undisturbed sites or sites with only very minor disturbance, so with minor pressures.

- Identify the **reference sites** for each Member State in each common IC type. Is their number sufficient to make a statistically reliable estimate?

**France and Spain**: 75 reference water bodies were selected and they were sufficient to make an estimate (Please check Annex II-France and Spain working document for the detailed list of reference water bodies).

- Explain how you have screened the biological data for impacts caused by pressures not regarded in the reference criteria to make sure that true reference sites are selected:

**France and Spain**: An expert judgement criteria includes several studies that ensures that true reference sites were selected.

- Give detailed description of **setting reference conditions** (summary statistics used)

**France and Spain common reference conditions**:

Chosen reference criteria were according to low values of LUSI (LUSI are based on land uses and continental pressures) and according with several expert judgement criteria. These criteria were indicative of undisturbed sites or sites with only very minor disturbance, so with minor pressures.

A hierarchical approach for defining reference conditions is suggested using the various methods in the following order (Reference: WFD CIS Guidance Document No. 5 Transitional and Coastal Waters–Typology, Reference Conditions and Classification Systems):

1. An existing undisturbed site or a site with only very minor disturbance (Spatial Data); or
2. historical data and information; or
3. models; or
4. expert judgement.

In case of spatial data, the network shall contain a sufficient number of sites of high status to provide a sufficient level of confidence about the values for the reference conditions, given the variability in the values of the quality elements corresponding to high ecological status for that surface water body type.

**France and Spain worked with spatial data.** Possible reference conditions were selected from the common data base. As **natural reference conditions** were available, alternative benchmark sites (coming from a lower part of the ecological gradient) were not necessary.

WFD CIS Guidance Document No. 5 suggests screening for unimpacted areas using pressure criteria and to identify areas with no or very minor morphological changes. Moreover, it suggests examining biological status of these areas alongside expert judgement to establish if these sites are at high status. For BQE phytoplankton, morphological changes were not taken into account. In France and Spain both, pressures (LUSI) and expert judgement, were taken into account when selecting reference sites.

To select the possible reference conditions several criteria were used. A water body can be chosen as reference WB if:

1. according with WFD CIS Guidance Document No. 5, the WB is an undisturbed site or a site with only very minor disturbance. Within NW Mediterranean coastal waters these characteristics are associated with LUSI values that not exceed 2 for Type III-W and Type Islands and with LUSI values that not exceed 3 for Type II-A, as this typology is naturally affected by freshwater inputs.
2. it was classified as a reference WB area previously to the 2nd IC process by expert judgement. This classification was based on different studies depending on the region. Some of these studies are: IMPRESS documents, high ecological status of others BQEs, high physicochemical status, no risk of breach the WFD environmental objectives, anthropogenic pressures, territory and population analysis, protected natural areas, historical data, etc.

**Intercalibration dataset contain sites in near-natural conditions in a sufficient number.** 75 water bodies were selected. The number of water bodies that fulfils each criteria by typology was variable.

**Table 6:** Number of water bodies that fulfil each criteria.

| Typology | LUSI | Expert judgement |
|---|---|---|
| Island-W | 39 | 4 |
| Type II-A | 8 | 2 |
| Type III-W | 26 | 9 |

The minimum - maximum ranges of 90th percentile Chlorophyll-*a* (µg/L) for each typology, taking into account each criteria were obtained.

Afterwards the minimum and maximum of each type were selected between the obtained values in order to obtain a general range for each typology. In consequence, reference conditions range for France and Spain were established.

**Table 7:** The minimum - maximum ranges of 90th percentile Chlorophyll-*a* (µg/L) for each type, taking into account the two criteria at the same time.

| Typology | Total Range | |
|---|---|---|
| | Min<br>90th percentile Chl-a (µg/L) | Max<br>90th percentile Chl-a (µg/L) |
| Island-W | 0.2 | 1.7 |
| Type II-A | 0.8 | 1.9 |

| | | |
|---|---|---|
| Type III-W | 0.2 | 2.6 |

Afterwards, reference conditions for each typology were established by expert judgement within these ranges as follows:

**Table 8:** Reference conditions in 90th percentile Chlorophyll-*a* (µg/L) for each type.

| Typology | Reference conditions 90th percentile Chl-a (µg/L) |
|---|---|
| Island-W | 0.6 |
| Type II-A | 1.9 |
| Type III-W | 0.9 |

Note that the reference condition for Type II-A could seem high. This is due because the majority of selected WB presented salinities, or densities, near the higher boundary of the definition of the type (Maximum Salinity=37.5). As a result not all the salinity range of the typology was covered by WB.

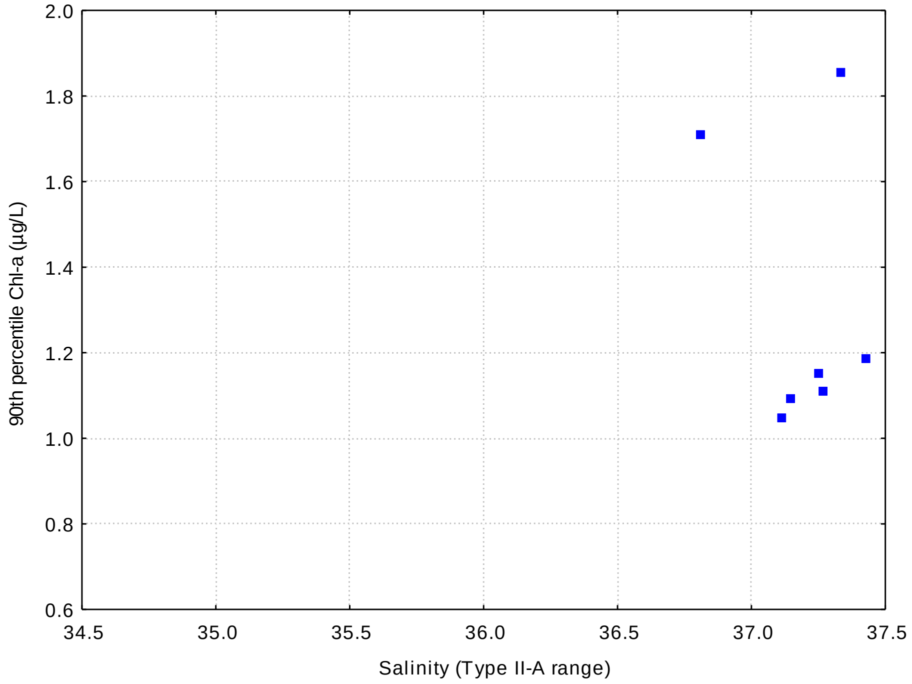



**Figure 14:** 90th percentile of Chlorophyll-*a* (µg/L) of selected WB against salinity range of Type II-A.

In consequence, the reference value for this typology was the maximum value with the established range, as if more WB were available covering all the salinity range of the typology higher values of 90th percentile Chlorophyll-*a* (µg/L) would be obtained.

For Type III-W and Type II-A the reference conditions are similar as those established within the 1st IC process, which were agreed and accepted by all member states. By using different data and different methodologies, the same range of values were obtained within both IC process, indicating the adequacy of the reference conditions obtained.

For France and Spain the reference conditions arise from a group of natural water bodies but are not linked with a concrete one. These water bodies are the following:

**Table 9:** Water bodies used to establish the reference conditions from France, Spain and Italy. They were selected by two criteria (LUSI and expert judgement; see text for more details).

| Country | Code | Water body | Type | LAT* UTM Yº | LON* UTM Xº |
|---|---|---|---|---|---|
| Spain | FO-10 | Entre Punta de ses Pesqueres y Punta de ses Pedreres | Island | | |
| Spain | FO-9 | Entre Punta de sa Gavina y Punta de ses Pesqueres | Island | | |
| France | FREC01ab | Pointe Palazzu - Sud Nonza | Island | 42.64722 | 8.94473 |

| Country | Code | Water body | Type | LAT* UTM Yº | LON* UTM Xº |
|---|---|---|---|---|---|
| France | FREC01c | Golfe de Saint Florent | Island | 42.71212 | 9.27699 |
| France | FREC02ab | Cap Est de la Corse | Island | 42.92184 | 9.47034 |
| France | FREC02c | Littoral Bastiais | Island | 42.61042 | 9.50308 |
| France | FREC02d | Plaine Orientale | Island | 42.02402 | 9.48983 |
| France | FREC03ad | Littoral Sud Est de la Corse | Island | 41.43362 | 9.29022 |
| France | FREC03c | Golfe de Santa Amanza | Island | 41.41940 | 9.23374 |
| France | FREC03eg | Littoral Sud Ouest de la Corse | Island | 41.44455 | 8.97469 |
| France | FREC03f | Goulet de Bonifacio | Island | 41.38975 | 9.15332 |
| France | FREC04ac | Pointe Senetosa-Pointe Palazzu | Island | 41.70907 | 8.68139 |
| Spain | IB-1 | Entre Punta des Jondal y Cap des Mossons | Island | | |
| Spain | IB-2 | Bahía de San Antoni | Island | | |
| Spain | IB-3 | Entre el Cap des Mossons y Punta Grossa | Island | | |
| Spain | IB-4 | Entre Punta Grossa y Cala Llenya | Island | | |
| Spain | IB-5 | Entre Cala Llenya y Punta Blanca | Island | | |
| Spain | IB-6 | Entre Punta Blanca y Punta des Andreus | Island | | |
| Spain | IB-7 | Entre Punta des Andreus y Punta de Sa Mata | Island | | |
| Spain | IBFO-8 | Els Freus de Eivissa y Formentera | Island | | |
| Spain | MA-1 | Entre Cala Falcó y Punta Negra | Island | | |
| Spain | MA-10 | Entre Punta des Jonc (Portocolom) y Cala Figuera | Island | | |
| Spain | MA-11 | Entre Cala Figuera y Cala Beltrán | Island | | |
| Spain | MA-13 | Entre Cala Beltrán y Cap de Regana | Island | | |
| Spain | MA-14 | Entre el Cap de Regana y el Cap Enderrocat | Island | | |
| Spain | MA-15 | Entre el Cap de Enderrocat y Cala Major | Island | | |
| Spain | MA-16 | Entre Cala Major y Cala Falcó | Island | | |
| Spain | MA-2 | Bahía de Santa Ponça | Island | | |
| Spain | MA-3 | Entre Punta Negra e Isla de Formentor | Island | | |
| Spain | MA-4 | Bahía de Soller | Island | | |
| Spain | MA-5 | Bahía de Pollença | Island | | |
| Spain | MA-6 | Entre el Cap Pinar y la Isla d´Alcudia | Island | | |
| Spain | MA-7 | Bahía de Alcudia | Island | | |
| Spain | MA-9 | Entre el Cap de Capdepera y Portocolom | Island | | |
| Spain | ME-1 | Entre el Cap de Bajolí y Punta Prima | Island | | |
| Spain | ME-2 | Bahía de Fornells | Island | | |
| Spain | ME-3 | Puerto de Mahón | Island | | |
| Spain | ME-4 | Entre Punta Prima y Punta de na Bruna | Island | | |
| Spain | ME-5 | Entre Punta de na Bruna y Cap de Bajolí | Island | | |
| Spain | 2 | Sierra de Irta | Type II-A | 4464195 | 782985 |
| Spain | 3 | 3 | Type II-A | | |
| Spain | C01 | Portbou-Llançà | Type II-A | 4698350 | 514395 |

| Country | Code | Water body | Type | LAT* UTM Yº | LON* UTM Xº |
|---|---|---|---|---|---|
| Spain | C03 | Cap de Creus | Type II-A | 4688066 | 516647 |
| Spain | C09 | L'Escala | Type II-A | 4665235 | 510008 |
| Spain | C12 | Pals-Sa Riera | Type II-A | 4650683 | 516428 |
| Spain | C15 | Blanes-Pineda de Mar | Type II-A | 4613452 | 482647 |
| France | FRDC05 | Côte Bleue | Type II-A | 43.27645 | 5.17304 |
| Spain | 11 | Cabo San Antonio-Punta de Moraira | Type III-W | | |
| Spain | 12 | Punta de Moraira-Peñón de Ifach | Type III-W | 4285399 | 770068 |
| Spain | 14 | 14 | Type III-W | | |
| Spain | 16 | 16 | Type III-W | | |
| Spain | 17 | 17 | Type III-W | | |
| Spain | 18 | 18 | Type III-W | | |
| Spain | 19 | 19 | Type III-W | | |
| Spain | 102 | Cabo de Palos-Punta Espada | Type III-W | | |
| Spain | 103 | Punta Espada-Cabo Negrete | Type III-W | | |
| Spain | 105 | La Manceba-Punta Aguilones | Type III-W | | |
| Spain | 108 | Cabo Tiñoso-Punta de la Azohía | Type III-W | | |
| Spain | 610019 | Cabo de Gata - Límite del PN Cabo de Gata | Type III-W | | |
| Spain | 610020 | Límite del PN Cabo de Gata - Limite demarcación mediterránea andaluza / Segura | Type III-W | | |
| Spain | C05 | Cap Norfeu | Type III-W | 4681369 | 523139 |
| Spain | C06 | Canyelles | Type III-W | 4675799 | 518148 |
| Spain | C10 | Montgrí | Type III-W | 4663309 | 512655 |
| Spain | C14 | Begur-Blanes | Type III-W | 4647151 | 517774 |
| Spain | C23 | Sitges | Type III-W | 4568361 | 410407 |
| Spain | C24 | Vilanova i la Geltrú | Type III-W | 4564508 | 398566 |
| Spain | C25 | Cubelles-Altafulla | Type III-W | 4561996 | 389298 |
| Spain | C26 | Tarragona Nord | Type III-W | 4554821 | 363189 |
| Spain | C28 | Cap de Salou | Type III-W | 4547705 | 347316 |
| Spain | C31 | Vandellós i L'Hospitalet de l'Infant | Type III-W | 4544221 | 329486 |
| Spain | C32 | L'Ametlla de Mar | Type III-W | 4531767 | 317912 |
| France | FRDC07a | Iles de Marseille hors Frioul | Type III-W | 43.19403 | 5.37378 |
| France | FRDC07b | Cap Croisette - Bec de l'Aigle | Type III-W | 43.17353 | 5.41776 |
| France | FRDC07h | Iles du soleil | Type III-W | 43.02925 | 6.32871 |
| France | FRDC08d | Ouest Fréjus - Pointe de la Galère | Type III-W | 43.42261 | 6.89776 |

* Note: France used the ETRS89 Projection.
º Note: Spain used the zone 30 in Valencia and the 31 in Catalonia, both in the N hemisphere.

