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Review of the methods, criteria and limit values for the assessment of Eutrophication (Biological Quality Element "Phytoplankton") as developed in the framework of the Intercalibration Exercise of the MED GIG (Mediterranean Eco-region) Water Framework Directive 2000/60 EC

UNEP/MAP
Athens, 2011

Review of the methods, criteria and limit values for the assessment of Eutrophication (Biological Quality Element “Phytoplankton”) as developed in the framework of the Intercalibration Exercise of the MED GIG (Mediterranean Eco-region) Water Framework Directive 2000/60 EC

FINAL REPORT

October 2011

PREAMBLE

The review of the methods, criteria and limit values for the assessment of Eutrophication, as developed in the framework of the Intercalibration Exercise of the MED GIG (Mediterranean Eco-region) Water Framework Directive 2000/60 EC, has been prepared by Dr. Franco Giovanardi, ISPRA, Italy, under the supervision of UNEP/MAP - MED POL.

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Introduction

The purpose of this report is then to review available scientific information on target values for eutrophication related parameters, in particular the work done by the Mediterranean Geographic Intercalibration Group (MED GIG), in the framework of the WFD 2000/60 EU.

In the following paragraphs, in addition to clarifying the meaning and objectives of the intercalibration (IC) exercise, still going on between the member countries (MSs) of the Mediterranean Eco-region, we tried to highlight the problems which arose and the difficulties encountered by national experts participating in the various GIGs, not only the Mediterranean one, but also the others IC Groups of the EU, the Baltic, the North East Atlantic and the Black Sea GIGs.

By the way, the technical reports that illustrate the achievements of the GIGs at the end of the first phase of the IC exercise, they can be downloaded from the CIRCA-JRC site (Internet clickable addresses are provided). These documents among other represent a valuable and updated collection of all the methods and criteria used (or still under development) by the European countries for the classification of ecological status of coastal waters, with particular reference to the Biological Quality Element (BQE) Phytoplankton.

The second part of this report ("*MED GIG BQE Phytoplankton common database elaborations*" and "*North Adriatic Sea: proposal for a trophic classification criterion of the coastal areas affected by the Po River inputs*" in Appendix 1), suggests procedures and methods developed by the undersigned in the working group Phytoplankton of the MED GIG. Even though the criteria and thresholds proposed, for the moment, may be subject to changes also of a substantial kind, before their validation for the purpose of publication in the EC Decision, nevertheless it is believed that these procedures are rigorous, consistent with the requirements of the WFD and of course "exportable" and applicable to other Mediterranean datasets.

Finally, for completeness of information, a technical note is reported in Appendix 2, concerning the assessment of the anthropogenic pressures on the coastal zone. This note has been prepared by the Spanish colleagues and introduces the LUSI index (Land Uses Simplified Index), an index that has already been used to test the sensitivity to the pressures of various BQE, including Phytoplankton.

The Intercalibration Exercise: I Phase

The European Directive 2000/60/EC (Water Framework Directive) established a framework in the field of water policy, and in particular requires the Member States (MS) to protect, enhance and restore all water bodies (including inland surface waters, transitional waters, coastal waters and groundwater) with the aim of achieving good surface water status at the latest 15 years after the date of its entry into force, subject to certain exceptions, in accordance with the provisions laid down in the Annex V.

The Intercalibration (IC) exercise has been coordinated by the Common Implementation Strategy (CIS) Working Group A - Ecological Status (ECOSTAT), which is responsible for evaluating the results of the IC exercise and making recommendations to the Strategic Co-ordination Group or WFD Committee.

The results of the first phase of the IC exercise were published in the Official Journal of the European Union as "Commission Decision 2008/915/EC - 30 October 2008". A Technical Report prepared by the Joint Research Centre in Ispra (Italy), provided an overview of the technical and scientific work that has been carried out specifically on the Coastal and Transitional Waters IC exercise, related to the ecological classification systems across the European Union, as required by the Water Framework Directive (WFD).

The IC process, being a key step in the definition of the ecological status, has been carried out at the level of each Biological Quality Element (BQE). The aim of the IC exercise was to ensure the comparability among MSs in relation to the biological monitoring results and to the adopted National classification systems (as stated in Section 1.2 of Annex V). All MSs (27 EC countries) were involved in this process, together with Norway, joined on a voluntary basis. The Geographical Intercalibration Groups (GIGs, i.e. groups of MSs that share the same water typologies), were identified in a number of 14.

In the Coastal and Transitional Waters IC exercise, 4 GIGs – Baltic, Black Sea, Mediterranean and North East Atlantic- were involved in the first phase (completed in 2007).

BQE Phytoplankton. As stated in the Annex V, for the ecological classification of coastal water quality by means of this BQE, the following parameters were requested: phytoplankton biomass and abundance, specific composition, bloom frequency and intensity. The wide range of hydrological (Salinity, Temperature, Transparency), physical-chemical (Dissolved Oxygen, pH, etc.), chemical (nutrients in their various forms) and biological (Chlorophyll *a*) parameters, were also strongly recommended as supporting elements.

Concerning a detailed review of the methods, criteria, limit values, etc., for the assessment of the Biological Quality Element "Phytoplankton", discussed among the WGs participating in the Intercalibration Exercise of the MED GIG, the following Documents are considered important.

- COMMISSION DECISION of 30 October 2008 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise. (*http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:332:0020:0044:EN:PDF*).

Article 1 of the Decision reads as follows: *For the purpose of section 1.4.1(iii) of Annex V to Directive 2000/60/EC, Member States shall use in their monitoring systems classification the values of the boundaries between classes that are set out in the Annex to this Decision.* About the Mediterranean Eco-region, the Technical Annex reports on pages L332/42-43, the description of types that have been intercalibrated (applicable specifically for phytoplankton), based on fixed values of salinity and/or density. The Ecological Quality Ratios (EQRs) and parameters values, expressed in µg/l of Chlorophyll *a* (as 90%ile), are then reported that apply to all countries sharing the types. In practice, this decision summarizes the state of the art at the end of the first phase of the IC exercise and sets out the results, not only for the EQRs values that have been intercalibrated, but also in relation to the various national methods of ecological classification that have been proposed for each BQE.

- Water Framework Directive intercalibration technical report. Part 3: Coastal and Transitional waters. (IC_TR_JRC51341-volumecoast, *Edited by Alessandro Carletti and Anna-Stiina Heiskanen)*

This Technical Report gives an overview of the technical and scientific work that has been carried out in the Coastal and Transitional Waters intercalibration of ecological classification systems across the European Union as required by the Water Framework Directive (WFD).
It includes descriptions on common and national coastal and transitional water types, national methods, common and national boundary setting protocols, the results of harmonisation of these boundaries between Member States as well as discussion of problems and way forward.
This report is available electronically at the following internet address:
http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library?l=/intercalibration_2&vm=detailed&sb=Title
The IC Report Part 3 on Coastal and Transitional Waters is accompanied by two Annexes, that report all the national methods included in the Intercalibration Exercise of Phase 1.

These annexes can be downloaded from the CIRCA site already cited above. In any case only the Phytoplankton national methods related to Mediterranean and Baltic GIGs are available (CoastAnnex2.3.1.pdf and CoastAnnexes.pdf, respectively). The available information in these documents appears to be comprehensive, not only to procedures and criteria adopted for

Phytoplankton-based classification (which always refer to a single metric, the chlorophyll, chosen as indicator for Phytoplankton biomass), but also for the methods, frequencies and strategies of the National monitoring programs, sometimes with very different strategies and objectives, from country to country.

The Intercalibration Exercise: II Phase

The result achieved at the end of the first phase, despite the efforts and the undoubted successes, it was still far from fully meet requirements of the Directive. Among other things, not all countries had participated in the IC working groups. Other countries, despite having participated in the work, proposed methods not adequate, or provided insufficient data. The Commission therefore decided to postpone the completion of the IC Exercise until 2011.

We summarize below the comments and observations made by ECOSTAT, concerning the lack of compliance of the methods and criteria proposed with the requirements of the Directive.

"About the BQE Phytoplankton, it is clear that not all aspects are covered as requested by the WFD. The Annex V outlines that: specific composition, abundance, frequency and intensity of algal blooms and biomass, are the required parameters for the BQE Phytoplankton in coastal waters. ECOSTAT stresses that, in cases where there are no credible justification for the use of chlorophyll as a single metric, these methods should not be included in the intercalibration exercise."

Compliance of the National Assessment Methods

- all 27 assessment methods (in all GIGs) include biomass (i.e. the Chlorophyll as indicator of biomass);
- in addition to Chlorophyll, 11 methods include also a total biomass metric (e.g.in Baltic and NEA GIGs, based on cell biovolume)
- 3 out of 27 methods include taxonomic composition: DE in Baltic, UK in NEA and BU in Black Sea.
- 5 out of 27 methods include abundance.
- 9 out of 27 methods assess frequency and intensity of algal blooms: 8 of 10 MS in NEA, 1 in Mediterranean (not intercalibrated).
- Bulgaria is the only MS that includes diversity in its phytoplankton method.

Justifications put forward by some MSs/GIGs

Although there was not a common position about the inclusion or exclusion of a given parameter, some MSs provided relevant justifications.

Baltic GIG:

- Lithuania highlights difficulties with consistent taxonomic identification.
- Latvia: algal blooms are not included in the assessment method due to the lack of data. No meaningful relations between pressure indicators and the taxonomic structure were however found. The latter was also concluded by Sweden.
- Estonia: lack of useful taxonomy-based evaluation systems for Baltic brackish coastal areas and difficulties with different counting strategies for cell abundance.

North Eastern Atlantic GIG:

- Ireland argues that taxonomic composition is subject to high natural variability but no further documentation is provided.
- Spain (Andalusia) argues that blooms are not found in Andalusian waters with enough frequency to use it as an indicator of disturbance. It is also argued that no significant relationship between species/taxonomical groups and eutrophication was detected.
- Norway takes into account and continues to work on the total biomass, including all kinds of phytoplankton taxa(autotrophic, mixotrophic, heterotrophic), like for instance, the large heterotrophic dinoflagellate species, which are however excluded from the chlorophyll analysis.
- France also continues to work on phytoplankton composition.