As two countries defined the same reference conditions, a common approach to compare, align and harmonise the criteria to select reference sites was not necessary.

**As a conclusion, reference conditions in 90th percentile of Chlorophyll-*a* are 0.6 µg/L for Typology Island-W, 1.9 µg/L for Type II-A and 0.9 µg/L for Type III-W for France and Spain.**

**Checking of compliance of reference conditions**

**Chosen reference conditions for France and Spain for all typologies are according with the normative definitions** (Definitions of the biological quality elements at high status in Annex V Table 1.2.3 and Table 1.2.4.).

The HIGH status normative definition for coastal water phytoplankton is:

*"Coastal Phytoplankton High status: The composition and abundance of the phytoplanktonic taxa are consistent with undisturbed conditions. The average phytoplankton biomass is consistent with the type-specific physico-chemical conditions and is not such as to significantly alter the type specific transparency conditions. Planktonic blooms occur at a frequency and intensity which is consistent with the type specific physicochemical conditions*" (Reference: WFD CIS Guidance Document No. 5 Transitional and Coastal Waters– Typology, Reference Conditions and Classification Systems).

***Test performed with minimum values from FR and SP as RC.***

The test with was performed using the lowest values of Chlorophyll-*a* for each type and using equidistant EQR boundaries. This serves as a comparison to the Croatian-Slovenian-Italian way of reference conditions derivation.

Table 1: Minimum reference conditions derived from the common data set.

| Type | 90th percentile Chlorophyll-*a* (µg/L) |
|---|---|
| Island-W | 0.16 |
| Type II-A | 0.80 |
| Type III-W | 0.20 |

Results of the quality assessment with these criteria are:

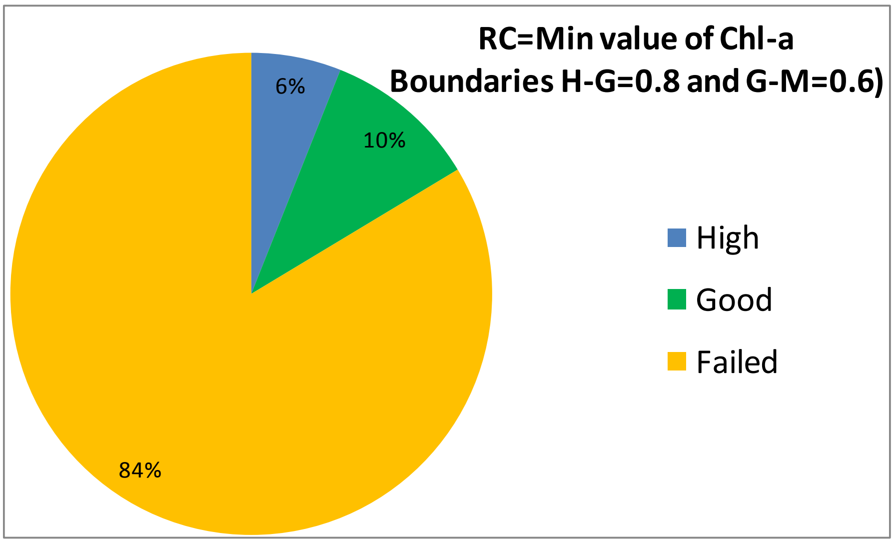



**Figure 1:** Quality assessment using minimum observed Chlorophyll-*a* concentrations as the reference.

The conclusion of this test is that the quality assessment using minimum observed Chlorophyll-*a* concentrations as the reference is not reflecting the FR and SP coastal quality according to expert judgement.

For comparison reasons, please find enclosed the graph of the quality assessment using RC and EQR agreed from FR and SP in the 2nd IC.

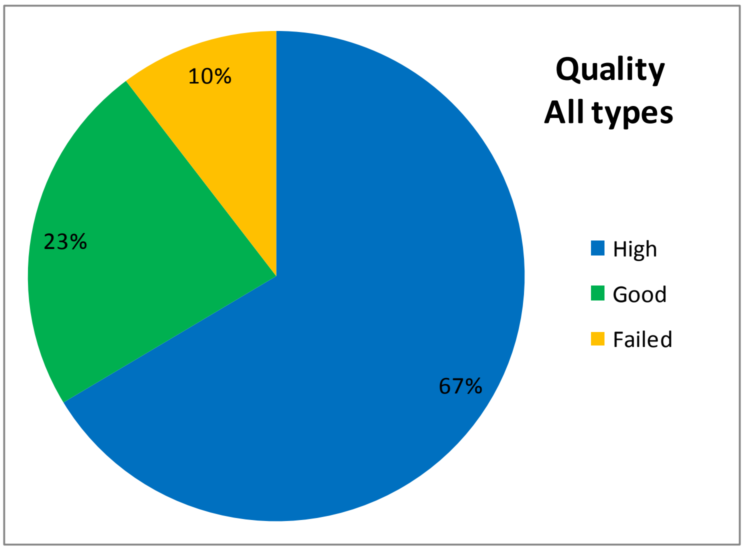


**Figure 2:** Quality assessment using RC and EQR agreed from FR and SP in the 2nd IC.
This is in clear agreement with the expert judgement regarding coastal quality based on the EQR phytoplankton.

## 3.4 BENCHMARK STANDARDIZATION

Before performing a common intercalibration is necessary to check that French and Spanish data are comparable. We based our investigation on some analysis performed with our data base and on what is widely known on Chlorophyll-*a* distribution in the W Mediterranean Sea. First, we will deal with literature available.

**What is known?**

**In the Mediterranean Sea, there is a known basin-scale east-west gradient in the Chlorophyll-a distribution.** We have an extremely oligotrophic Eastern basin and a more productive Western side. Furthermore, **within the Western basin this is also a noticeable gradient.** For further explanations, please find these references:

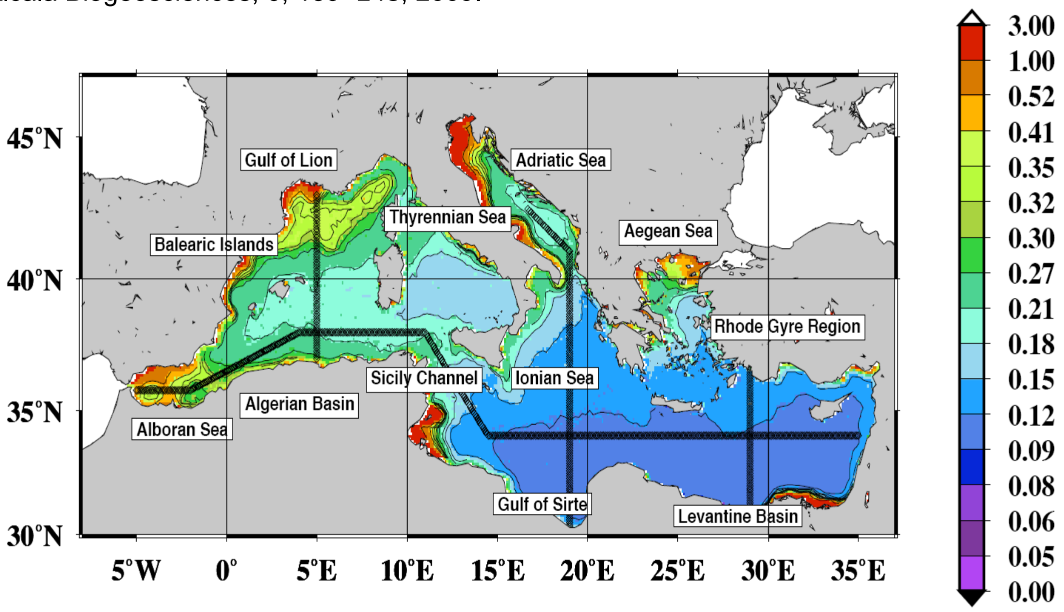
Algal blooming patterns and anomalies in the Mediterranean Sea as derived from the SeaWiFS data set (1998–2003). V. Barale, J. Jaquet, M. Ndiaye. Remote Sensing of Environment 112 (2008) 3300–3313:

- Fig. 2. CZCS-derived (1979–1985) climatological Chlorophyll monthly means, from the OCEAN database, for the Mediterranean Sea.
- Fig. 3. SeaWiFS-derived (1998–2003) climatological Chlorophyll yearly mean, Mediterranean Sea. Note: a 3D enhancement has been applied to the colour coded data, in order to better highlight Chlorophyll patterns and gradients.
- Fig. 4. SeaWiFS-derived (1998–2003) climatological Chlorophyll monthly means, for the Mediterranean Sea.
- Fig. 12. Selected SeaWiFS-derived Chlorophyll daily images, north-western near-coastal area (upper row, from left to right: 25–29 April and 4-14-16 May, 2002) and south-eastern near coastal area (lower row, from left to right: 11-13-18-20-22 June, 2001) of the Mediterranean Sea.

Plankton in the open Mediterranean Sea: a review. I. Siokou-Frangou, U. Christaki, M. G. Mazzocchi, M. Montresor, M. Ribera d'Alcala, D. Vaque, and A. Zingone Biogeosciences, 7, 1543–1586, 2010

- Fig. 6. Spatial distribution of the seven bioprovinces derived from the analysis of the SeaWiFS Chlorophyll-*a* dataset (D'Ortenzio and Ribera d'Alcala, 2009).

On the trophic regimes of the Mediterranean Sea: a satellite analysis. F. D'Ortenzio and M. Ribera d'Alcala Biogeosciences, 6, 139–148, 2009.




- **Figure 1:** Ten years climatological mean map of the Chlorophyll concentration in mg/m3, with, over-imposed, the geographical locations of the regions cited in the text. Bold lines indicate the position of the four transects used to extract satellite data. *Source: D'Ortenzio and M. Ribera d'Alcala, 2009. On the trophic regimes of the Mediterranean Sea: a satellite analysis. Biogeosciences, 6, 139–148.*

- Fig. 2. Hoevmoeller diagram on the West-East transect of Normalized Chlorophyll concentration (see Fig. 1 for the geographical position of the transect). Normalized Chlorophyll is calculated normalizing the values along the transect by the maximum value of the transect.

**This work clearly delimitate regions or bio-regions based on the Chlorophyll-*a* data. One of the regions, the NW Mediterranean region, includes Spain and France.**

Links to download the files:

- https://webmail.csic.es/bigfiles/descarga.php?l=46653043g&t=1309504046&f=Dortenzio_2009_Biogeoscience.pdf
- https://webmail.csic.es/bigfiles/descarga.php?l=46653043g&t=1309504046&f=Barale_2008.pdf
- https://webmail.csic.es/bigfiles/descarga.php?l=46653043g&t=1309504046&f=SiokuFrangou_2010_Biogeosciences.pdf

Moreover temporal variability has already been described for the Spanish and French area and it is of the same order of magnitude between both countries. For more information please find Morales Blake, A. 2006. Distribución horizontal y estacional de los niveles tróficos en el MNO, obtenidos a partir de composiciones mensuales climatológicas de la clorofila superficial del mar. PhD.

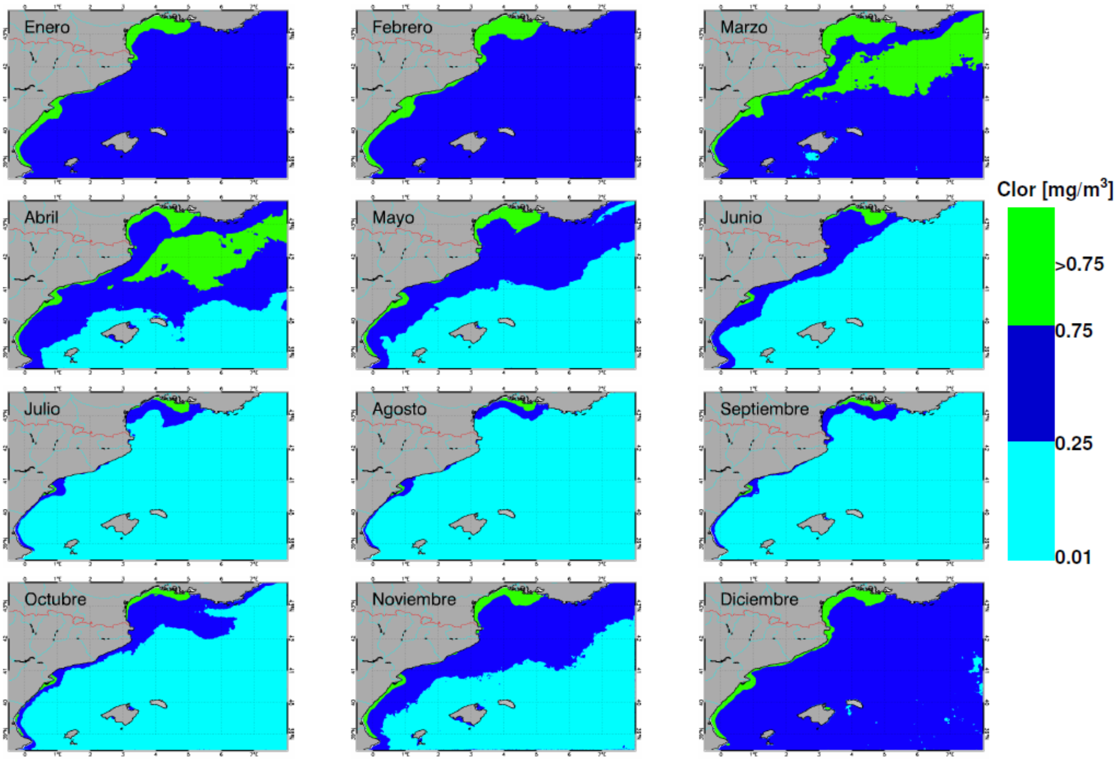



**Figure 2:** Distribución horizontal y estacional de los niveles tróficos en el MNO, obtenidos a partir de composiciones mensuales climatológicas de la clorofila superficial del mar. Source: Morales Blake, A. 2006. Distribución horizontal y estacional de los niveles tróficos en el MNO, obtenidos a partir de composiciones mensuales climatológicas de la clorofila superficial del mar. PhD.

**There is temporal variability within Spanish and French areas and it shows the same magnitude within each country.**

**In situ measured concentrations of Chlorophyll-*a* of France and Spain are according with satellite data and literature.**

## Descriptive statistics of French and Spanish data

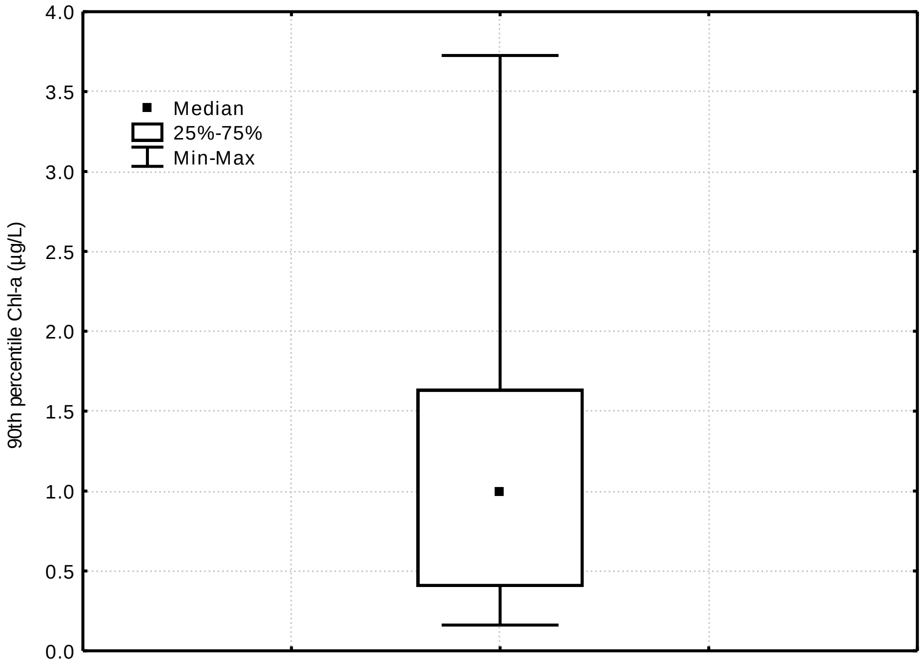



**Figure 3:** Descriptive statistics of 90th percentile Chlorophyll-*a* (µg/L) of the common data base (n= 151)

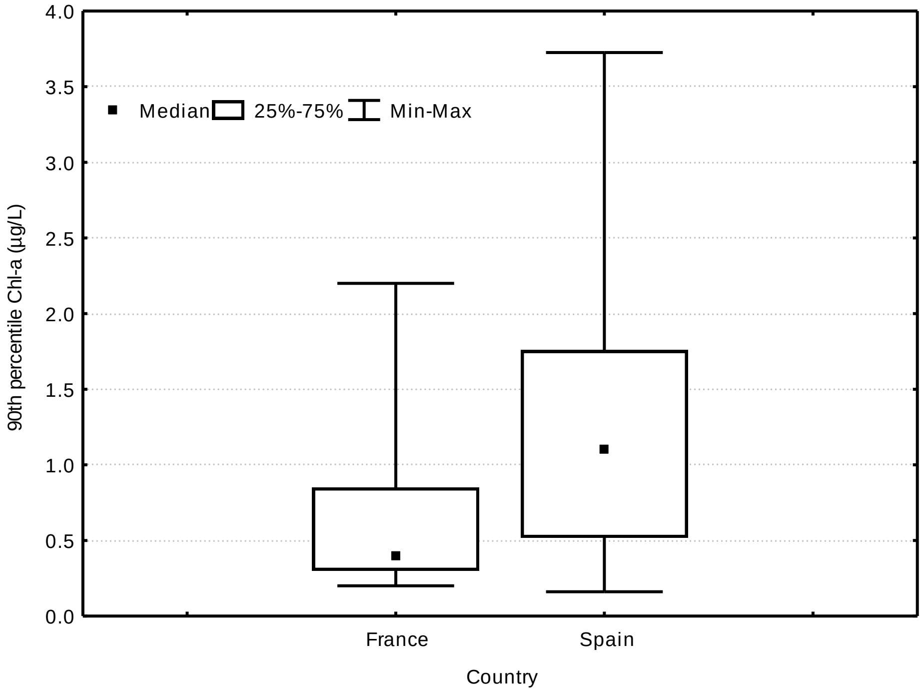



**Figure 4:** Descriptive statistics of 90th percentile Chlorophyll-*a* (µg/L) of the common data base by country.

**Taking in to account all the values, even though maximum French values are lower than maximum Spanish values, ranges (25%-75%) from both countries overlap.**

## Coefficients of variation

The coefficients of variation are used as a statistical measure of the dispersion of data.

**Table 4:** variation coefficients of 90th percentile Chlorophyll-*a* (µg/L) of the common data base by countries.

| Country | N | CV of P90 |
|---|---|---|
| Spain | 92 | 70.66 |
| France | 24 | 85.06 |

**Both countries show similar values of CV.**

**<u>Test of normality of the common data set</u>**

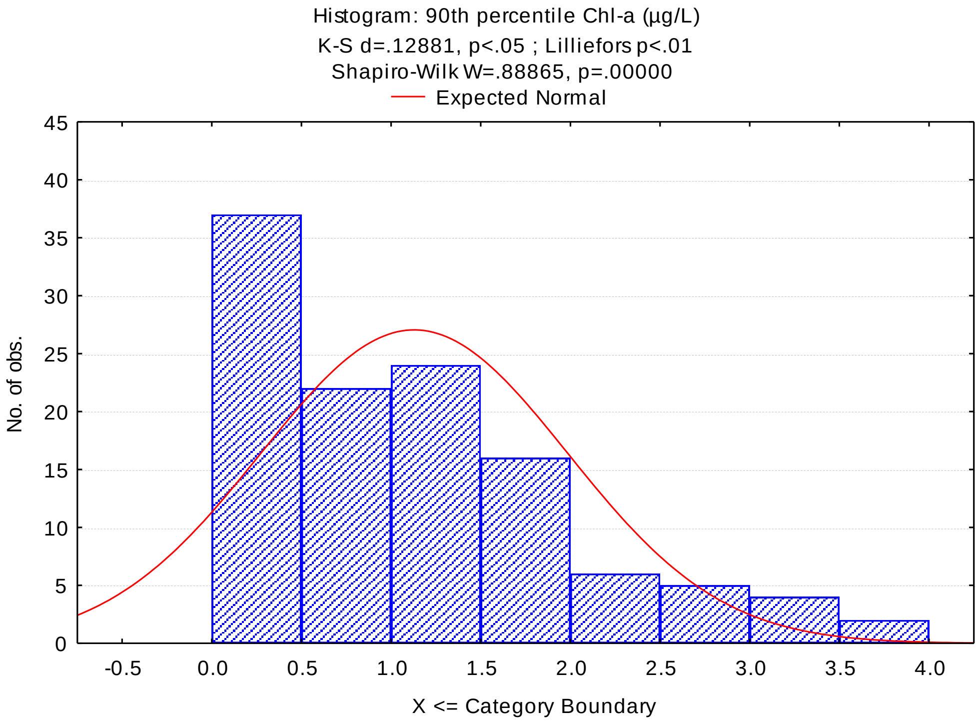



**Figure 5:** Histogram of 90th percentile Chlorophyll-*a* (µg/L) of the common data set. Results of tests of normality are shown. STATISTICA Software was used.

**Distribution of 90th percentile Chlorophyll-*a* (µg/L) is not normal, thus non parametric statistics should be used for data analysis.**

**<u>Evaluating differences between groups using non parametric tests</u>**

Non parametric tests were performed, using Bray Curtis similarity, in order to check if there were significant differences between country data. Primer Software was used.

A One-Way Analysis of Similarities (One-Way ANOSIM) was performed with the following results:

> <u>Test:</u>
> Sample statistic (Global R): 0,08
> Significance level of sample statistic: 2,7%
> Number of permutations: 999999 (Random sample from a large number)
> Number of permuted statistics greater than or equal to Global R: 26852

**There are no significant differences between 90th percentile (µg/l Chlorophyll-*a*) data from Spain and France.**

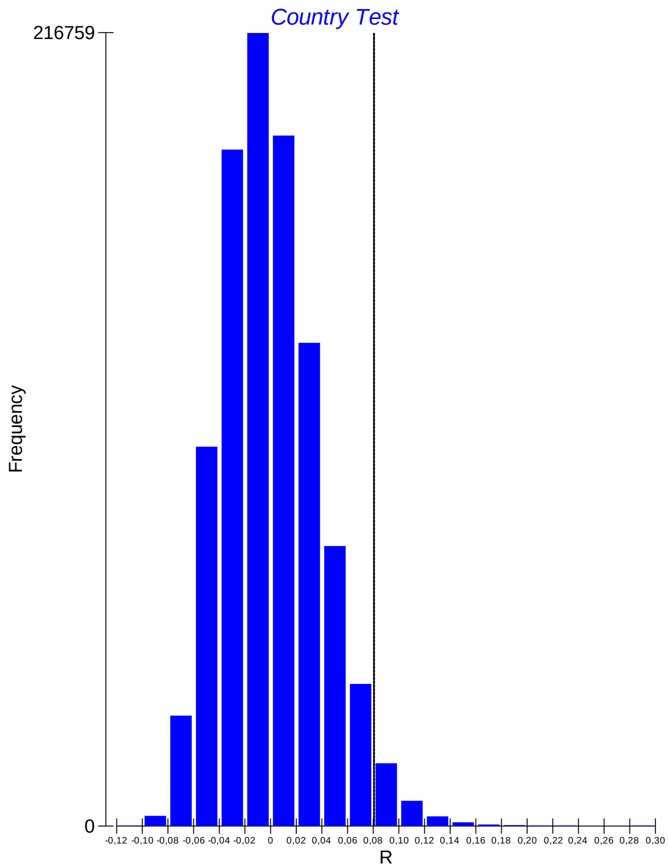



**Figure 6**: Histogram of expected values and sample statistic.

In addition a Hierarchical Cluster analysis was also performed to corroborate the conclusions. In this case Group average was used.

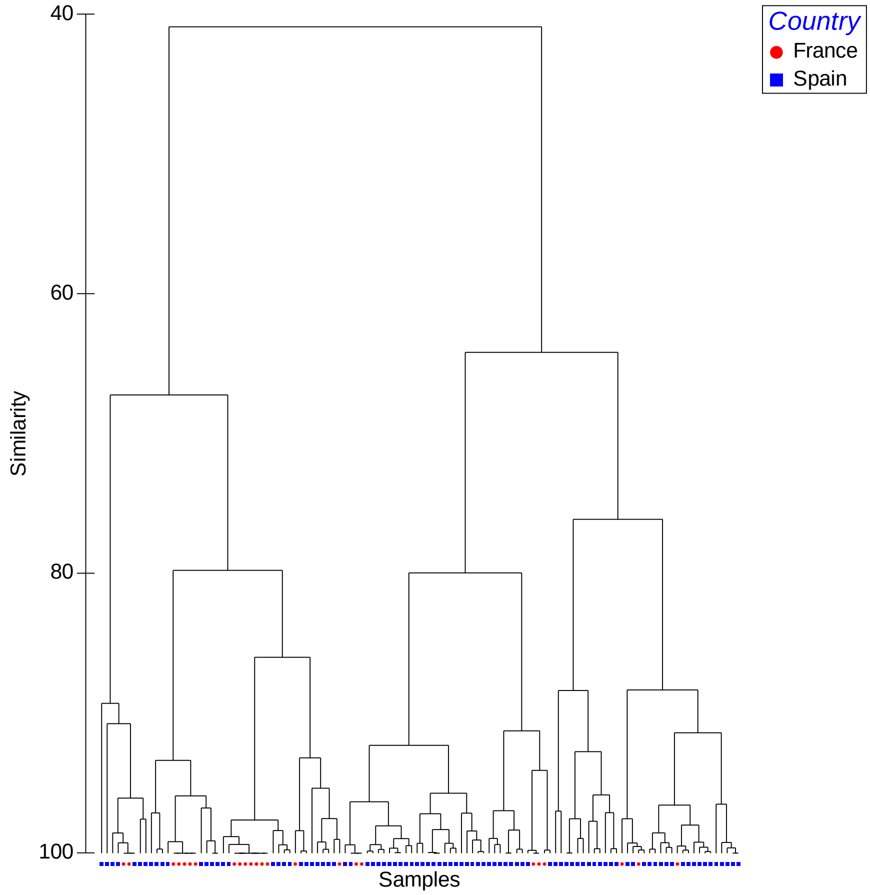



**Figure 7**: Cluster analysis of the common data base. Country was specified.

Cluster analysis visually confirm previous conclusion: there are no differences in the Chlorophyll-*a* data between countries.

**Summarizing, there is a gradient within the Western Mediterranean basin but there are no significant differences between 90th percentile (µg/l Chlorophyll-*a*) data from the two countries.**

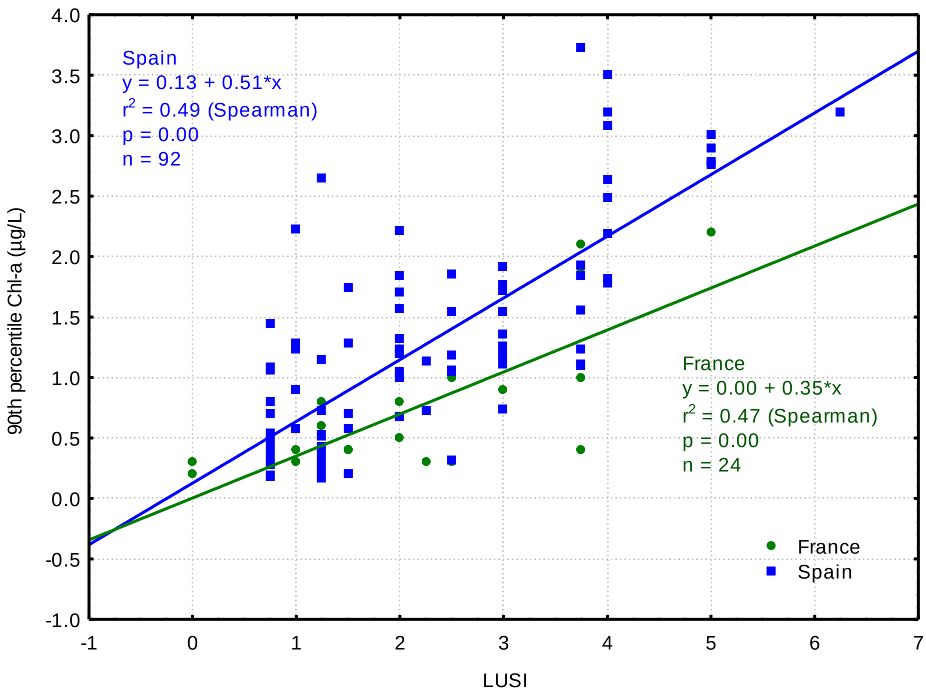



**Figure 10**: Relationship between pressure (LUSI) and impact data (90$^{th}$ percentile µg/l Chlorophyll-*a*) by countries.