Mediterranean GIG:

- Spain argues that there is general agreement about the link between mean value of nutrients and mean values of chlorophyll *a* in the coastal waters. Spain still argues that "phytoplankton bloom frequency and phytoplankton community composition indices", in absolute terms, are not synonymous of environmental quality, and do not add more information than chlorophyll-a. They conclude that those indices depend on many factors, and their use cannot be considered for management purposes.
- France: abundance was not considered as a metric to be intercalibrated, since the functional relationships with the pressures are not yet well known. France is however developing a composition index to complement its phytoplankton indicator currently adopted.

ECOSTAT conclusions:

~ There is no consensus on which parameter is useful and if this parameter has some added value for the assessment of BQE Phytoplankton. There are different approaches among Member States (from fully compliant methods, up to methods that includes only one parameter), and among the GIGs:

- Mediterranean Sea GIG: only chlorophyll *a* (except FR)
- Baltic Sea GIG: only chlorophyll *a,* supplemented with total biomass (except DK)
- NEA GIG: chlorophyll *a* and frequency and intensity of blooms
- Black Sea: chlorophyll *a* supplemented with total biomass (and other parameters for BU)

~ ”It is recommended that methods based only on the chlorophyll, are not to be included in the final results of IC, where useful scientific proposals have been made, but not respected.
Methods that consider only the chlorophyll will be accepted only when a common position within the GIG will be agreed and scientific supporting documentation will be improved, including the reliability of the G/M boundary based on the chlorophyll as unique parameter.”

IC Exercise Phase 2 - BQE Phytoplankton. Criteria and limit values for the assessment of the Biological Quality Element "Phytoplankton", discussed among the WGs participating in the Intercalibration Exercise of the MED GIG (Mediterranran Eco-region), as required by the Water Framework Directive 2000/60 EC.

1. Preamble

Among all the methods and procedures proposed by the MED GIG MSs participating in the intercalibration exercise, different sampling strategies have been pointed out among the MED GIG MSs. In practice two main sampling strategies can be highlighted: samples affected by salinity gradients and those not affected, as represented in Fig. 1. Differences in Chlorophyll-Dilution factor relationships, depend greatly on the portion of water body sampled. E.g., Spain data are to be referred to the Coastal Inshore Waters (CIW) and clearly fall outside the other MS ranges. The Spanish experts have developed their own approach trough LUSI Index (See Appendix 2: Assessment Pressure Methodology -Land Uses Simplified Index - LUSI - *(Eva Flo, Jordi Camp, Esther Garcès, 2011*), in order to address the effects of human-induced pressures mainly on this type of waters (CIW), rather than on the whole CW water body.

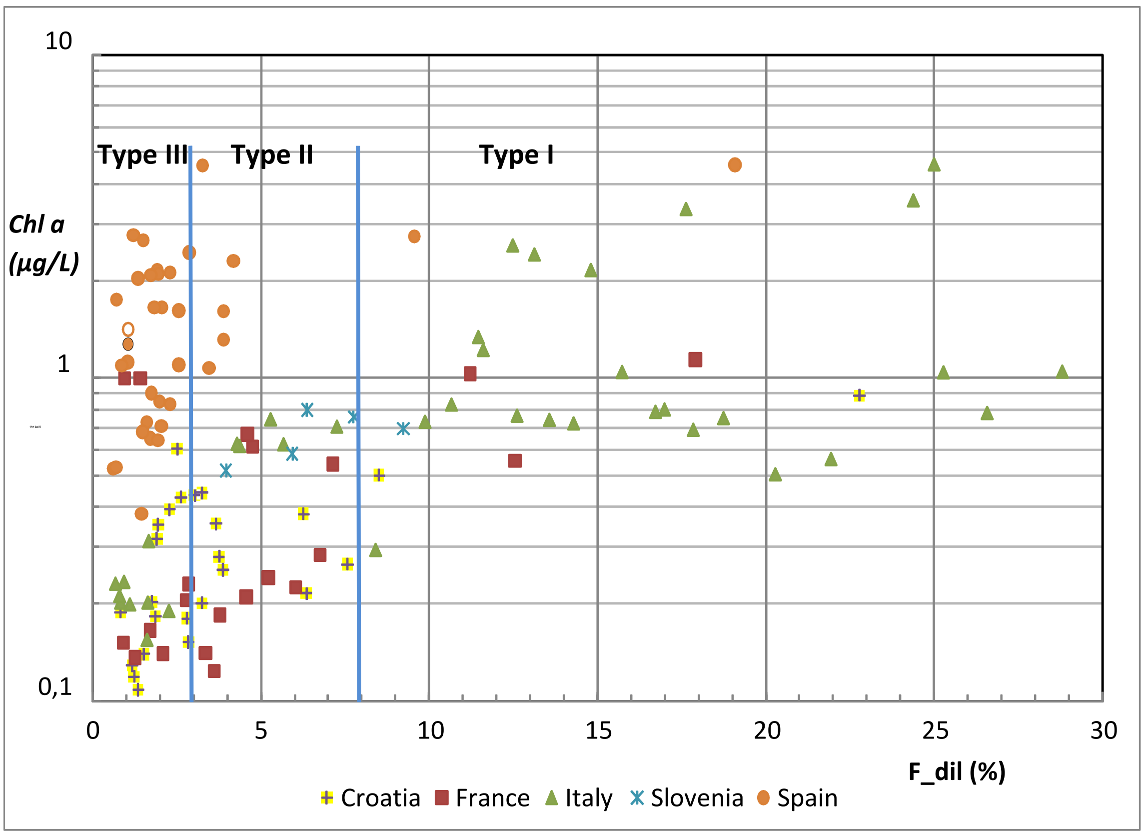


Figure 1. Chlorophyll *a* concentration vs. dilution factor (Chl *a* annual geometric mean per station). (Source: MED GIG Milestone 4b - F.Giovanardi, R.Precali and C.Mazziotti: Elaborations on the common Data Base)

Note: the typologies represented in the diagram are evaluated by considering the density parameter of the seawater, as sigma_t, to say: Type I<25, Type II between 25 and 27, Type III > 27. The F_dil % is calculated as follows: $(Sal_{open\ sea} - Sal_i)/ Sal_{open\ sea} *100$

Concerning the whole coastal water body and its trophic level, the Mediterranean experience shows that coastal waters are those directly or indirectly affected by the freshwater inputs from the continent (contribution of large and minor rivers, industrial and urban settlement discharges and so on). This is in agreement with the salinity/dilution factor approach.

In this regard, it is important to treat the question of the trophic levels in the light of an integrated systems conception, which considers both scientific and socio-economic issues as interrelated. Accordingly, coastal waters, their connected hinterland and related human activities, the catchment areas with the related nutrient loads, represent the total system, of which the natural and socio-economic components are the respective subsystems.

This is the conceptual framework that has guided and still guides the approach to the problem of the control of trophic levels, against the risk of the Eutrophication (O.E.C.D. - Vollenweider, 1982. Vollenweider et al., 1992. Unep MED POL, 1996).

In more practical terms, Vollenweider suggested to adopt a methodology of nutrient load assessment based on the different sources of N and P, to prepare an inventory of all these sources, according to types and quantity released, in terms also of pathways of transfer and input-output fluxes. In this way it should be finally possible to reach a first realistic evaluation of N and P mass balance across the various productive compartments, which impact the coastal zone.

Regarding the MED GIG common data set, at the moment, as well as temperature and salinity data, we have available a range of parameters such as: Chla *a*, dissolved oxygen, DIN, TP, orthophosphate and orthosilicate. These parameters are also on the MEDPOL list as fundamental trophic status indicators, just because they can be directly connected to the loads coming from the continent.

Due to the particular nature of these 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 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 appropriate 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 tha 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, the annual geometrical means have been adopted (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.

In this phase, due to the lack of all information on nutrients loadings, at the Mediterranean scale, the data processing has been mainly directed to find functional relationships between chlorophyll *a* and the other parameters, in terms of concentration, by using linear models (i.e. Multiple Regression Techniques).

As far as the use of chlorophyll as phytoplankton biomass indicator, the choice of chlorophyll was mandatory, since it was not available no other more representative indicator. We had to keep in mind that Chl a isn't a true state parameter of system, but rather a roughly estimator of biomass. The real indicators are the phytoplankton species and the related abundances.

2. Reference conditions.

In order to fix a reference value for Chl a, the approach already mentioned above has been followed. Consider Fig. 2. On the basis of all the available data for the Mediterranean coastal environment (EC Countries), in a Chl a vs F_dil (%) diagram, a realistic lower demarcation for the scattering area will be provided by the curve that separates the data points from the empty area below, assuming that the line represents the natural conditions, or better said, the best conditions attainable depending on the freshwater inputs.

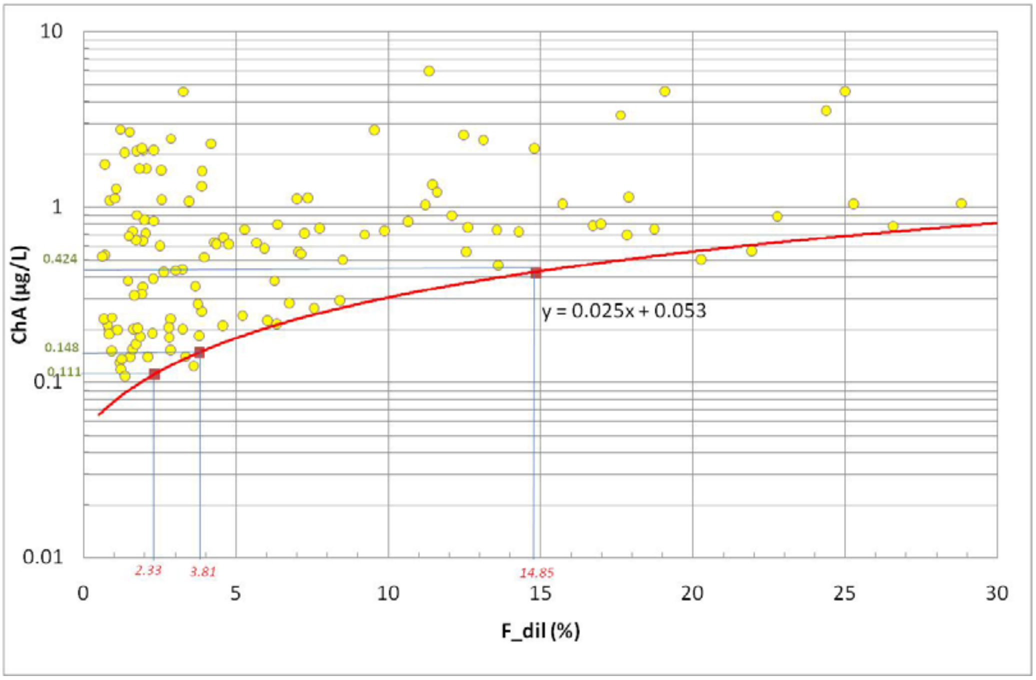


Fig. 2 (Source: MED GIG Milestone 4b - F.Giovanardi, R.Precali and C.Mazziotti: Elaborations on the common Data Base)

This line will be meant as the minimum threshold for the chlorophyll concentration, to be assigned to the geometric means per sampling station, as a function of the dilution factor.