**Common data shows a significant relationship between pressure and impact by countries.**

Afterwards a statistical test was performed in order to check if there were significant differences between the slopes and intercepts of impact-pressure relationships between countries. Prism software was used and the method used is equivalent to an Analysis of Covariance (ANCOVA).

> <u>Test:</u>
> **There were no significant differences between slopes of impact-pressure relationships from the two countries (F = 2.75, p=0.10) but there were significant differences between intercepts (F = 12.78, p=0.00).**

There is a variability range of Chlorophyll-*a* values due to natural factors within the Western Mediterranean basin. This range could be slightly different from one area to another but these values are low and are related to non disturbed conditions. On the contrary, high values of Chlorophyll-*a* are typically due to continental pressures related to human activities. In general, problematic values of Chlorophyll-*a* are much more higher than natural background values. Therefore, impact-pressure relationships from France and Spain are similar and their slopes are not significantly different.

**The Intercalibration is feasible in terms of pressures-impact relationship based on French and Spanish data.**

However, as there were differences between intercepts and after the Validation Workshop of the WFD intercalibration, held in Italy last November, these relationships were plotted by country and type (we excluded Type Island-W) at the same time, in order to detect potential biogeographical differences taking into account both factors:

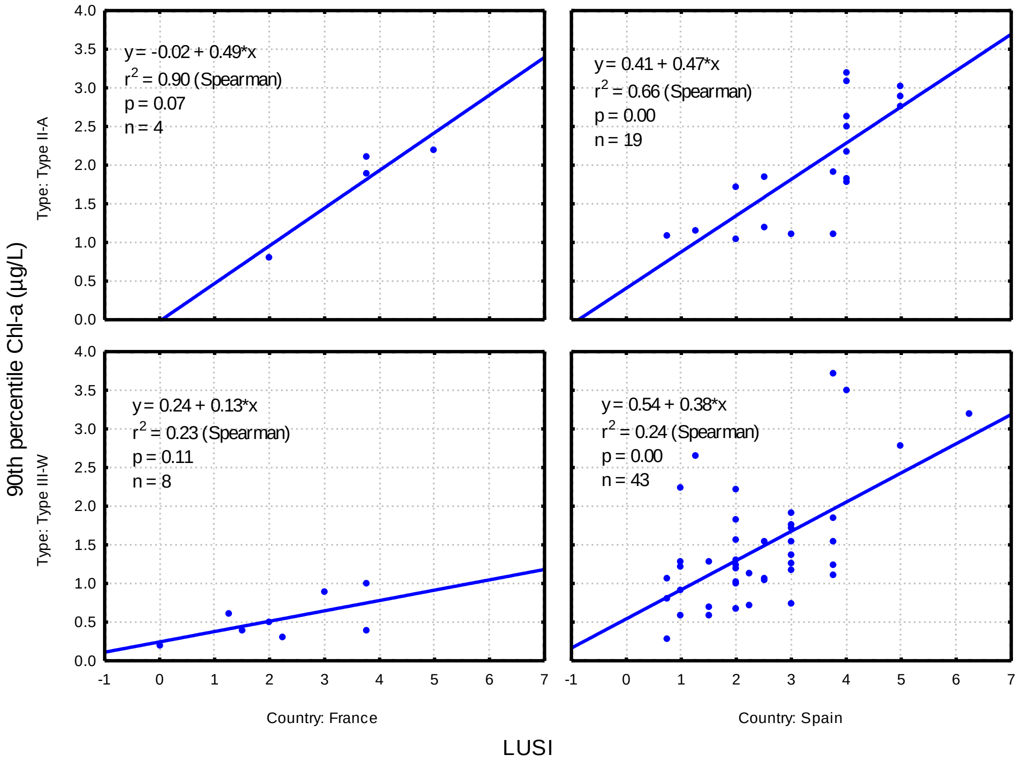



**Figure 11**: Relationship between pressure (LUSI) and impact data ($90^{th}$ percentile µg/l Chlorophyll-*a*) by countries and typologies.

In this case also several tests were performed in order to check if there were significant differences between the slopes and intercepts of impact-pressure relationships between countries by typologies.

> **Regarding, Type II-A, there were no significant differences between slopes of impact-pressure relationships from the two countries (F = 0.01, p=0.94) and there were no significant differences between intercepts (F = 1.85, p=0.19).**

**Regarding, Type III-W, there were no significant differences between slopes of impact-pressure relationships from the two countries (F = 1.58, p=0.21) but there were significant differences between intercepts (F = 12.69, p=0.00).**

These preliminary results seemed to show that there were potential biogeographical differences between countries taking into account Type III-W. In consequence, France and Spain explored and apply the methodology of continuous benchmarking using a generalized linear model (GLM) in order to determine the offsets between countries and the common model and be able to perform the boundary comparison and, if necessary, the boundary harmonisation (*cf* annex ?).

Even if this procedure seemed not necessary for Type II-A it was performed for both typologies. The conclusion of these statistical exercise is explained in this document, but finally recected as justified, as agreed during the ECOSTAT meeting at JRC Ispra, in March 2012.

# 4 COMPARISON OF METHODS AND BOUNDARIES

## 4.1 IC OPTION AND COMMON METRICS

- IC option: Option 1 with common national boundary setting.

- Explanation for the choice of the IC option:

**France and Spain** use the same assessment method, same data acquisition and same numerical evaluation. Common boundary setting procedure was worked out by France and Spain at the scale of common IC types using **IC Option 1 (same assessment method, same data acquisition and same numerical evaluation).**

During all that process, there were parallel discussions about which was the most appropriate metric to use within the water quality assessment based on BQE Phytoplankton. The use of geomean has some constrains for the main goal of the IC, as geomean discards some high values of chlorophyll-a that must be taken into account for the assessment of WB Quality. We have done a study comparing the evaluation of the quality based on chlorophyll-a using mean and geomean. We found that the evaluation done with means is more adequate as reflects better the phytoplankton biomass. Moreover, working with geomean implies the risk of dismiss situations that are not very common but that have to be taking into account in the assessment of the water body quality (e.g. considering blooms as outliers). Since i) Spain is able to work with 90th percentile and means indistinctly and ii) France is using 90th percentile; the final decision is to use 90th percentile for the intercalibration between France and Spain.

**The IC for France and Spain is based on 90th percentile of Chlorophyll-*a* in µg/l.**

## 4.2 DESCRIPTION OF BOUNDARY SETTING PROCEDURE SET FOR THE COMMON IC TYPE

Summarize how boundaries were set following the framework of the BSP:

– Description how the full procedure is applied (use of discontinuities, paired metrics, equidistant division of continuum)

**France and Spain:**

Before reaching the final conclusions we have performed different tests with data provided from France and Spain following the guidance document Nº14 on the intercalibration process

Briefly, there were not discontinuities in the relationship between the metric and the gradient of impact represented by the data set (Step 4). France and Spain were not able to use paired metrics to assess class centres or class boundaries (Step 6). Afterwards (Step 8), France and Spain divided the continuum of impact below the high-good boundary into four equal width classes but the values of the metric of the quality element represented at the good and moderate status class boundaries did not agree with the normative definitions. Finally, France and Spain revised the boundaries by expert judgement until values represented in the good and moderate status classes were consistent with the normative definitions.

Boundary values obtained are the result of a combination of both historical data analysis and expert judgment. Specific approach for H/G boundary was derived from metric variability at high and good status by expert judgement according with normative definitions, and specific approach for G/M boundary was derived from expert judgement.

**Table 10:** High-Good and Good-Moderate boundaries in terms of Ecological Quality Ratios and Chlorophyll-*a* for each typology.

| Typology | Ecological Quality Ratios | | Chlorophyll-*a* (based on 90th percentile in µg/l of Chlorophyll-*a*) | |
|---|---|---|---|---|
| | High-good boundary | Good-moderate boundary | High-good boundary | Good-moderate boundary |
| Type II-A | **0.80** | **0.53** | **2.38** | **3.58** |
| Type III-W | **0.80** | **0.50** | **1.13** | **1.80** |
| Type Island-W | **0.80** | **0.50** | **0.75** | **1.20** |

**Boundaries in terms of 90th percentile Chlorophyll-*a* are according with the chosen RC.**

**Boundaries in terms of EQR are distributed along the 1-0 gradient.**

**Good-moderate EQR boundary values are positioned with coherence within the gradient (0 and 1), being around 0.5.**

- Provide pressure-response relationships (describe how the biological quality element changes as the impact of the pressure or pressures on supporting elements increases)

**France and Spain:** To assess pressure France and Spain have used LUSI values (LUSI are based on land uses and continental pressures. Please find below Annex II- for further explanations of LUSI). At higher values of LUSI higher values of 90th percentile of Chlorophyll *a* in µg/L.

Please check ***Annex II* - Assessment Pressure methodology - Land Uses Simplified Index (LUSI)** for further explanations of LUSI.

**Common data shows a significant relationship between pressure and impact.**

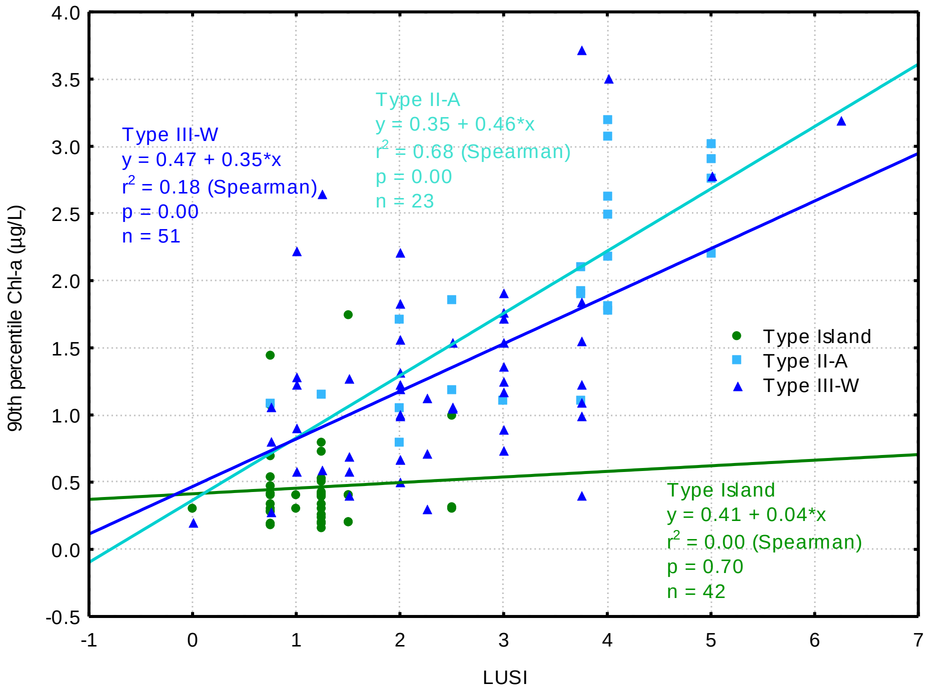



**Figure 9**: Relationship between pressure (LUSI) and impact (90th percentile µg/l Chlorophyll-*a*) data by typologies.

**Common data shows a significant relationship between pressure and impact by typologies, except for type Island-W.**

Island typology does not show a significant relationship between pressure and impact, as Islands receive a major influence from oceanic than continental factors.

– Provide a comparison with WFD Annex V, normative definitions for each QE/ metrics and type

For all IC types, definitions of the metric used (representing Chl *a* biomass) at high, good and moderate status are according the normative definitions.

**Assessment based on intercalibration results**

To check if the water quality assessment based on BQE phytoplankton was adequate, all water bodies of the common database were assessed and compared with knowledge of the areas based on expert judgement.

**Table 12:** Reference conditions and EQR boundaries needed to assess water bodies' quality based on BQE Phytoplankton.

| Type | | Island-W | Type II-A | Type III-W |
|---|---|---|---|---|
| Reference conditions 90th percentile Chlorophyll-*a* in µg/l | | 0.60 | 1.90 | 0.90 |
| Boundaries (EQR) | H/G | 0.80 | 0.80 | 0.80 |
| | G/M | 0.50 | 0.53 | 0.50 |
| | Failed | < 0.50 | < 0.53 | < 0.50 |

The formula of the EQR used was:

$$EQR = \frac{Chl - a \quad reference\_P90}{Chl - a \quad waterbody\_P90}$$

Quality assessment of French and Spanish water bodies results are:

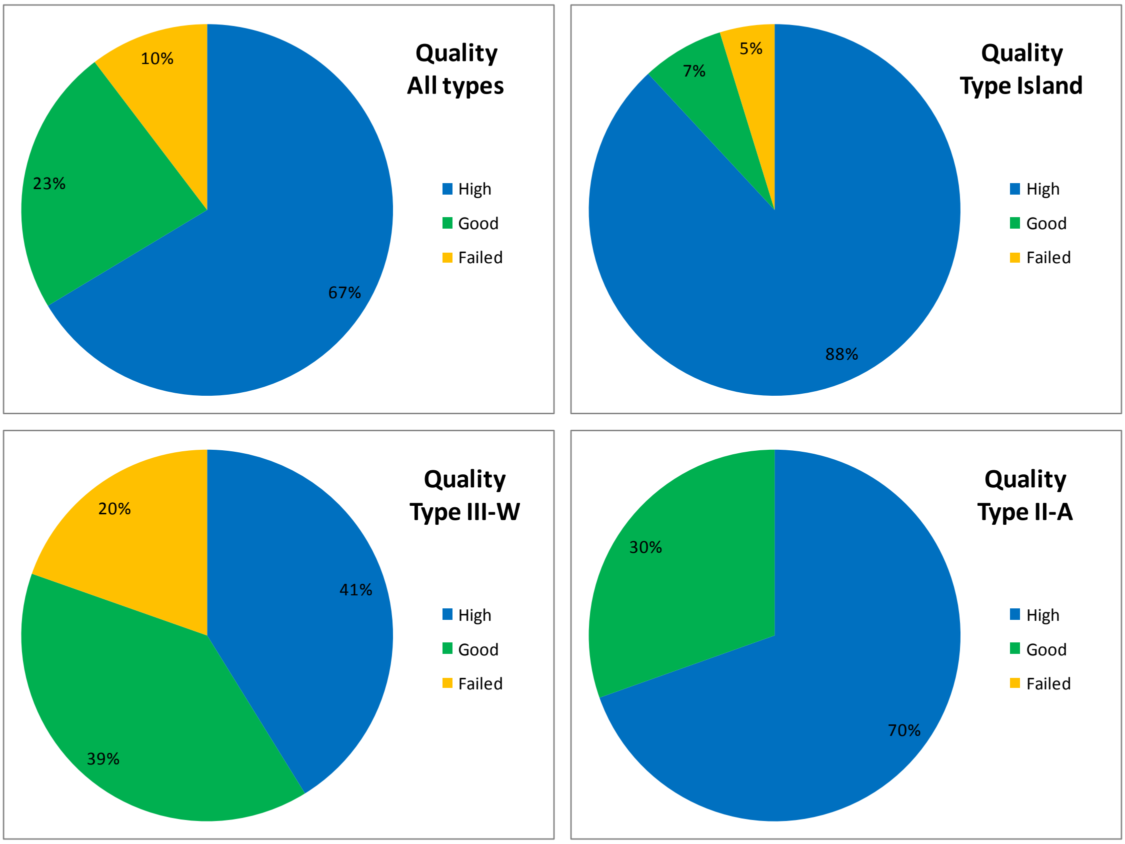


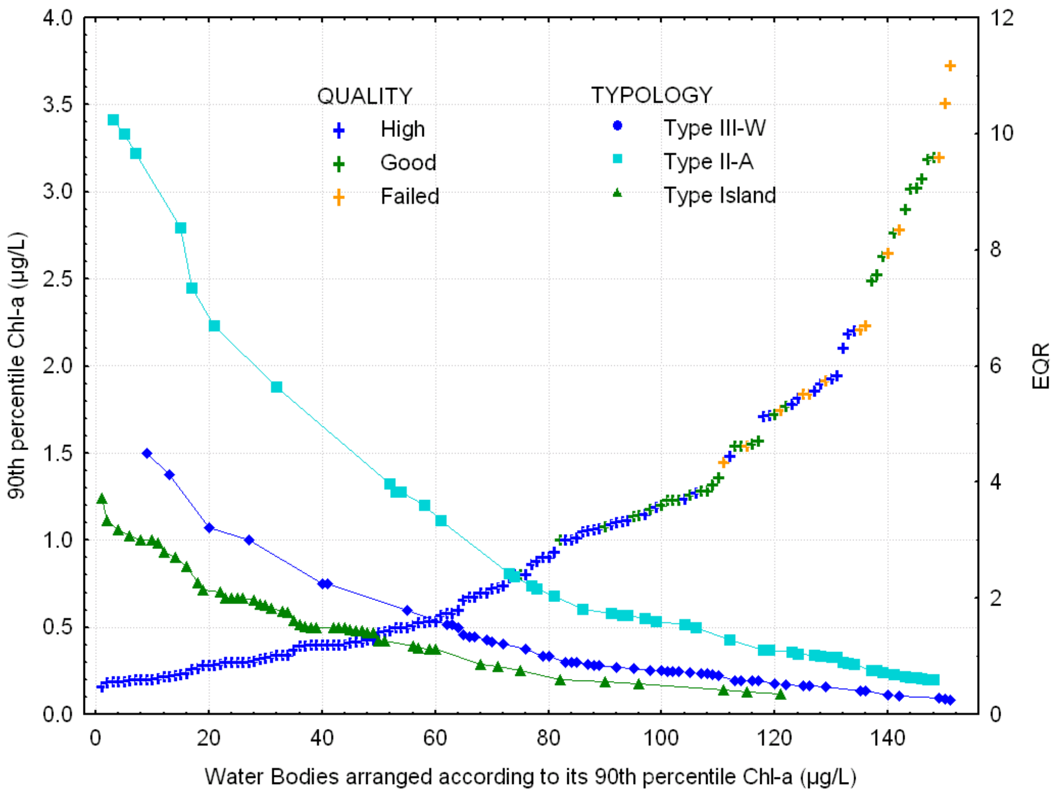

**Figure 15:** Results of the assessment of French and Spanish water bodies using the assessment procedure established during the 2nd IC process.

**These results are in agreement with expert judgement of studied areas, confirming that selected RC and boundaries are adequate.**

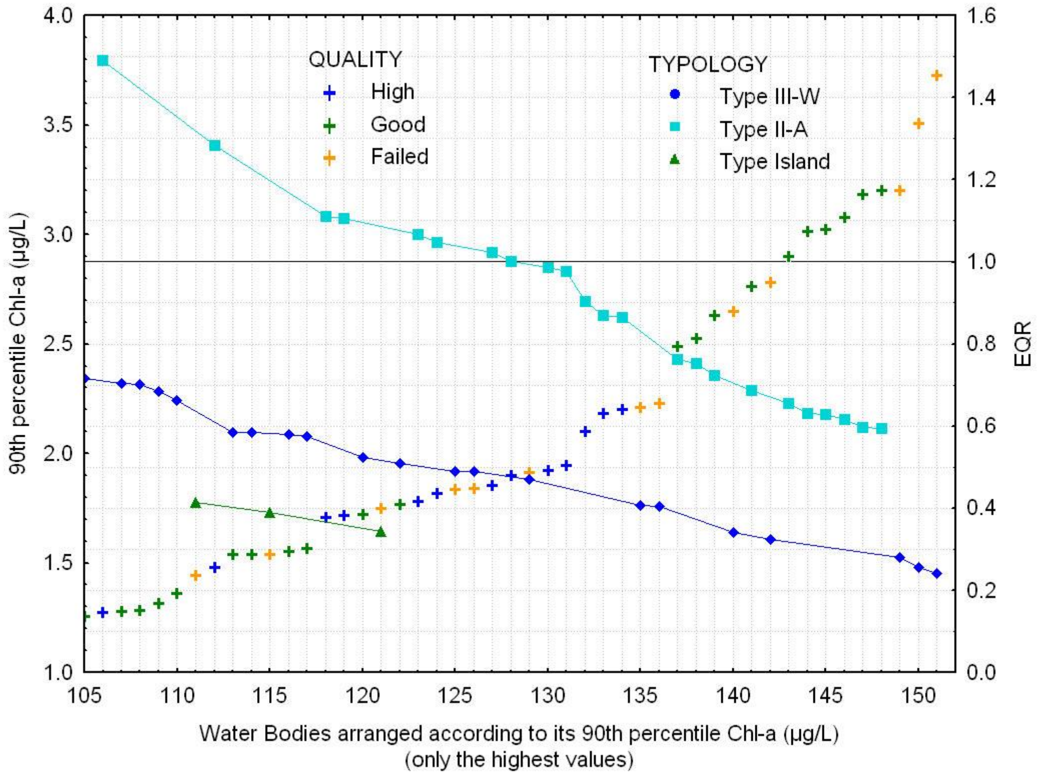
By using this assessment EQR>1 are obtained, as Chlorophyll-*a* values could be lower than the RC. Even though the WFD CIS Guidance Document No. 5 states that EQR should be between 0 and 1, **we suggest the use of EQR > 1 for the BQE Phytoplankton.**

As previously stated, there is a variability range of Chlorophyll-*a* values due to natural factors within the Western Mediterranean basin. This range could be slightly different from one area to another but usually these values are low and are related to non disturbed conditions, so high values of EQR are obtained. On the contrary, high values of Chlorophyll-*a* are typically due to continental pressures related to human activities; in these cases low values of EQR are obtained. In general, problematic values of Chlorophyll-*a* are much more higher than natural background values. This is highly important for management as problematic water bodies could be identified by their low EQR values. Moreover, the EQR value would be an estimation of the degree of anthropogenic impact at which the water body has been submitted. In contrast, non disturbed water bodies would present high EQR values. In this case, the exact value of EQR would be not important as these water bodies would present an acceptable quality status; in addition, only water bodies classified with High status would present EQR higher than 1.

Working with French and Spanish data base, all water bodies classified with a non acceptable quality status (Failed) present high values of 90th percentile of Chlorophyll-*a* and low EQR and only water bodies classified with a High status present EQRs higher than 1 (see figure 12a and b).




a)




b)

**Figure 16:** French and Spanish water bodies arranged by its 90th percentile of Chlorophyll-a (left). For each water body its EQR (right), quality status and typology are indicated: a) all water bodies and b) only water bodies with highest 90th percentile of Chlorophyll-a (including all water bodies classified as Failed).

To obtain maximum EQR=1, the minimum values of Chlorophyll-*a* should be used as reference conditions. **Why is not adequate to use the minimum values of Chlorophyll-*a* as reference conditions?** Oceanic waters (included some nearshore waters), usually low affected by anthropic pressures, shows high variability of Chlorophyll-*a* due to natural, climate and oceanographic causes. This high variability could be observed not only between different geographical areas, but also between annual cycles within the same area, as it was discussed above. Using the minimum value of Chlorophyll-*a* available as a reference conditions results in a breaching of the WFD environmental objectives for a great number of water bodies. Moreover, the major part of these water bodies are not affected by anthropogenic pressures and are assessed as high by others BQE and by expert judgement. A test performed with minimum values from France and Spain data set as RC demonstrate the inadequacy of this criteria and reinforce the process proposed by France, Spain and Italy. This test is detailed in **Test performed with minimum values from France and Spain as reference conditions.**

However, **the option to transform all EQR values that are > 1 to the value of 1 and obtain an EQR range between 0 and 1 is an acceptable option** for two reasons: a) is in agreement with WFD CIS Guidance Document No. 5; and b) the obtained quality assessment is reflecting the FR and SP coastal quality according to expert judgement, so there is no risk of breaching of the WFD environmental objectives.

## 4.3 BOUNDARY COMPARISON AND HARMONISATION

Describe comparison of national boundaries, using comparability criteria (see Annex V of IC guidance).

**France and Spain**, following the suggestions raised out at the Validation Workshop of the WFD intercalibration, held in Italy last November, tested the methodology of continuous benchmarking (see annex ?) using a generalized linear model (providing a data doubling step) in order to determine the offsets between countries and the general model for Type III-W and Type II-A. Afterwards, for each country and typology, these offsets were compared with the corresponding class acceptance value in order to determine if the corresponding boundaries should be adjusted.
After this statistical exercise, the boundaries were not changed due to several weaknesses of the process that are explained below.

**However, there are several weaknesses identified within this process.**

First of all, the pressure-impact relationship of Type III water bodies from France does not have an enough number of observations and presents a relatively high p value in the Spearman regression to be considered statistically acceptable.

Secondly, this separation of the original data set into two groups, splitting French data by one side and Spanish data from the other, is not even conceptually acceptable. There are not significant differences between 90th percentile (µg/l Chlorophyll-a) data from the two countries, as it has been already showed with statistical procedures and reviewed literature, including satellite images of Chlorophyll-a concentration in the Mediterranean Sea. The data variability that exists cannot be addressed at this moment. Accordingly, data from Spain and France should be considered as one unique dataset in order to perform a common boundary setting.

Therefore, the results of the continuous benchmarking procedure are not acceptable for France and Spain, and the use of a correction factor to assess the quality of the Type III French water bodies is not adequate and statistically relevant.

In conclusion, France and Spain should use the Option 1, same assessment method, same data acquisition and same numerical evaluation, to intercalibrate its water bodies.

Considering the above explanations the IC results from France and Spain are:

| Typology | Ecological Quality Ratios | | Chlorophyll-*a* (based on 90th percentile in µg/l of Chlorophyll-*a*) | | |
|---|---|---|---|---|---|
| | High-good boundary | Good-moderate boundary | Reference Conditions | High-good boundary | Good-moderate boundary |
| Type Island-W | **0.80** | **0.50** | **0.60** | **0.75** | **1.20** |
| Type II-A | **0.80** | **0.53** | **1.90** | **2.38** | **3.58** |
| Type III-W | **0.80** | **0.50** | **0.90** | **1.13** | **1.80** |

– Do all national methods comply with these criteria? (Y/N) Yes
– If not, describe the adjustment process:

## TYPE III - E GREECE AND CYPRUS

The coastal waters of Cyprus are classified as **Type III** (no freshwater input – density greater of 27), due to their hydrographical features and the prevailed physicochemical characteristics; in fact mean salinity of coastal waters of Cyprus is 39.1. The annual mean of chl α for the years 2007 to 2010 was 0.05 μg/L while, the calculated 90$^{th}$ percentile ranged from 0.09 to 0.11 respectively. The overall average level of Chl *a* for the entire period, 2007 to 2010, was 0.05 and the respective 90$^{th}$ percentile was 0.10. These values were used for the assessment of the ecological status of the coastal waters of Cyprus according to the Eutrophication Scale, which was developed by Ignatiades *et al*. (1992) and Karydis (1999), and further modified by Siokou & Pagou, 2000; Pagou, 2000) based on nutrient and phytoplankton data collected from several coastal and marine areas from Greece.