The Table below (Tab. 1) shows the reference values for Chlorophyll, for each of the three typologies. The g-means, the minimum and maximum values are reported, taking into consideration that the 10Log-transformation of the original data approximates the Chl distribution to the normality, with a Standard Deviation of the 10Logs nearly constant, between 0.35 and 0.40. ([1])

Tab. 1	**type I**			**type II**			**type III**		
	G_Mean	Min	Max	G_Mean	Min	Max	G_Mean	Min	Max
Chl as µg/L Ref. Value	0.42	0.09	1.94	0.15	0.03	0.68	0.11	0.02	0.51

3. Testing the sensitivity of the Chlorophyll to the Pressure Indicators.

The sensitivity of the Chlorophyll variability has been tested against the nutrient concentrations, the Oxygen % saturation (expressed as aD_O, absolute % deviation from the saturation)([2]), the Dilution factor F%, etc.

From the common data set, the following procedure has been adopted:

- Calculation of the averages values per each sampling station (g-means for the nutrients and Chl concentration, and arithmetic means for Oxygen and F_dil (that in fact do not require Log-transformation, being expressed as %).

- Three sub-files have been then created corresponding to the three different typologies (Type 1: sigma_t< 25. Type 2: 27>sigma_t>25. Type 3: sigma_t>27)

- Analysis of the statistical distributions, by means of the Johnson method (Giovanardi et al., 2006), in order to highlight different behaviours among the variables, depending on different typology of coastal waters.

- Multiple linear regression analysis (Linear models) applied to Type 1 and Type 2 sampling stations averages data, in order to test the variability of the Chlorophyll depending on different pressure indicators. (Note: for Type 3 sampling stations, it was not possible to apply this procedure, because of a poor and not significant sample size).

3.1. Cumulative distribution curves.

This kind of representation offers the advantage of synthesize in an unique diagram a lot of statistical properties of the data, such as the ranges of a single distribution, the difference between types and the difference in the shape of single distributions. As an example, we report here below two cases related to Dilution factor and to Chlorophyll. (Figs. 3 and 4)

([1]) It is worth to recall that in a normalized distribution of log-transformed data, the min and max values can be empirically evaluated by subtracting or adding to the log-mean two times the S.D.(discarding the few data points outside this range) and then reconverting into numbers the values obtained in this way. Analogously, we can calculate also the distribution percentiles, e.g. 95th percentile of a normal distribution corresponds to: Mean+1.645*S.D. and so on.

([2]) This parameter represents a rough estimation of intensity of primary production and it is meant to be proportional both to the intensity of synthesis processes (with Oxygen production and consequent oversaturation) and/or to the intensity of biomass degradation and mineralization (with Oxygen consumption and consequent undersaturation).(Vollenweider et al., 1998)

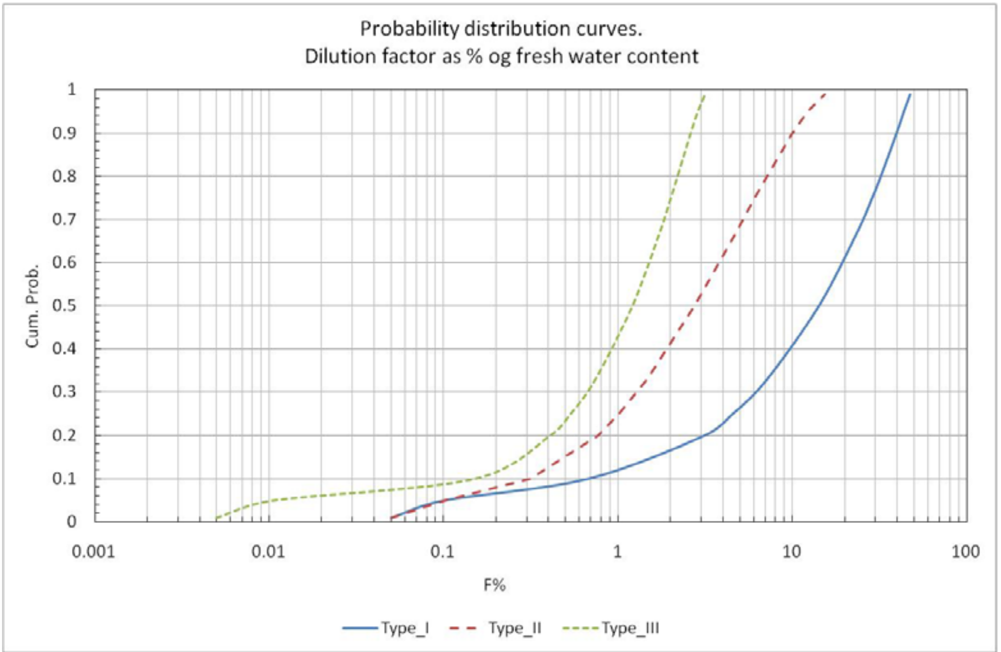


Figure 3: Probability distribution curves in relation to dilution factor (%)

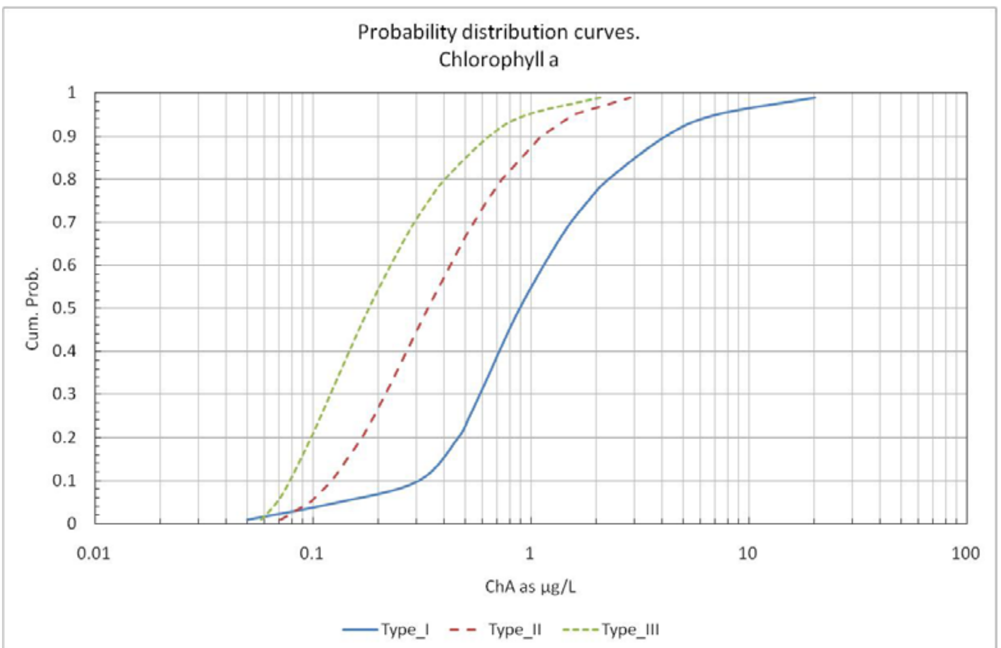


Figure 4 Probability distribution curves in relation to chl-a (µg/l) (Source: MED GIG Milestone 4b - F.Giovanardi, R.Precali and C.Mazziotti: Elaborations on the common Data Base)

The "sigma" shape for the three types of chlorophyll distributions on a Log-scale proofs the goodness of the preliminary Log-transformation performed on the original data. On the contrary the F_dil curve shapes demonstrate that log-transformation is not fully adequate for a variable expressed as a percentage.