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, 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). Recent studies made on phytoplankton biomass in the deeper waters of eastern Mediterranean reveal that prevailing oligotrophic conditions result in low chlorophyll *a* concentrations ranging from 0.1 to 0.2 μg/L (Krom et al., 1992). It has also been shown that chlorophyll *a* concentrations off the coast of Cyprus are among the lowest in the region and ranged from 10 to 90 ng/L (Bianchi et al., 1996). Recent studies along the coastal waters of Cyprus confirmed its oligotrophic status (Argyrou, 2005, 2006).

The group has defined reference conditions.

**Reference conditions**

- Common approach for setting reference conditions (true reference sites or indicative partial reference sites, see Annex III of the IC guidance):

| |
|---|
| **Greece and Cyprus** selected pristine undisturbed areas (Natura 2000) as reference sites. LUSI values were indicative of the non-disturbance of reference sites. |

- Detailed description of **reference criteria** for screening of sites in near-natural conditions (abiotic characterisation, pressure indicators):

| |
|---|
| **Greece and Cyprus** chosen reference criteria were according to low levels of eutrophication (Greek Eutrophicaion Scale) and low values and LUSI which are based on land uses. (see ANNEX II for further explanations of LUSI). |

- Identify the **reference sites** for each Member State in each common IC type. Is their number sufficient to make a statistically reliable estimate?

| |
|---|
| **Greece and Cyprus:** 2 reference sites for Cyprus: Code CY_25-C3-S1, WB name Cape Greco (pristine area, Natura 2000 site) and Code CY_5-C1-S1, WB name Akamas (pristine area, proposed Natura 2000 site). |

- Explain how you have screened the biological data for impacts caused by pressures not regarded in the reference criteria to make sure that true reference sites are selected:

| |
|---|
| |

- Give detailed description of **setting reference condition**s (summary statistics used)

| |
|---|
| |

**Description of boundary setting procedure set for the common IC type**

Summarize how boundaries were set following the framework of the BSP:

– Provide a description how you applied the full procedure (use of discontinuities, paired metrics, equidistant division of continuum)

| |
|---|
| **Greece and Cyprus** Boundary values resulted from modification of the Greek eutrophication scale in line with expert judgement. An eutrophication scale was developed specified for the Greek seas and based on nutrient concentration ranges and phytoplankton parameters including Chl a concentrations (Boundary Setting for Type III-E BQE Phytoplankton (Simboura, *et al.*, 2005). The original scale included four levels of eutrophication: eutrophic, higher, esotrophic, lower mesotrophic and oligotrophic, which |

were modified in order to fit the five-step ecological status scale of the WFD. The lower mesotrophic range was split into two using the median value of the two boundary limits (0.1-0.6), resulting into the Good quality class (0.1-0.4) and Moderate quality class (0.4-0.6). Consequently, H/G boundary derived from boundary between oligotrophic and lower mesotrophic class and G/M boundary from an equidistant split of the lower mesotrophic class, where the median is taken as the G/M boundary.

- Provide pressure-response relationships (describe how the biological quality element changes as the impact of the pressure or pressures on supporting elements increases)

**Greece and Cyprus**
**Cyprus:** The application of the newly proposed Pressure Index LUSI (Flo, Camp & Garces, 2011) elicited a positive and significant correlation between 90th percentile Chl *a* (μg/L) for 10 sites-Water Bodies (17 stations) and the corresponding LUSI values ($N=17$, $r^2= 0.33$ Pearson´s $r= 0.52$, $p<0.05$).
To assess pressure LUSI values were used. Further explanations on LUSI are given in ANNEX II. There is a significant positive correlation between LUSI values (pressure) and Chl *a* (μg/L) concentrations (response).

- Provide a comparison with WFD Annex V, normative definitions for each QE/ metrics and type

For all IC types, definitions of the metric used (representing Chl *a* biomass) at high, good and moderate status are according the normative definitions.

### 3.4 BENCHMARK STANDARDIZATION

No data were provided/analysed in the 2nd phase by Greece, in order to check biogeographical differences, including differences with Cyprus.

## 4 COMPARISON OF METHODS AND BOUNDARIES

### 4.1 IC OPTION AND COMMON METRICS

- IC option: Option 1 with common national boundary setting.

- Explanation for the choice of the IC option:

**Greece and Cyprus** use the same assessment method, same data acquisition and same numerical evaluation.

### 4.2 RESULTS OF THE REGRESSION COMPARISON

Not needed in an Option 1.

### 4.3 COMPARABILITY CRITERIA

Not needed in an Option 1.

# 5 FINAL RESULTS TO BE INCLUDED IN THE EC DECISION

## 5.1 TABLE WITH EQRS

| Member State | Classification Method | Ecological Quality Ratios High-good boundary | Good-moderate boundary |
|---|---|---|---|
| France and Spain - Type IIA | Biomass - Chlorophyll *a* (based on 90th percentile of Chlorophyll *a* in µg/L) | 0.80 | 0.53 |
| France and Spain - Type III-W | Biomass - Chlorophyll *a* (based on 90th percentile of Chlorophyll *a* in µg/L) | 0.80 | 0.50 |
| France and Spain - Type Island-W | Biomass - Chlorophyll *a* (based on 90th percentile of Chlorophyll *a* in µg/L) | 0.80 | 0.50 |
| Italy - Type I | Biomass - Chlorophyll *a* (based on annual geometric mean of Chlorophyll *a* in µg/L) | 0.78 | 0.59 |
| Italy - Type IIA Tyrrhenian | Biomass - Chlorophyll a (based on annual geometric mean in µg/l of Chlorophyll-a) | 0.76 | 0.59 |
| Croatia, Italy and Slovenia - Type IIA Adriatic | Biomass - Chlorophyll *a* (based on annual geometric mean of Chlorophyll *a* in µg/L) | 0.75 | 0.58 |
| Greece and Cyprus – Type III-E | Biomass - Chlorophyll *a* (based on 90th percentile of Chlorophyll *a* in µg/L) | 0.80* | 0.20* |

*EQRs same as in the IC1 exercise included in the 2008/915/EC Commission Decision

**Biological Quality Element** | **Phytoplankton**

**Description of types for coastal waters that have been intercalibrated (applicable for phytoplankton only)**

| Type | Description | Density (kg/m³) | Annual mean Salinity (psu) |
|---|---|---|---|
| **Type I** | Highly influenced by freshwater input | <25 | <34.5 |
| **Type IIA, IIA Adriatic and IIA Tyrrhenian** | Moderately influenced by freshwater input (continent influence) | 25-27 | 34.5-37.5 |
| **Type IIIW** | Continental coast, not influenced by freshwater input (Western Basin). | >27 | >37.5 |
| **Type IIIE** | Not influenced by freshwater input (Eastern Basin) | >27 | >37.5 |
| **Type Island-W** | Island coast, not influenced by freshwater input (Western Basin). | >27 | >37.5 |

**Countries sharing the types**
**Type I:** France, Italy
**Type IIA:** France, Spain
**Type IIAdriatic: Croatia,** Italy, Slovenia
**Type Island-W:** France, Spain
**Type IIIW**: France, Spain
**Type IIIE:** Greece, Cyprus

**Phytoplankton: parameter indicative of biomass (Chlorophyll a)**
**Results coastal waters: Ecological quality ratios and parameter values**
Parameter values are expressed in µg/l of Chlorophyll *a*, for the 90$^{th}$ percentile calculated over the year in at least a five year period. The results relate to geographic areas within the types as described in the technical report.

| Type | Ecological Quality Ratios | | Values (µg/l, 90%ile) | |
|---|---|---|---|---|
| | *High-Good boundary* | *Good-Moderate boundary* | *High-Good boundary* | *Good-Moderate boundary* |
| ***Type I*** | | | | |
| Italy | 0.78 | 0.59 | 7.00 | 17.30 |
| ***Type II-A*** | | | | |
| France | 0.80 | 0.53 | 2.38 | 3.58 |
| Spain | 0.80 | 0.53 | 2.38 | 3.58 |
| ***Type II Adriatic*** | | | | |
| Croatia | 0.75 | 0.58 | 1.58 | 3.81 |
| Italy | 0.75 | 0.58 | 1.58 | 3.81 |
| Slovenia | 0.75 | 0.58 | 1.58 | 3.81 |
| ***Type II Tyrrhenian*** | | | | |
| Italy | 0.76 | 0.59 | 1.06 | 2.19 |
| ***Type Island - W*** | | | | |
| France | 0.80 | 0.50 | 0.75 | 1.20 |
| Spain | 0.80 | 0.50 | 0.75 | 1.20 |
| ***Type III-W*** | | | | |
| France | 0.80 | 0.50 | 1.13 | 1.80 |
| Spain | 0.80 | 0.50 | 1.13 | 1.80 |
| ***Type III-E**** | | | | |
| Cyprus | 0.80 | 0.50 | 0.1 | 0.4 |
| Greece | 0.80 | 0.50 | 0.1 | 0.4 |

*Chl-a vales same as in the IC1 exercise included in the 2008 Commission Decision (see below**)

** For IC2 Cyprus (Type IIIE) followed the WFD definitions similar to other MSs in the group:

Phytoplankton Parameter for: Chlorophyll *a* concentration (same as in IC1 and other Types).

Sampling and analysis: (same as IC1 and other TYpes)

**New Data provided for IC2: YES** (from ongoing monitoring programme)

Reference conditions: Type, number and location of RC same as in IC1 (**new: extended period of RC - new historical data added to RC dataset). Criteria: LUSI values (new)** but still in agreement with initial IC1 criteria. New CY data agree with ref values obtained within IC1 process. Chosen reference conditions for CY for Type IIIE are according with the normative definitions

Boundary Setting: in the IC1 Boundary Setting protocol with Greece, based on modification of the Greek eutrophication scale in line with expert judgment and consensus with other Mss**. In IC2 method tested against pressures using the new LUSI index verifying the H/G boundary of 0,1 µg/l (Comm. Decision 2008/915/EC), because CY stations are in a narrow trophic window not reaching down to the G/M boundary (ultra-oligotrophic Levantine Sea). Almost all CY stations fall in the H/G class (IC1 data and IC2 new data).**

Typology: same as in IC1 (Type IIIE: SAL ≥ 37,5), same as other MSs

Metric: 90th percentile of Chlorophyll-a in µg/l (same as IC1, same as other MSs).

**Pressure indicators (new in IC2): LUSI index**

**Established relationship between Pressure-Metric: YES (new in IC2)**

Assessment concept: same as in IC1 (same as other MSs)

**Overall: Cyprus phytoplankton parameter assessment is in a narrow trophic window (H/G less than 0.1 µg/l)), due to the ultra-oligotrophic nature of the Levantine. This is a well known fact in the scientific literature and has been demonstrated both in IC1 and IC2 with new Chl-a data**

## 5.2 CORRESPONDENCE COMMON TYPES VERSUS NATIONAL TYPES

- Present how common intercalibration types and common boundaries will be transformed into the national typologies/assessment systems (if applicable)

## 5.3 GAPS OF THE CURRENT INTERCALIBRATION

**We consider this intercalibration phase finished for all countries.**

# 6 ECOLOGICAL CHARACTERISTICS

## 6.1 DESCRIPTION OF REFERENCE COMMUNITIES

Description of the **biological communities** at reference sites or at the alternative benchmark, considering potential biogeographical differences:

See the section on the setting of reference conditions.

## 6.2 DESCRIPTION OF GOOD STATUS COMMUNITIES

**Description of IC type-specific biological communities representing the "borderline" conditions between good and moderate ecological status**, considering possible biogeographical differences (as much as possible based on the common dataset and common metrics).

90th percentile of Chl *a* (µg/L):
**France and Spain:**
Type II-A: 3.58
Type III-W: 1.80
Type Island: 1.20

**Croatia, Italy and Slovenia:**
Type I: 17.3
Type IIA Adriatic: 3.81
Type IIA Tyrrhenian: 2.19

**Greece and Cyprus:**
Type III-E: 0.4

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**ANNEX I: Trophic state classification criterion based on TRIX Index**

As a direct measure of the trophic levels of the NW Adriatic coastal waters, a TRophic IndeX (TRIX) was proposed (Vollenweider et al., 1998, Giovanardi and Vollenweider, 2004). TRIX Index formulation is the following:

$$TRIX = (Log10\ [ChA\ a \times aD\%O \times minN \times TP] + k) / m.$$

The four components of the Index represent the fundamental trophic state variables, to say:

a) factors that are direct expression of productivity:
- ChA = chlorophyll *a* concentration, as μg/L;
- aD%O = Oxygen as absolute % deviation from saturation;

b) nutritional factors:
- minN = mineral nitrogen: dissolved inorganic nitrogen, DIN = N(as N-NO3+N-NO2+N-NH4), as μg/L;
- TP = total phosphorus, as μg/L.

The parameters k = 1.5 and m =12/10 = 1.2, are scale coefficients, introduced to fix the lower limit value of the Index and define the extension of the Trophic Scale, from 0 to 10 TRIX units. Log-transformation was considered proper to normalize variables that generally vary in an exponential way (Giovanardi et al., 2006), and also meets the assumption that with increasing absolute component values, the compounded effects tend to flatten out.

Among the array of all conceivable and measurable trophic indicators for constructing an index, the factors listed above encompass the main characteristics of the planktonic community (such as phytoplankton biomass (Chl *a*), its metabolic activity (aD%$O_2$), nitrogen and phosphorus), thought to have primary causative bearing on trophic conditions. Table 1 reports the numerical scale for TRIX as well as the corresponding water quality conditions, based on the experience gained in over twenty years of observations and monitoring of the Adriatic coastal area. The TRIX Index has been also adopted by UNEP-MEDPOL (2003), for coastal waters trophic classification, to be used in other areas under Eutrophication risk of the Mediterranean Sea.

A revisitation of the TRIX index in the light of the European Water Framework Directive (WFD, 2000/60/EC) and new TRIX derived tools have been also discussed in Pettine et al. (2007). In this paper, a number of Italian coastal sites were grouped into different types based on a thorough analysis of their hydro-morphological conditions, and type-specific reference sites were selected. Unscaled TRIX values (UNTRIX) for reference and impacted sites were calculated and UNTRIX-based classification procedures were proposed. The authors concluded that "*these procedures, to be validated on a broader scale, could provide users with simple tools that give an integrated view of nutrient enrichment and its effects on algal biomass (Chl a) and on oxygen levels*".

**Tab. 1** Reference values for annual TRIX means, corresponding trophic state and related coastal water quality conditions.

| TRIX annual means | Trophic Status | Water quality Conditions |
|---|---|---|
| **<4** | **Elevated (oligotrophy)** | • Scarcely productive waters.<br>• Good water transparency.<br>• Absence of anomalous water colour.<br>• Absence of Oxygen under-saturation conditions in the bottom waters. |
| **4-5** | **Good (mesotrophy)** | • Moderately productive waters.<br>• Occasional water turbidity.<br>• Occasional anomalous water colour.<br>• Occasional bottom water hypoxia. |
| **5-6** | **Mediocre (eutrophy)** | • Very productive waters.<br>• Low water transparency.<br>• Frequent anomalous water colour.<br>• hypoxic and occasional anoxic episodes in the bottom layers.<br>• Some degradation of benthic communities. |

| | | |
|---|---|---|
| **>6** | **Bad (hypereutrophy)** | • Strongly productive waters.<br>• High water turbidity.<br>• Diffuse and persistent anomalies in water colour.<br>• Diffuse and persistent hypoxic/anoxic episodes in the bottom waters.<br>• High mortality rate of benthic organisms.<br>• Alteration of the benthic communities and strong decrease of the biodiversity |

*(From Rinaldi and Giovanardi, 2011)*

The following figure shows an example of trophic classification based on TRIX Index, as a final result of the monitoring data elaboration.

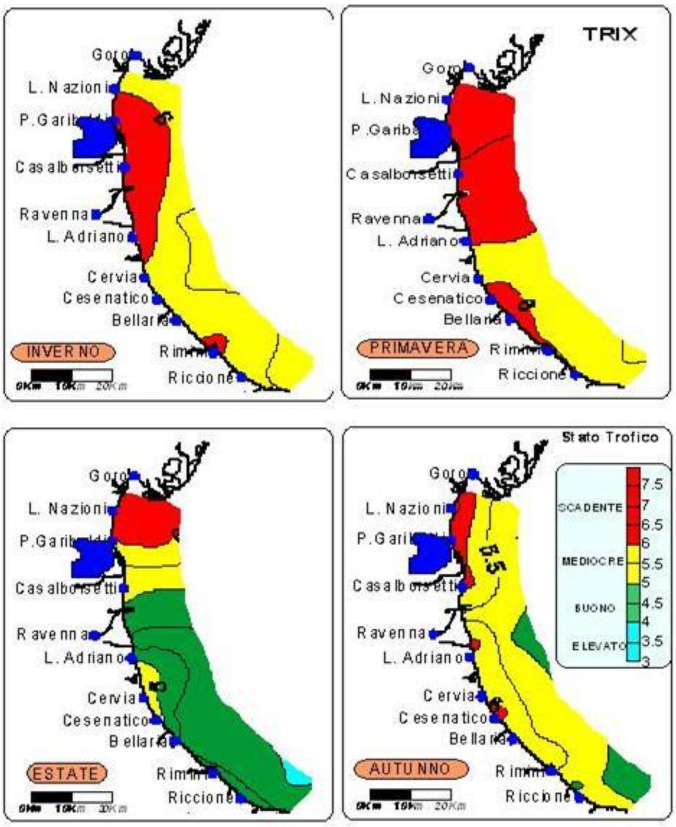


**Fig. 1.** *Distribution Maps of the TRIX Index along the coasts of the Emilia Romagna Region.* (From in-shore to 10 km off-shore. Year 2009. Seasonal averages) (Source: Annual reports of the S.O.D. – Struttura Oceanografica Daphne of the ARPA ER).

Before the WFD 2000/60 was received with Decree Law 152/2006, the classification criterion used in Italy to set objectives to be reached and/or maintained for coastal water trophic status, was based on TRIX scale. Among the many Regions of the Northern Adriatic, the plans for protection of coastal waters adopted and under development, are often still based on the TRIX. In particular, the objectives of the sanitation plans of the Emilia Romagna region fix the achievement of a good trophic status (i.e. TRIX<5), in the coastal area south of Ravenna, while in the area immediately behind the Po Delta, the achievement of good status for the moment seems to be unrealistic. In this regard we must remember that previous assessments on the percent removal of nutrient loads from all over the Po basin, which was considered necessary to bring the Po-Adriatic System to a level of pristine naturalness (> 50% removal), was not sufficient to achieve a good status in the Po Delta area, although the risks of anoxia in the bottom waters were significantly reduced. (Giovanardi and Tromellini, 1992).

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## ANNEX II: Assessment Pressure methodology - Land Uses Simplified Index (LUSI)

Eva Flo, Esther Garcés and Jordi Camp

### RATIONALE

The assessment of the anthropogenic pressures on the coastal zone is essential to develop management plans required for compliance of the Water Framework Directive. We need to work on: identifiable inland pressures, which may be related to the impact on the coastal zone and these pressure-impact relationships occur through known mechanisms.

The coastal zone is subject to continuous population growth which is one of the main drivers of continental pressures. We must understand these pressures and their global and local effects on the environment, in order to understand the processes and interactions and guide effective management actions. We must provide an objective, comparable and reproducible information and evaluation. Most experts dealing with environmental management agree that the main pressures on the coastal zone are related to the population density, tourism, urbanization, industry, agriculture, fisheries and maritime transport.

A simple assessment of human pressures on the coastal zone (LUSI) is based on quantitative evaluation of government census data or from satellite images that reflect the land use, according to the following principles:

1. It is known that different land uses generate different qualities of inland waters. Although this is very variable, there is a gradient of nutrient richness from the contributions that range from a minimum in land in its natural state up to urban areas. The gradient is: i) natural-rainfed agriculture, ii) irrigated agriculture, iii) residential-industrial (very variable), iv) urban. This applies to the area of influence of a particular stretch of coast without rivers.
2. In the vicinity of a river the main impacts are the uses of its watershed. Because of the Water Framework Directive, there is a direct control over the quality of river water, which makes the problem easy or at least takes it to another level of discussion and area of responsibilities. In the case of the influence of a river on the coastal area, it is well reflected in the fresh water content, i.e. salinity. From here, we have used the typology of the water mass with a high, low or no river influence.
3. Other factors should also be taken in to account. The continental influence is maximized in concave areas of the coast (a lot of land in a little water inflow with low removal times, e.g. bay) and minimized in convex areas (high inflow with more dilution e.g. headland) which suggest an influence based on the morphology of the coast.

From these principles, we constructed a simple index, LUSI, which can be applied from land use maps or satellite images (Google Earth). There are other similar indexes based on government census data or satellite images of land use. For example, Lopez and Royo et al. 2009 and 2010 use similar indexes and apply them in four regions of Italy. The authors conclude that the application of these methods allows the evaluation of pressure in a simple and repeatable way in time and space.

### METHOD

Land Uses Simplified Index (LUSI) is a specific combination of pressures that influences a Water Body.

The selected pressures are related to main characteristics and uses of land that could have an influence on phytoplankton growth:

- Urban
- Industrial
- Agricultural (only irrigated land)
- Rivers (Typology based on salinity is used)

Each pressure has been categorized in two or three categories and each category has a score.

For urban, agricultural (irrigated) and industrial pressures, categories have been created depending on the % of surface used for this activity (Catalan land uses study of 1997). An area comprised between the coast line and 1,5 km inland and between the limits of each water body has been taken into account to associate a category of each pressure to each water body.

For river pressure, categories have been created depending on salinity, thus each water body has been assigned a category depending on its typology.

Categories and scores of each pressure are:

| Urban | Agricultural (irrigated) | Industrial | River (Typology) | Score |
|---|---|---|---|---|
| | <10% | <10% | Type III | 0 |
| <33% | 10 a 40% | >10% | Type II | 1 |
| 33 a 66 % | >40% | | Type I | 2 |
| >66% | | | | 3 |

For each water body all scores are summed. Afterwards, a correction is applied to the sum in order to take into account the degree of confinement that could emphasize or diminish the effect of these pressures on the water body. Depending on the shape of the coastal line the sum is multiplied by the correction number:

| Confinement | Correction number |
|---|---|
| Concave | 1.25 |
| Convex | 0.75 |
| Straight | 1.00 |

Finally LUSI is obtained as follows:

**LUSI= (Score urban + score agricultural + score industrial + score typology) * Correction number**

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To perform LUSI calculation, France used Corine Land Cover (http://www.eea.europa.eu/publications/COR0-landcover) using the 15 items nomenclature of CLC information from the year 2006, and applied the following equivalences for the calculation of LUSI index:

| LUSI item | CLC Code | CLC item |
|---|---|---|
| **Urban** | **11** | **Urban fabric** |
| **Commercial and industrial** | **12** | **Industrial, commercial and transport units** |
| | **13** | **Mine, dump and construction sites** |
| **Agricultural** | **21** | **Arable land** |
| | **22** | **Permanent crops** |
| | **23** | **Pastures** |
| | **24** | **Heterogeneous agricultural areas** |

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In one Spanish region (Valencia) a modification of LUSI has been performed. It has been named LUSIval. The selected pressures that could have an influence on phytoplankton growth are the same that the original LUSI, but they have been calculated in another form and a new pressure has been added. The pressures are:

- Urban
- Industrial
- Agricultural (only irrigated land)
- Rivers (Typology based on salinity is used)
- Others significant pressures

For urban and agricultural (irrigated) pressures, two equations are used:

**Score urban** = $3.333 * 10^{-6}$ * Population number in littoral cities
**Score agricultural** = $4.286*10^{-5}$ * $m^2$ cultivates in agriculture basin area

For industrial pressures, different categories have been created depending on the % of surface used for this activity in areas near the coast.

For river pressure, different categories have been created depending on salinity, thus each water body has been assigned to a different category depending on its typology.

| Industrial | River (Typology) | Score |
|---|---|---|
| <10% | Type III | 0 |
| >10% | Type II | 1 |
| | Type I | 2 |
| | | 3 |

For others significant pressures, different aspects have been taking into account. These are:

- Rivers, channels… that significantly affect, Score = 1
- Harbours that significantly affect, Score = 1
- Influence of adjacent water bodies that significantly affect, Score = 1

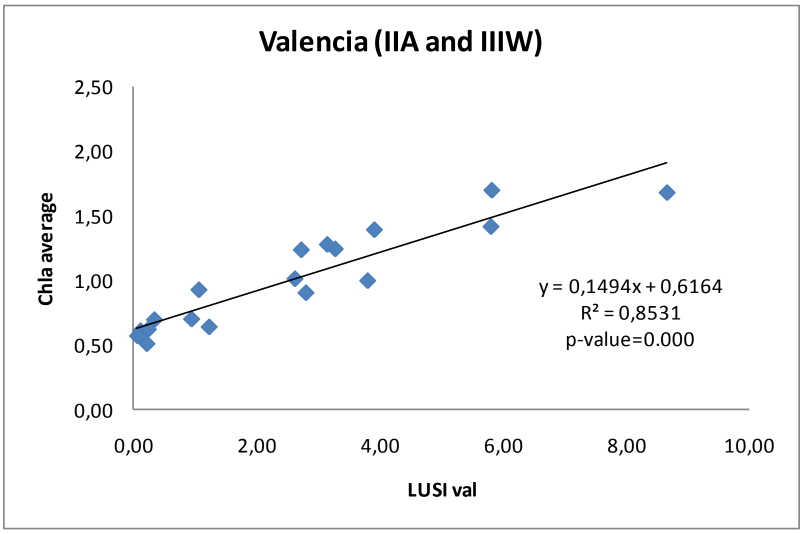
For each water body all scores are summed. Afterwards, a correction is applied to the sum in order to take into account the degree of confinement that could emphasize or diminish the effect of these pressures on the water body. Depending on the shape of the coastal line the sum is multiplied by a correction number as in the original LUSI:

| Confinement | Correction number |
|---|---|
| Concave | 1.25 |
| Convex | 0.75 |
| Straight | 1.00 |

Finally LUSIval is obtained as follows:

**LUSIval= (Score urb + score agric + score indust + score typology + Others significant pressures) * Correction number**

Then when LUSI has been estimated at different levels of detail. Figure 1 illustrate the LUSIval index with data of the Valencia region.



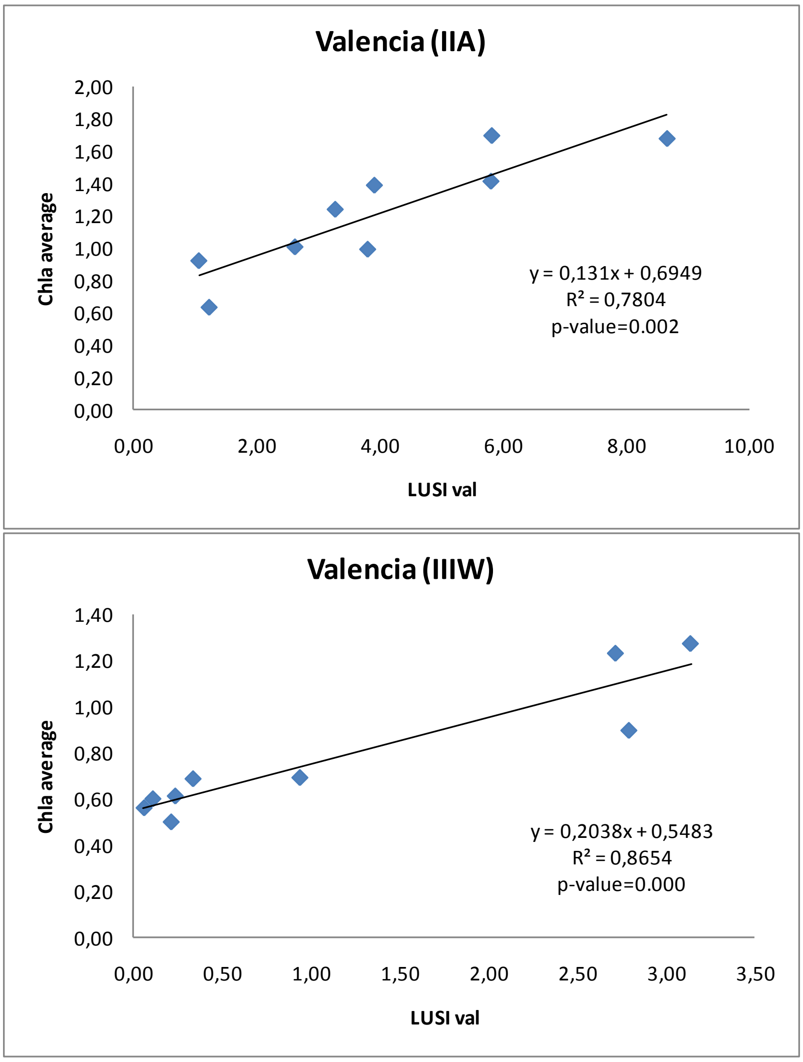



Figure 1. Relationship between pressure and impact of Spain data only in Valencian region. Pressure gradient is calculated according to LUSIVal (n=18 for all types; 9 for type IIA and 9 for type IIIW).