3.2. Multiple linear regression analysis

- Type 1

The considered sampling stations belonging to Type 1 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_1)
```

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

Tab. 2	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 Chlorophyll; the other regressors, although highly significant, have lowest effects. The fitted LM explains at least the 86% of the total Chl variability and the tests performed on the residuals insure us that the remaining Chl variability is not affected by other independent variables, not considered in the adopted linear model.

It is interesting to remark that Phosphorus not only represents the main pressure indicator that affects Chlorophyll variation, but it assumes also the role of limiting factor for the algal growth, as well highlighted in the following diagram (Fig. 5), where the N/P ratios (i.e. the ratio between the available nitrogen –DIN- and the soluble orthophosphate) are plotted vs Chl g-means, for each sampling station belonging to Type 1.

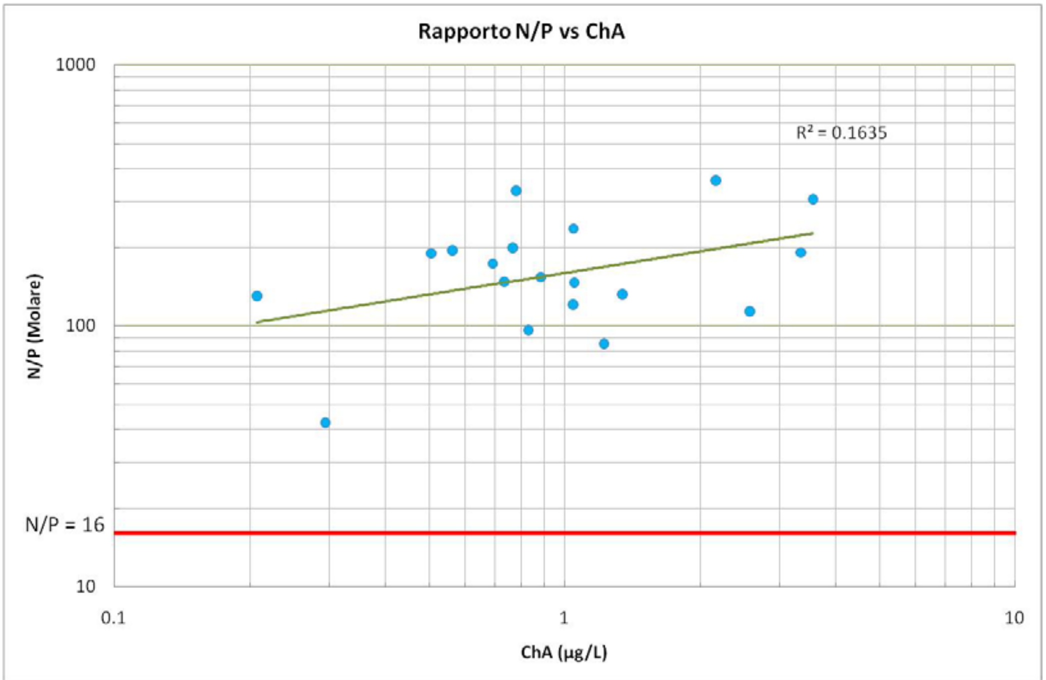


Figure 5: Type 1 - N/P ratios against Chl g-mean concentration, per sampling station. (Source: MED GIG technical document - F.Giovanardi, R.Precali and C.Mazziotti: Elaborations on the common Data Base)

Finally, the next diagram(Fig. 6) shows the quantitative relationships between Chlorophyll and Total Phosphorus, obtained both from a direct regression TP vs Chl (R2=0.60) and from solving the Inverse of the Linear Model described above, by means of linear programming techniques.

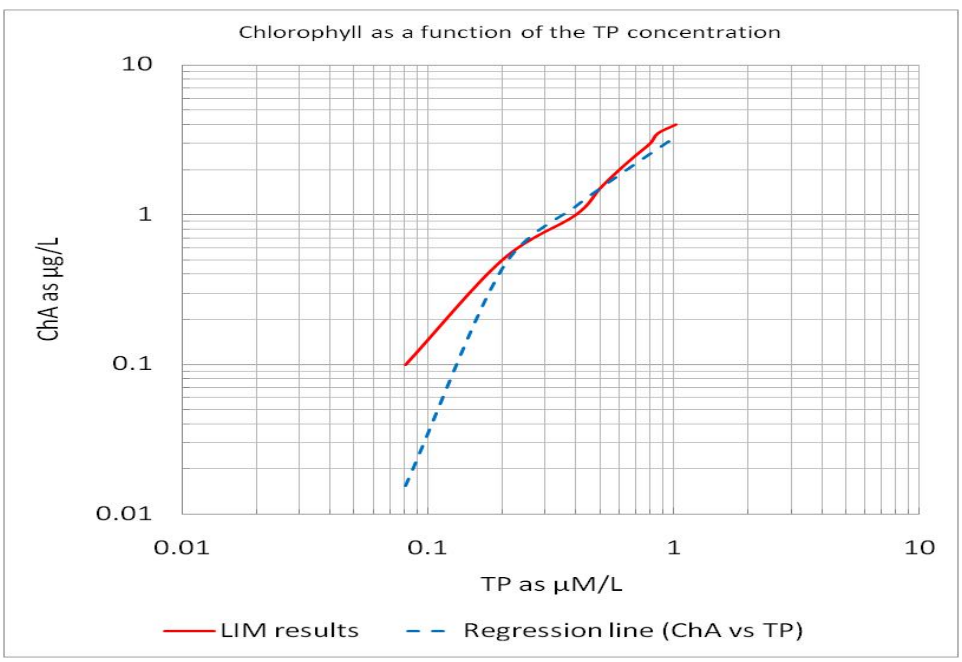


Figure 6: Chlorophyll as a function of the TP concentration (Source: MED GIG technical document - F.Giovanardi, R.Precali and C.Mazziotti: Elaborations on the common Data Base)

- Type 2

The considered sampling stations belonging to Type 2 were 30.

The linear model adopted by the Stepwise Regression Technique was the following:

lm(formula = ChA ~ f_dil + TP, data = Type_2)

The multiple regression analysis provided the following results:

Tab. 3	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 2 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 Chlorophyll explained by the model is ca 77%. The N/P ratio among the Type 2 sampling stations confirms that also in this case Phosphorus is the limiting factor, except for two Tyrrhenian Sea sampling stations, as shown in the next diagram (Fig. 7).

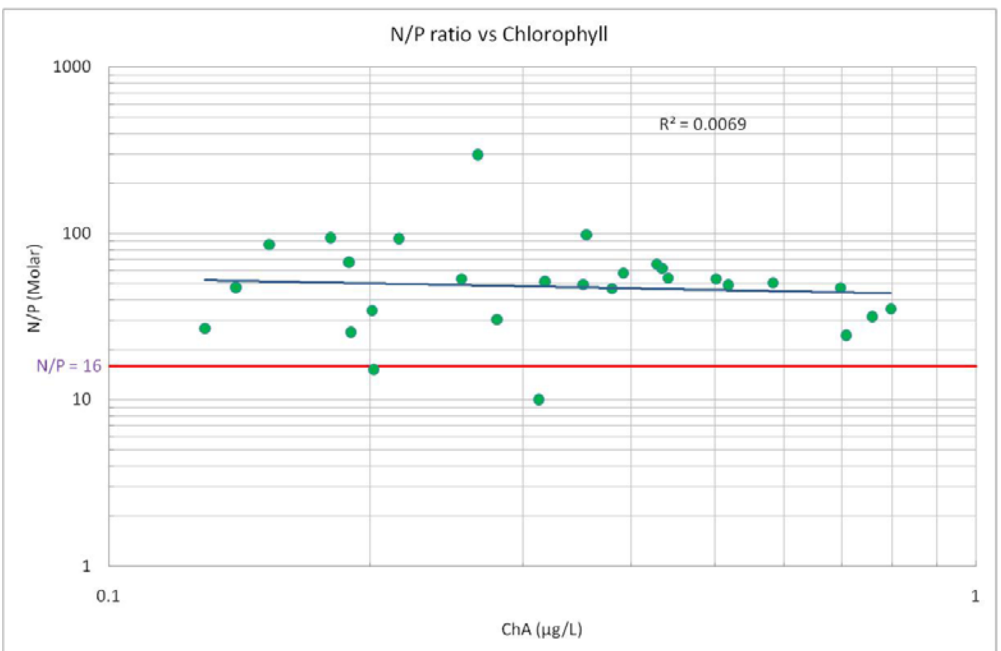


Figure 7: Type 2 - N/P ratios against Chl g-mean concentration, per sampling station. (Source: MED GIG technical document - F.Giovanardi, R.Precali and C.Mazziotti: Elaborations on the common Data Base)

The functional relationships between Chlorophyll and the related regressors, are reported in the following diagram (Fig. 8).

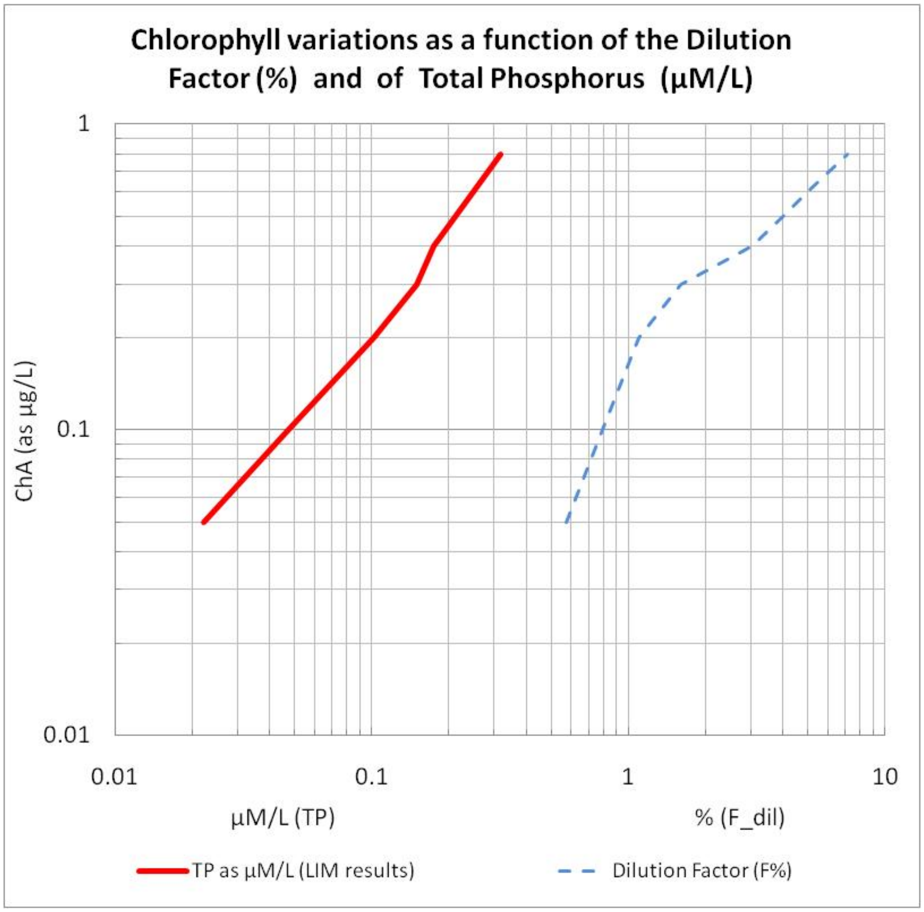


Figure 8: Chlorophyll variations as a function of the dilution factor (%) and of Total Phosphorus (μM/L) (Source: MED GIG technical document - F.Giovanardi, R.Precali and C.Mazziotti: Elaborations on the common Data Base)

4. Effects of the pressures on the coastal marine environment.

In order to test the effects of nutrients on the coastal marine environment and to fix benchmarks for Chlorophyll boundaries in the classification criterion, the behaviour of the Oxygen, depending on the gradient of TP and Chl, has been analysed.

The results of this investigation are represented in the next diagrams (Fig 9).

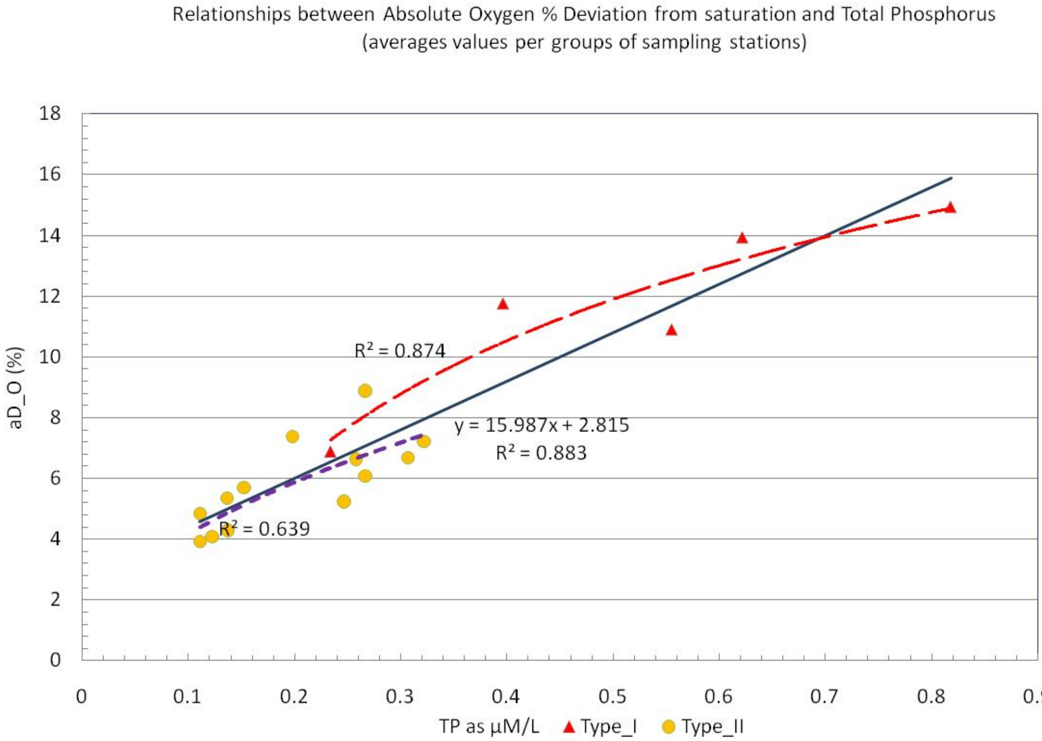


Figure 9: Relationships between absolute Oxygen % deviation from saturation and Total Phosphorus (Source: MED GIG technical document - F.Giovanardi, R.Precali and C.Mazziotti: Elaborations on the common Data Base)

There is good agreement between the increase of the parameter aD_O (take note: referred to the surface layers), and the corresponding annual averages TP concentrations: we have for type 1, a change of aD_O from 8 to 15%; for type 2, from 4 to 8%.

The analysis of the rich database of the ARPA-ER (Daphne), related to Northern Adriatic coastal waters, shows that for Type 1, the risk of anoxia (O2< 1 mg/L) in the bottom waters during the summer and autumn, becomes real (see Table below), when the average value of the aD_O in the surface layers exceeds 10%. Analogously, for Type 2, the risk of ipoxia (O2< 3 mg/L) in the bottom waters becomes real when the aD_O in the surface layers exceeds 6%.

Table 4: Northern Adriatic coastal waters database

Year	**O2 (mg/L) Bottom**	**aD_O%**	**Annual g-mean Cl (µg/L)**	**Annual g-mean TP (µg/L)**	**Annual g-mean TP (µM/L**	**Sample size**
2008	<1	10.84	4.75	23.71	0.77	66
2007	<1	12.5	7.25	27.16	0.88	37
2006	<1	12.6	9.6	26.7	0.86	33
2005	<1	13	9.5	30.5	0.98	105
2004	<1	11.5	4.7	29.42	0.95	63
2008	1-3	9.92	3.49	20.58	0.66	43
2004	1-3	8.8	4.74	29.53	0.95	48

5. Approach to the classification for Type I and Type IIA.

As already mentioned above, we have tested the sensitivity of the Chlorophyll variability against the nutrient concentrations, the Oxygen % saturation (expressed as aD_O, absolute % deviation from the saturation), the Dilution factor F%, etc.

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.