Finally, we want to keep the attention to the reader that a further step need to be explored when LUSI index has been estimated in different ways. A normalization of the different index has to be discussed.

**ANNEX , : Statistical exercise between France and Spain in order to check the continuous benchmarking approach after the validation workshop in Ispra (November 2011)**

**This approach of continuous benchmarking is presented here. But since the relationships between pressures and indicator are not statistically significative, they are not applicable.**

Models were performed using the R software and providing a data doubling step. The results were the following:

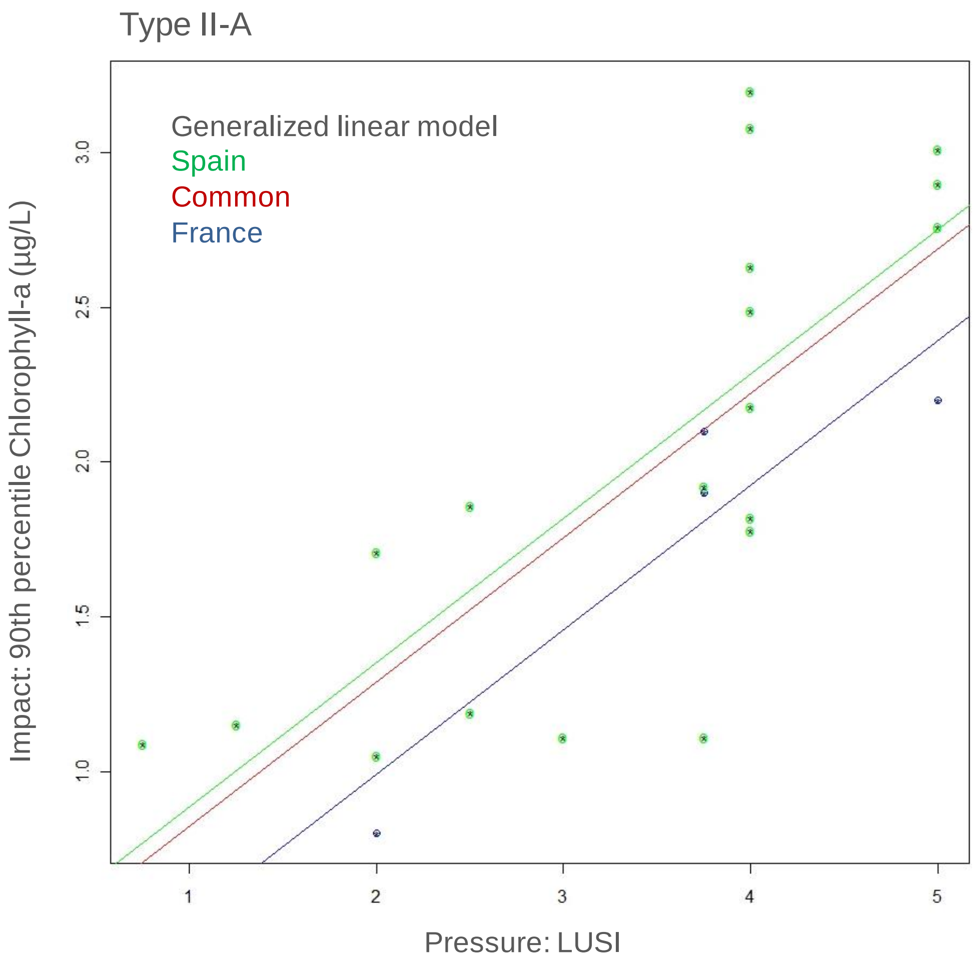



**Figure 12**: Relationship between pressure (LUSI) and impact data (90th percentile (µg/l Chlorophyll-*a*) by countries and for Type II-A. The GLM results are indicated (common in red, French in blue and Spanish in green).

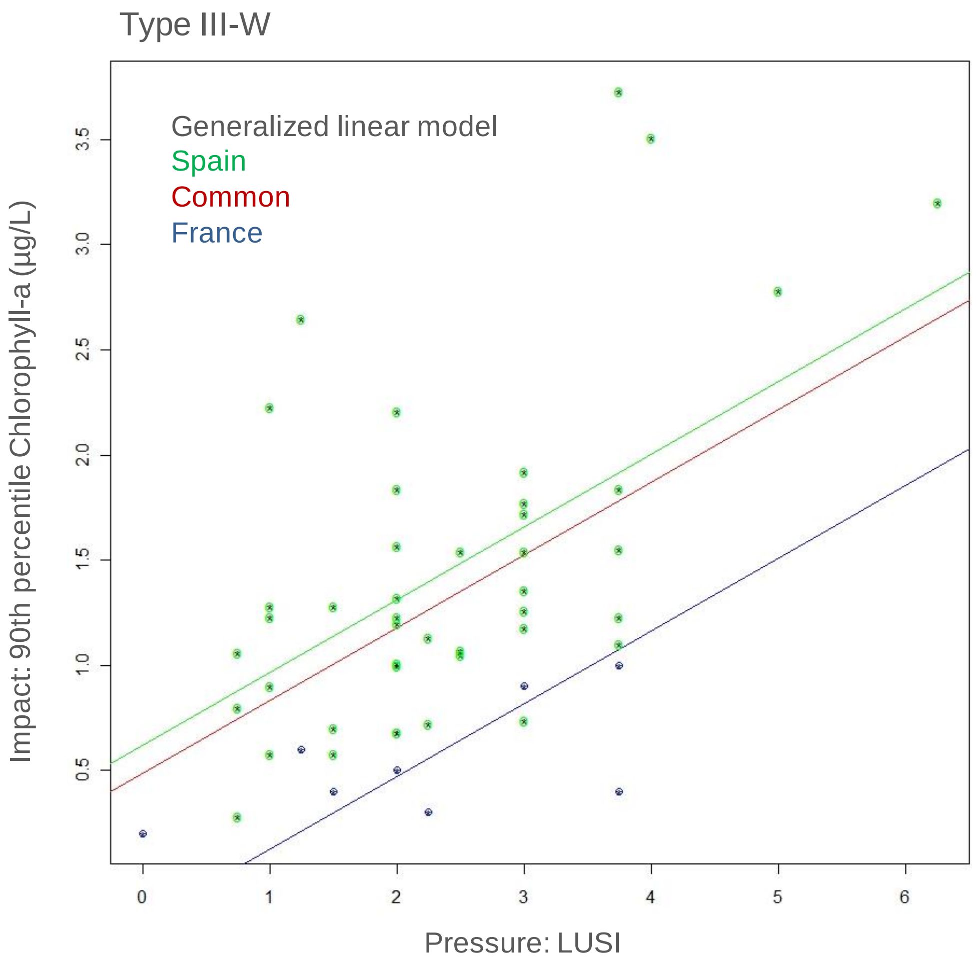



**Figure 13**: Relationship between pressure (LUSI) and impact data (90$^{th}$ percentile (µg/l Chlorophyll-*a*) by countries and for Type III-W. The GLM results are indicated (common in red, French in blue and Spanish in green).

**Table 5:** Offsets obtained by GLMs for Type II-A and Type III-W for France and Spain.

| Country | Offsets | |
|---|---|---|
| | Type III-W | Type II-A |
| France | **-0,71** | **-0,30** |
| Spain | **0,13** | **0,06** |

## <u>Type III-W</u>

As noted in validation workshop of November 2011 in ISPRA, the pressure-impact relationship for Type III-W seemed to show some biogeographical differences between France and Spain (See section Pressures). In consequence, France and Spain were recommended to apply the methodology of continuous benchmarking using a generalized linear model (GLM) in order to determine the offsets between countries and the general model for Type III-W. The offset for France was -0,71 (so below the general model) and the offset for Spain was 0,13 (so above the general model). These results are showed in Figure 13 and they demonstrate that for a given value of 90th percentile (µg/l) Chlorophyll-a, the pressure value associated to this value is higher for France and lower for Spain. Therefore, France is less restrictive and Spain is more restrictive than the general model.

After the offsets were set, the mathematical procedure was:

Firstly, the High class width was calculated as the common High-Good boundary minus the lowest value of 90th percentile (µg/l) Chlorophyll-a:

High class width = 1.13 - 0.20 = 0.93

The High class acceptance value was the quarter of the class:

High class acceptance value = 0.93 / 4 = 0.23

Secondly, the Good class width was calculated as the common Good - Moderate boundary minus the common High-Good boundary:

Good class width = 1.80 - 1.13 = 0.67

The Good class acceptance value was the quarter of the class:

Good class acceptance value = 0.67 / 4 = 0.17

Thirdly, the Moderate class width was calculated. As the assessment method only distinguish among High, Good and Failed, the width of the Failed Class, which included the Moderate, Poor and Bad classes was calculated as the Maximum value of 90th percentile (µg/l) Chlorophyll-a minus the common Good -Moderate boundary:

Moderate, Poor and Bad classes width = 3.73 - 1.80 = 1.93

The Moderate, Poor and Bad classes were considered to have the same width, so the Moderate class width was the above width divided by 3:

Moderate class width = 1.9 / 3 = 0.64

The Moderate class acceptance value is the quarter of the class:

Moderate class acceptance value = 0.64 / 4 = 0.16

Fourthly, the absolute values of the offsets of each country were compared to those corresponding class acceptance values in order to determine if boundaries should be adjusted. As the offset for France (-0,71) was below the common boundaries, the absolute value of this offset was compared with the High class acceptance value and with the Good class acceptance value. Besides, as the offset for Spain (0,13) was above the common boundaries, the absolute value of this offset was compared with the Good class acceptance value and with the Moderate class acceptance value.

France offset (0,71) > High the class acceptance value (0.23)
France offset (0,71) > Good class acceptance value (0.17)
Spain offset (0,13) < Good class acceptance value (0.17)
Spain offset (0,13) < Moderate class acceptance value (0.16)

In consequence France should adjust the High - Good and the Good - Moderate boundaries and Spain should not further adjust these boundaries.

Finally, the correction coefficient to adjust France boundaries was established. There were two possibilities:

a) the absolute value of the offset minus the High class acceptance value:

High correction coefficient = 0.71 - 0.23 = 0.48

b) the absolute value of the offset minus the Good class acceptance value:

Good correction coefficient = 0.71 - 0,17 = 0.54

Using the higher correction coefficient, it was possible to adjust High - Good and Good - Moderate boundaries at the same time, so the chosen correction coefficient for France was:

**French correction coefficient for Type III-W = 0.54**

In conclusion, **boundaries had been compared and harmonized**. According with the methodology of continuous benchmarking, Spain and France can use the common High - Good and Good - Moderate boundaries to assess the quality of their water bodies of Type III-W; Spain can do this assessment directly, without using any specific correction coefficient, and France should add the specific correction coefficient to their values of 90th percentile Chlorophyll-a (µg/l) of their Type III-W water bodies before to calculate the EQR value and afterwards assess its quality using the common boundaries.

## Type II-A

As noted in validation workshop of November 2011 in ISPRA, the pressure-impact relationship for Type II-A seemed to show biogeographical differences between France and Spain (See section 2.4. Pressures). In consequence, France and Spain have applied the methodology of continuous benchmarking using a generalized linear model (GLM) in order to determine the offsets between countries and the general model for Type II-A. The offset for France was -0,30 (so below the general model) and the offset for Spain was 0,06 ( so above the general model). These results are showed in Figure 12 and they demonstrate that for a given value of 90th percentile (µg/l) Chlorophyll-a, the pressure value associated to this value is higher for France and lower for Spain. Therefore, France is less restrictive and Spain is more restrictive than the general model.

After the offsets were set, the mathematical procedure was:

Firstly, the High class width was calculated as the common High-Good boundary minus the lowest value of 90th percentile (µg/l) Chlorophyll-a:

High class width = 2.38- 0.80 = 1.58

The High class acceptance value was the quarter of the class:

High class acceptance value = 1.58 / 4 = 0.40

Secondly, the Good class width was calculated as the common Good - Moderate boundary minus the common High-Good boundary:

Good class width = 3.58 - 2.38 = 1.20

The Good class acceptance value was the quarter of the class:

Good class acceptance value = 1.20 / 4 = 0.30

Thirdly, the Moderate class width was not calculated, as in Type III-W. As it was commented before, not all the salinity range of the typology was covered by WB and in consequence, if more WB were available covering all the salinity range of the typology higher values of 90th percentile Chlorophyll-*a* (µg/L) would be obtained.

Fourthly, the absolute values of the offsets of each country were compared to those corresponding class acceptance values in order to determine if boundaries should be adjusted. As the offset for France (-0,30) was below the common boundaries, the absolute value of this offset was compared with the High class acceptance value and with the Good class acceptance value. Besides, as the offset for Spain (0,06) was above the common boundaries, the absolute value of this offset was compared with the Good class acceptance. This Spanish offset should also be compared with the Moderate class acceptance value, but as it was not possible to determine this value, it was not possible to perform this comparison.

France offset (0, 30) < High the class acceptance value (0.40)
France offset (0, 30) = Good class acceptance value (0.30)
Spain offset (0, 06) < Good class acceptance value (0.30)
Spain offset (0, 06) < Moderate class acceptance value (--)

It was consider that the offset of 0.06 was very little and it was supposed that, if a Moderate class acceptance value would exist, this last value would be higher than the offset. In consequence, France and Spain should not adjust their boundaries.

In conclusion, **boundaries had been compared** and Spain and France can use the common High - Good and Good - Moderate boundaries to assess the quality of their water bodies of Type III-W; both countries can do this assessment directly without using any specific correction coefficient.