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 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 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. 10). For this reason we have made distinction between Type IIA (Adriatic Sea) and Type IIA (Tyrrhenian Sea) as Type IIA Adriatic and Type IIA Tyrrhenian .

The following proposals can be therefore extended to all the coastal water bodies classified as Type I and both Type IIA.

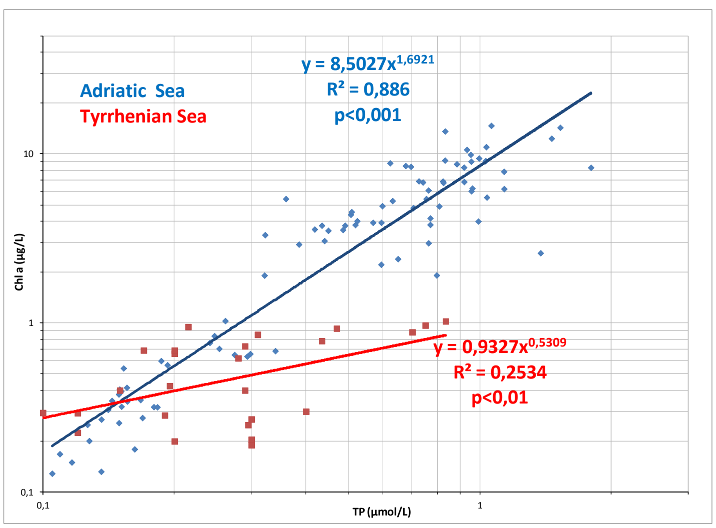


Figure 10. G-mean of Chlorophyll a (Chl a) vs. Total Phosphorous (TP) concentration in Adriatic and Tyrrhenian Sea. ((Source: MED GIG technical document - F.Giovanardi, R.Precali, J. France and C.Mazziotti: Elaborations on the common Data Base)

6. Approach to the classification for Type IIIW

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 chlorophyll 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: 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 Chlorophyll data available are mainly related to the oligotrophic Tyrrhenian coastal waters of Sardinia, Calabria, etc. The annual geometric means of chlorophyll 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 chlorophyll 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 chlorophyll 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 chlorophyll is not a suitable indicator, but as requested by the directive, the EQB Phytoplankton should 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.

7. Boundary setting procedures based on TRIX Index and proposed boundaries.

For management purposes, TRIX index (Vollenweider et al. 1998), as such, is more functional and useful to represent the ecological status of the BQE Phytoplankton, rather than a single indicator such as Chlorophyll 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. (see also Appendix 1, attached below).

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.

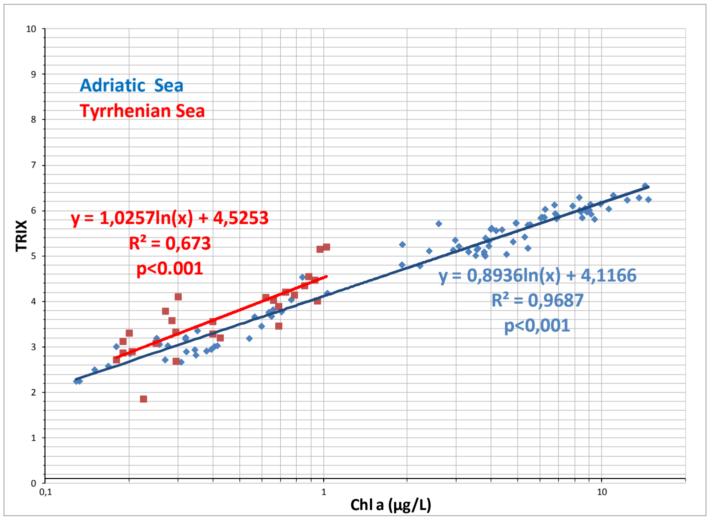
The use of TRIX as common metric for Chl *a* and Total Phosphorus, as mentioned above, reflects a “Management Approach“ more than an “Eco-system approach“. In the case of BQE Phytoplankton, it is believed that the ecosystem approach promoted by the Directive, at the current state of knowledge, it is still premature. Preliminary studies about the effects of trophic level increase on the biodiversity expressed by phytoplankton, have shown promising results. They have been in fact identified the 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 are becoming more and more frequent blooms of phytoplankton species characterized by small size (<3 μm), with a large number of cells/L. 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. 11).

The boundaries for all the types are reported in the Table 5.



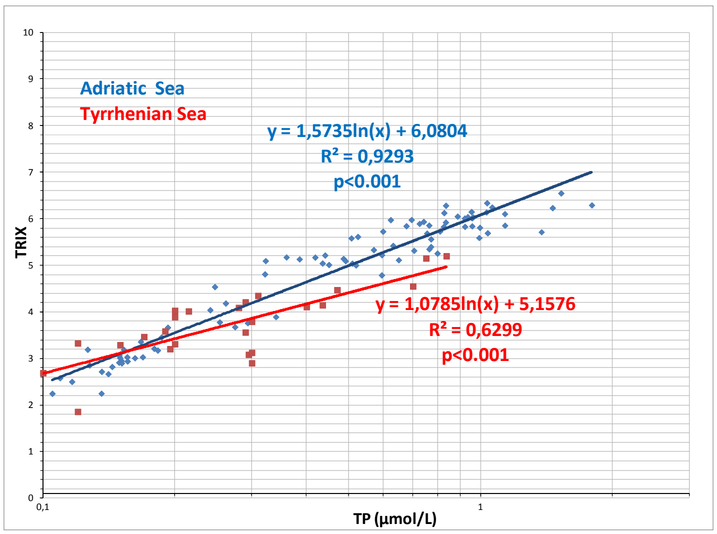


Figure 11. TRIX vs Chl a and TP for the various types with relationship curves. (Source: MED GIG technical document - F.Giovanardi, R.Precali, J. France and C.Mazziotti: Elaborations on the common Data Base)

Table 5. Boundaries for TRIX, Chl a (Geometric Mean and 90th %le) and Total Phosphorous (TP) by Type. (Source: MED GIG technical document - F.Giovanardi, R.Precali, J. France and C.Mazziotti: Elaborations on the common Data Base)

Type I

Boundaries	TRIX	Chl a annual g.means (µg/L)	Chl a 90th percentile (µg/L)	TP annual g.means (µM/L)
Ref. Values	-	0.8	2.3	0.24
H/G	5.0	2.5	7.0	0.4
G/M	5.7	6.2	17.3	0.6
M/P	6.4	15.1	42.5	0.9
P/B	7.1	37.1	104.4	1.6

Type IIA- Adriatic sea

Boundaries	TRIX	Chl a annual g.means (µg/L)	Chl a 90th percentile (µg/L)	TP annual g.means (µM/L)
Ref. Values	-	0.15	0.36	-
H/G	3.7	0.65	1.58	0.23
G/M	4.5	1.57	3.81	0.37
M/P	5.3	3.79	9.20	0.61
P/B	6.1	9.14	22.17	1.01

Type IIA- Tyrrhenian sea

Boundaries	TRIX	Chl a annual g.means (µg/L)	Chl a 90th percentile (µg/L)	TP annual g.means (µM/L)
Ref. Values	-	0.15	0.36	-
H/G	3.7	0.4	1.06	0.26
G/M	4.5	0.9	2.19	0.54
M/P	5.3	1.9	4.51	1.14
P/B	6.1	3.8	9.30	2.40

8. Concluding remarks.

The procedure used to test the effect of pressures on the coastal systems in terms of increase of Chl concentrations, demonstrated the different behavior of the Adriatic coastal waters with respect to the Tyrrhenian waters (See Fig. 10). In fact,with the same nutrients availability, the Tyrrhenian coastal system seems to respond less “efficiently”, producing less biomass (as measured by Chlorophyll, the main indicator of phytoplankton biomass).

On these differences of “trophodynamic”kind is therefore based the additional identification of two subtypes: to say the type IIA “Adriatic” and the Type IIA “Tyrrhenian”, for which a specific classification criterion has been proposed, as shown above.

The steering group of experts at the JRC (Ispra), however, have questioned the choice of keeping separate the Tyrrhenian type from the remaining part of the western Mediterranean Sea (France and Spain coastal waters). Better said, it was pointed out that was still missing a benchmarking analysis between the Tyrrhenian and the rest of the Type IIA waters, with the LUSI or any other pressure indicator.

For this purpose, the Spanish and French common data base of Chl data and the corresponding LUSI values, was integrated with the Tyrrhenian data. Spanish and French experts are still working on this data, in order to proceed to the intercalibration, using the LUSI as pressure indicator, in view of the approval in the next “validation meeting” set for mid-November 2011at the IRC at Ispra (Italy).

In any case we can already be sure of the following results:

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

(Source: MED GIG Working document. **France**: C. Belin, R.Buchet and V. Derolez. -. **Spain**: E. Flo, E. Garcés (Spanish coordinator), E. Garcia, F. Orozco, I. Romero, F. Salas and J. Camp.- **Italy**: F. Giovanardi).

As you can see, the reference values and the limits between the classes are expressed as 90^{th} percentile. For the Type IIA less stringent limits have been proposed (the values of the H/G and G/M boundaries), if compared with the corresponding Type IIA "Tyrrhenian" reported above. We have however to consider that the new criterion take into account the whole pressure and concentrations gradient that can be encountered along the Mediterranean coasts of France and Spain.

Concerning the Type III W waters, we observe that a new sub-type has been introduced (Type Island-W), with the aim of keeping separate and better preserve the island coastal environment (Balearic, Corsica and Sardinia islands).

Finally, taking into consideration what already highlighted in the above paragraph on Type III W about the the Discrimination Limits, we can assign to the G/M boundary Chl values as 90^{th} %le, the meaning of unique treshold-value that can not be exceeded, but this represents already subject of debat and discussion in the planned consultation meeting on the Med Pol monitoring activity.

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Appendix 1

BQE Phytoplankton - North Adriatic Sea: the use of TRIX to assess the trophic levels of the coastal areas affected by the Po River inputs.

Introduction: the Po River-Adriatic System

As it is well known, NW Adriatic Sea represents a unique case in the Mediterranean basin, due to the massive algal blooms occurring in that area. The intensity of the blooms and the related values of chlorophyll and phytoplankton biomass, cannot be found in any other part of the Mediterranean Sea. (Vollenweider et al., 1992a, Vollenweider et al. (eds), 1992b)

The reasons for this phenomenon, well studied and monitored for over thirty years, are to be mainly referred to the nutrient inputs coming from the catchment of the River Po. On average the loading of nutrients entering the Adriatic coastal system can be estimated at more than 110.000 tonn/year of Nitrogen and 7000 tonn/year of Phosphorus *(Source: Po River Basin Authority. A.d.B. f. Po, Parma)*.

The Po River Valley represents a basin of a national importance, with an extension of almost a fourth of the whole national territory and with a population of 16 million people. In the basin are concentrated over a third of Italian industries and agricultural production, as well as more than half of the livestock and farms. This makes the Po River basin a strategic area for the entire Italian economy and one of Europe with the highest concentration of population, industries and commercial activities.

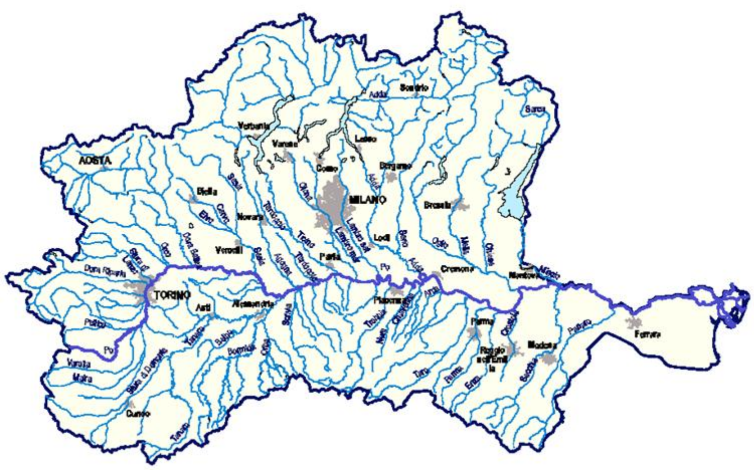

Fig.1 *The Po River Basin: hydrography*. (Source: Po River Basin Authority – A.d.B. f. Po, Parma - Italy).

To the nutrients contribution from the River Po we have then to add those from the other basins of the Northern Adriatic Sea: Adige, Piave, Brenta, Tagliamento, Venice and Grado-Marano lagoons, etc. The low and sandy coasts and the prevailing current framework, determine optimal conditions for coastal Eutrophication occurrence. The general current that goes up along the Adriatic Sea in a counterclockwise direction, as a rule, tends to "push" the freshwater inputs from the Po River against the coasts of the Emilia Romagna Region.

The coastal system proves highly efficient in using available nutrients and then produces large quantities of phytoplankton biomass. In general, the algal cycles are already beginning from January to February, with large blooms of Diatoms, in response to the high flows of the River Po (winter floods). These winter blooms often provide a strong contribution in determining elevated annual Chlorophyll averages.

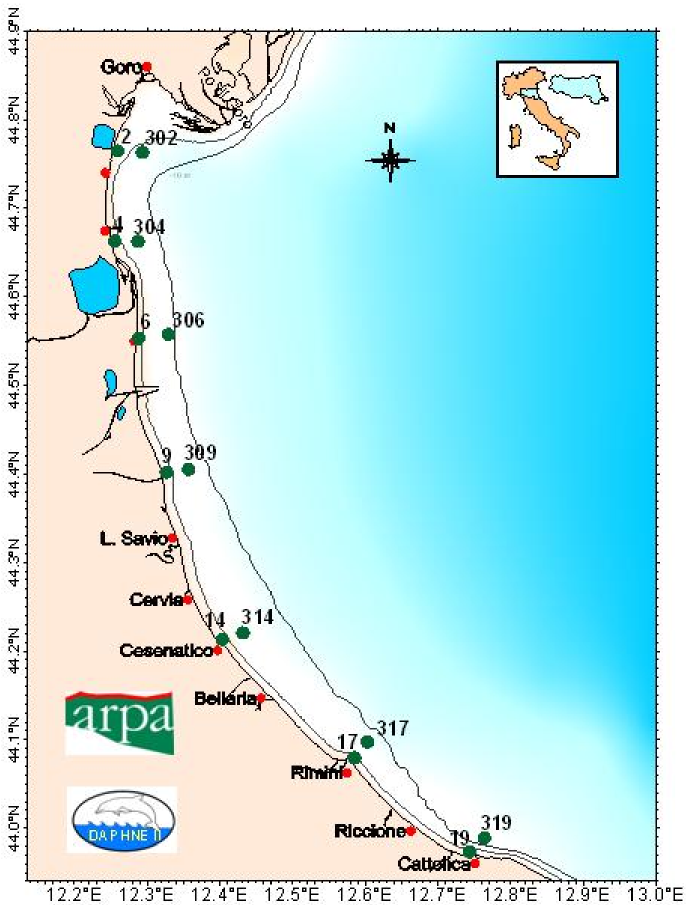

Fig.2 *NW Adriatic Sea – The coastal belt of the Emilia Romagna Region, directly affected by the Po River inputs*. (Source: Annual reports of the S.O.D. – Struttura Oceanografica Daphne of the ARPA ER).

During the summer months, we assist to a strong decrease of algal production, with much less intense blooms, mostly related to Dinoflagellates.

It is worth to remark that the N/P ratios result always very high (Phosphorus-limitation) during winter and springtime, due to the high Po River flows. In the summer months this ratio decreases, coinciding with the low flows of the Po, up to provide in some cases, Nitrogen-limitation situations (N/P <16, as molar ratio) (Innamorati and Giovanardi, 1992).

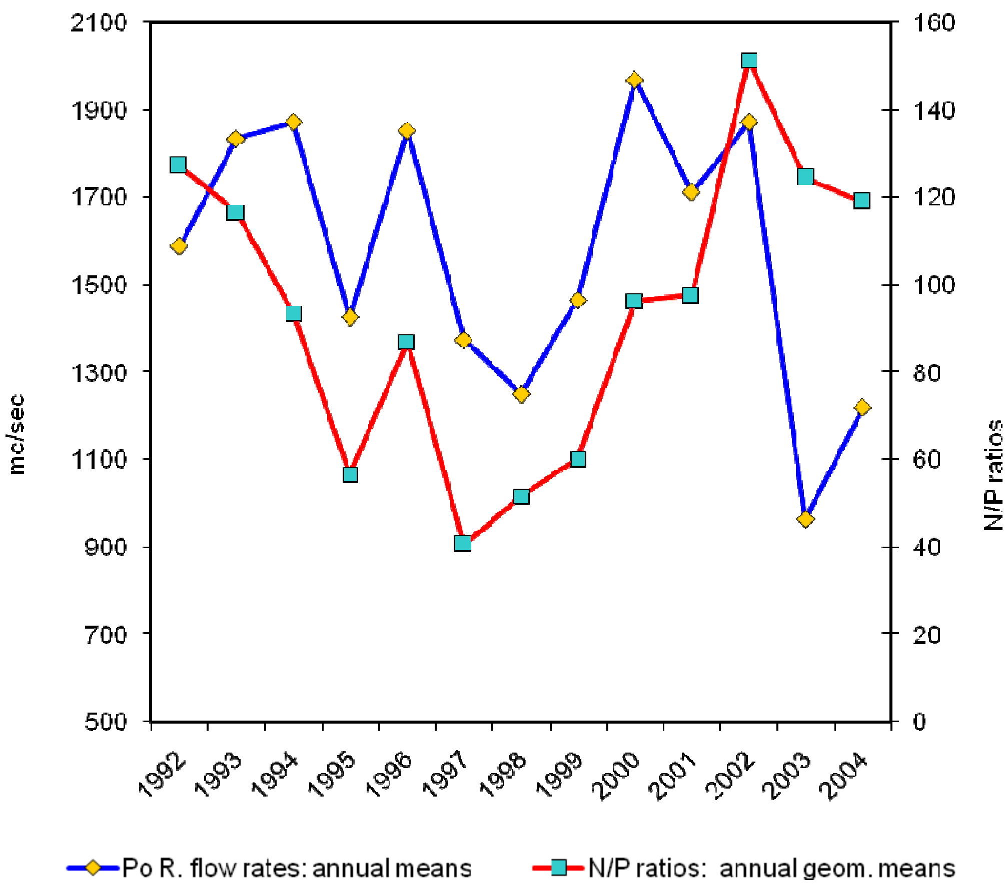

Fig.3 *Interannual variations of the Po R. flow rates and N/P ratios changes (St. 14: Cesenatico, ca. 50 km from the Po River mouth)* (Source: Annual reports of the S.O.D. – Struttura Oceanografica Daphne of the ARPA ER).

Due to the October and November rains on the Po basin, new algal cycles can take place and determine a new rise in Chlorophyll and biomass values.

Concerning the harmful consequences of the Eutrophication, to say ipoxia and anoxia in the bottom layers, it is necessary to point out that such a phenomenon takes place almost every year: areas more or less large of the coastal NW Adriatic Sea are affected, with greater frequency and intensity near the Po delta and, in the most southern zone, in the range of three kilometers from the shore.

The Oxygen demand deriving from the oxidation processes of the large amount of organic matter accumulated in the bottom waters, can become so high as to cause anoxic crises, resulting in the death of all the benthic organisms.

The persistence of this phenomenon is mainly due to the occurrence in the coastal belt of a marked thermohaline stratification, with weak coastal currents and calm sea. These conditions are typical of the late-summer season and autumn, when also the Po River flows are low and the coastal system shows the minimum values of Chlorophyll in the surface waters.

For some significant years, the evolution of the phenomenon and the affected coastal areas, are reported in the next figure.

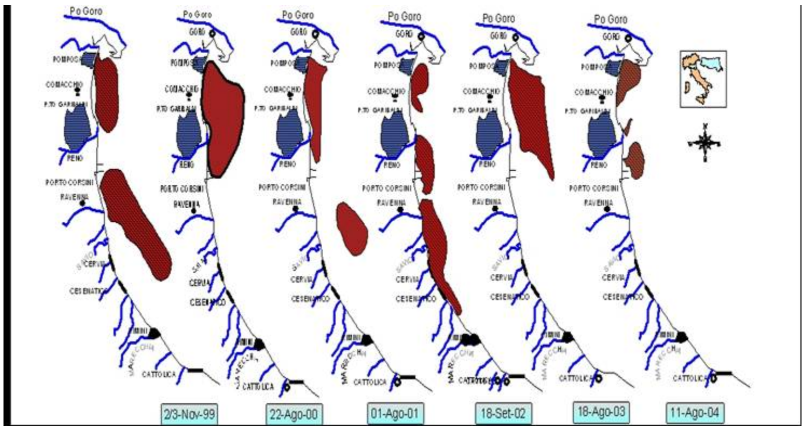

Fig. 4 *NW Adriatic Sea: anoxia in the bottom waters.* (Source: Annual reports of the S.O.D. – Struttura Oceanografica Daphne of the ARPA ER).

The framework briefly outlined above is however very complex and may have numerous variants. It should also considered that the interannual variability is high (years with heavy rainfall and high flow rates of the River Po or *vice versa*). This has led, and still implies, difficulties in correctly interpreting the monitoring data, when it comes to checking the possible effects of the sanitation policies put in place, at the scale of the Po basin.

In this regard we must remember that eutrophication control policies adopted by the Adriatic Coastal Regions and promoted by the Po River Authority, were always directed to the removal of nutrients and to the control of sources of nitrogen and phosphorus, both point sources (by means of interventions of structural type at the wastewater treatment plants: denitrification and tertiary treatment for nitrogen and phosphorus removal from waste waters), and diffuse sources (adoption of adequate regulations and best practice codes in agriculture to limit the spreading of chemical fertilizers and organic manure) (O.E.C.D. – Vollenweider and Kerekes (Eds), 1982. Haddrill et al. 1983).

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. • Good water transparency. • Absence of anomalous water colour. • Absence of Oxygen under-saturation conditions in the bottom waters.
4-5	**Good (mesotrophy)**	• Moderately productive waters. • Occasional water turbidity. • Occasional anomalous water colour. • Occasional bottom water hypoxia.
5-6	**Mediocre (eutrophy)**	• Very productive waters. • Low water transparency. • Frequent anomalous water colour. • hypoxic and occasional anoxic episodes in the bottom layers. • Some degradation of benthic communities.
>6	**Bad (hypereutrophy)**	• Strongly productive waters. • High water turbidity. • Diffuse and persistent anomalies in water colour. • Diffuse and persistent hypoxic/anoxic episodes in the bottom waters. • High mortality rate of benthic organisms. • 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.

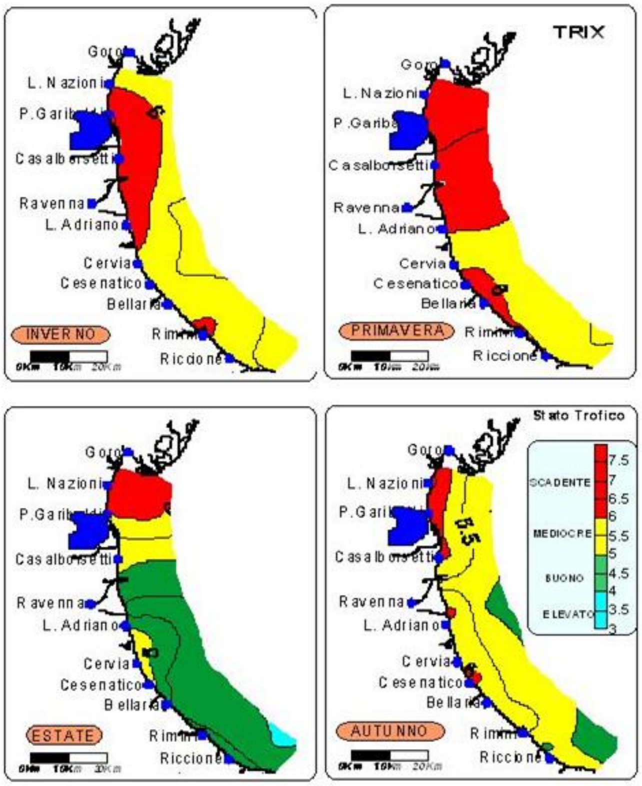

Fig. 5. *Distribution Maps of the TRIX Index along the coasts of the Emilia Romagna Region.* (From in-shore to 10 km off-shore. Year 2009. Seasonal averages) (Source: Annual reports of the S.O.D. – Struttura Oceanografica Daphne of the ARPA ER).

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([3]), 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).

([3])The quays of the port of Ravenna, that go out into the sea for some km, mark in fact the boundary between two distinct coastal water bodies, the Northern Area, close to the Po Delta and the Southern area, from Ravenna to Cattolica (see the map in Fig. 2).

TRIX-Chlorophyll relationships

As agreed among the UE MS experts involved in the Intercalibration Exercise, the chosen indicator for a classification criterion related to BQE Phytoplankton, is represented by the Chlorophyll *a*. By analyzing the long time series of this parameter (Data-base DAPHNE of the Env. Agency of the Emilia Romagna Region – ARPA ER), we have a direct confirmation of the extreme variability of the coastal system, as mentioned above. The interannual variability, in terms of changes in annual average Chl a, is reported in the next figure.

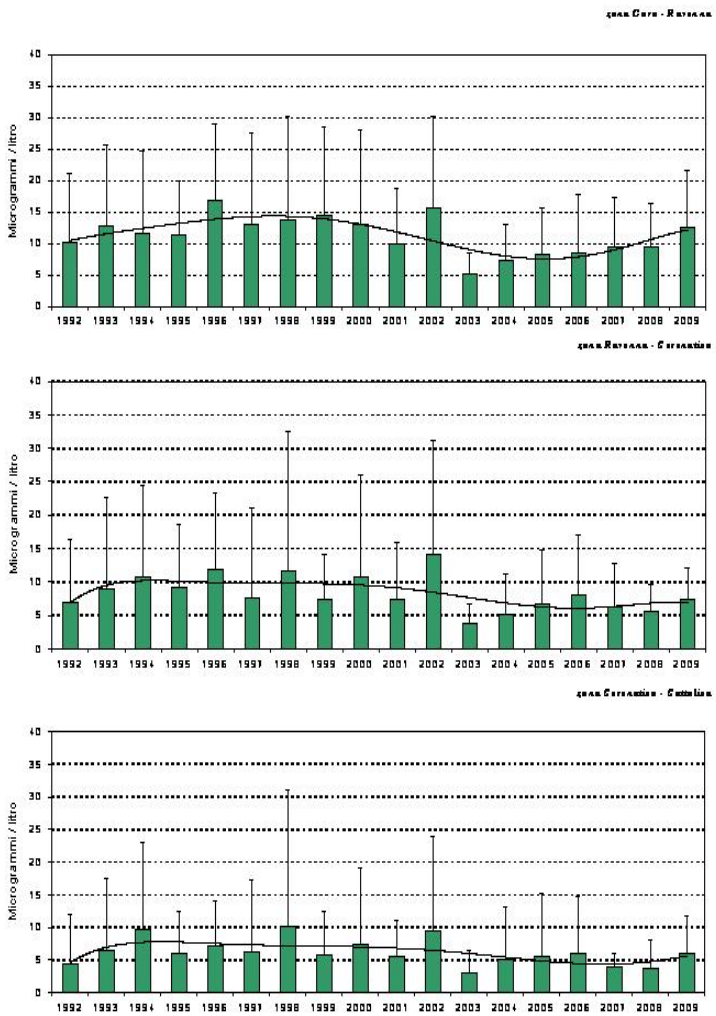

Fig. 6 Changes in annual averages of Chlorophyll values, from 1992 to 2009, in three coastal areas of the NW Adriatic Sea, from inshore to 3 km offshore. (From Goro to Ravenna: Northern Area, close to the Po River mouth (north of the jetties of the port of Ravenna). From Ravenna to Cesenatico: Intermediate Area. From Cesenatico to Cattolica: Southern Area, 60 km and more from the Po River delta.) (Source: Annual reports of the S.O.D. – Struttura Oceanografica daphne of the ARPA ER).

A quick inspection of the diagrams shows us minimum values of Chl a (ca. 5 and 3 μg/L, in the northern and in the southern zone, respectively), related to the year 2003. This low values reflect the behaviour of the coastal system in response to the low input of nutrients from the Po, since the hydrological year 2003 was strictly unusual if compared with others, because of the lack of significant floods for all the first 10 months of the year (Fig. 7).

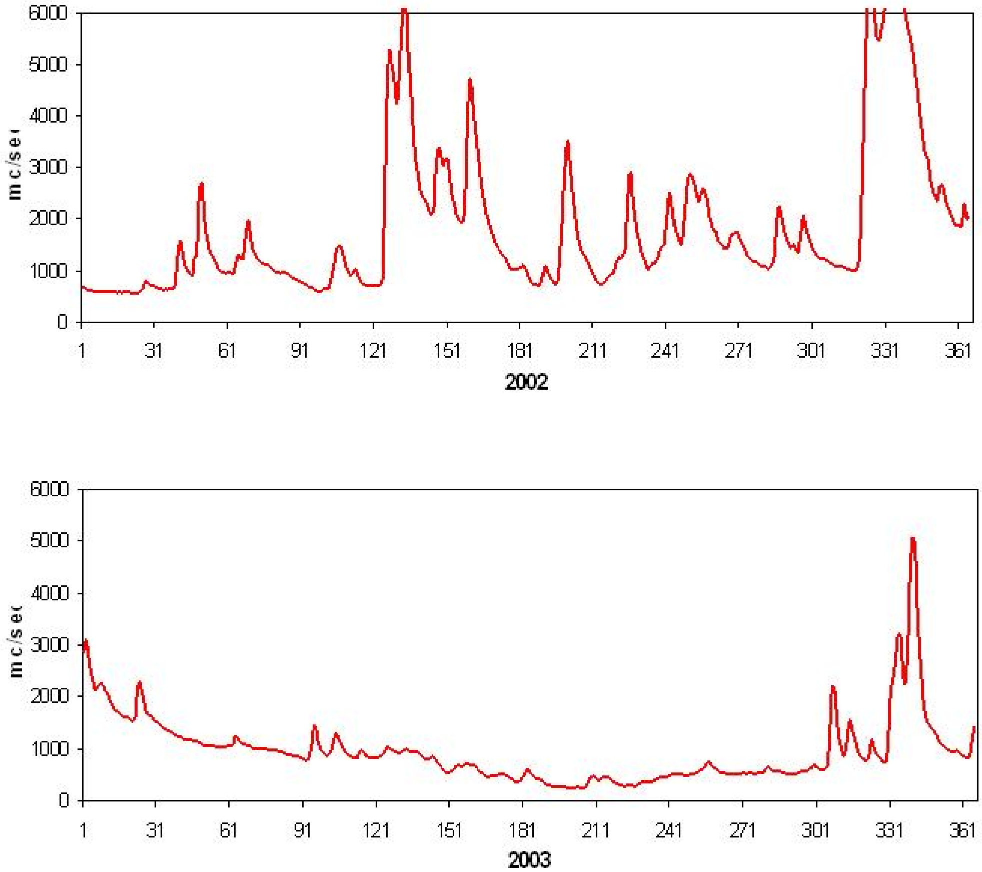

Fig. 7 Flow rates of the River Po, recorded at Pontelagoscuro (FE), in 2002 and 2003. (Source: Po River Basin Authority – A.d.B. f. Po, Parma - Italy).

By definition, Chlorophyll *a*, as 10Log, represents a component in the TRIX formulation, in such a way that the relationship between annual geometric means of Chl and corresponding TRIX mean values, can only provide a high correlation.

In the following diagram (Fig. 8), the data points are referred to two distinct periods, before and after 2001. The sole purpose of this graph is to verify the effects on the TRIX Index derived from the increase of Chlorophyll. The plotted lines (of a Log type), are, in practice, coincident and the oscillations of the TRIX data points around these lines, are to be mainly referred to the effects of the other original components on the variability of TRIX, to say nutrients and dissolved oxygen.

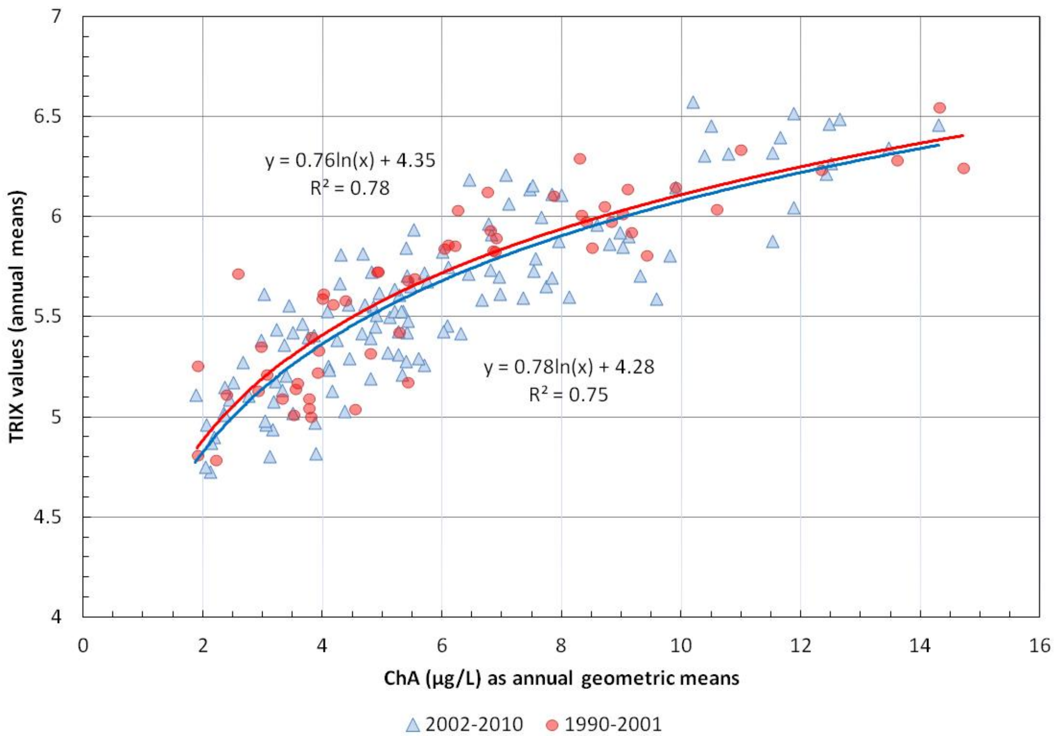

Fig. 8 *NW Adriatic Sea: TRIX index values against Chlorophyll annual geom. averages*. (The data points are referred to sampling stations 0.5 km off-shore, from Goro to Cattolica. Source: Adriatic Data Base of the S.O.D. – Struttura Oceanografica Daphne - ARPA ER).

We can interpret these trends as a substantial stability of the system Po-Adriatic over twenty years, in maintaining the same degree of efficiency in the use of available nutrients to produce phytoplankton biomass. The lack of a noticeable shift between the two curves, is a matter of reflection about the great inertia of the Po-Adriatic System to oppose the changes of trophic kind, despite the actions carried out in these two decades to combat eutrophication.

From the diagram in Fig. 8, we can draw the correspondences between TRIX and Chlorophyll([4]):

TRIX ref. value	ChA (µg/L) (as annual geometric mean)	Upper limit ChA (µg/L) (90-th percentile)	Lower limit ChA (µg/L) (10-th percentile)
5	2.5	7.1	0.9
5.5	5	14	1.8
6	9	24.6	3.1
6.5	17	47.6	6.0

As can be seen from the table, single point Chlorophyll values can vary for a huge range, from 0.9 to 47.6 µg/L, i.e. using the traditional terminology, from oligotrophic to hyper-eutrophic conditions. This is exactly what occurs in the Northern Adriatic coastal areas, where not only Chlorophyll, but also the other physical and chemical-biological characteristics, due to fluctuating freshwater supply,

([4]) The upper and lower limits of Chlorophill, as 90-th and 10-th percentiles, have been calculated under the hypothesis of Chl log-normal distributions.

intermittent longitudinal currents and inshore-offshore water exchanges, vary spatially and temporally at scales of kilometres and of days to weeks, respectively. The monitoring data therefore are often characterized by an extreme variability (Giovanardi and Vollenweider, 2004).

It is just for these reasons that Vollenweider introduced the TRIX Index (Vollenweider et al., 1998.), having concluded that, for coastal marine areas of this kind *"…a purely numeric scale that scores the trophic properties of surface and subsurface seawaters, station by station and/or sequential in time, would be preferable to some preconceived categorical denominators.*"

Conclusion

A classification criterion based on TRIX 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 WFD 2000/60 EC Directive, at the current state of knowledge, it is still premature.

Preliminary studies about the effects of trophic level increase on the biodiversity expressed by phytoplankton, have shown promising results. They have been in fact identified the 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 are becoming more and more frequent blooms of phytoplankton species characterized by small size (<3 μm), with a large number of cells/L. 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 WFD 2000/60.

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Appendix 2

BQE Phytoplankton - Assessment Pressure Methodology: Land Uses Simplified Index (LUSI) Spain-Catalunia. ***(Eva Flo, Jordi Camp, Esther Garcès, 2011)***

Note: this technical document was distributed as proposal for human-induced pressure evaluation and benchmarking purposes. The LUSI index has been adopted for IC exercise in the case of other BQEs, as e.g. Macroalgae (MA_LUSI). This BQE is in fact very sensitive to local impacts and local pollution sources. At the moment, a paper on the LUSI has been prepared and it is being published in the journal "Ecological Indicators".

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

In some Spanish regions (Valencia, Spain) 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

In conclusion, 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.

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