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Agenda item 3: State of Play of Integrated Monitoring and Assessment Programme (IMAP) Implementation with Regards to EO5 and EO9, MEDPOL Monitoring Programme and Way Forward

Guidance on Application of the Water Typology and related Monitoring and Assessment Aspects (Draft)

UNEP/MAP
Athens, 2019

Note by the Secretariat

The Mediterranean is one of the most oligotrophic seas in the world and most of its biological productivity takes place in the euphotic zone (UNEP, 1989, UNEP/MAP, 2012). The development of nutrient/phytoplankton concentration scales has been a difficult task for marine scientists because of the seasonal fluctuations of nutrient and phytoplankton concentrations, phytoplankton patchiness and small-scale eutrophication phenomena. Although long-term scientific research (UNEP/FAO/WHO1996; Krom *et al.*, 2010) has shown that the main body of the Mediterranean Sea is in good condition, there are coastal areas, especially in enclosed gulfs near big cities in estuarine areas and near ports, where marine eutrophication is a serious threat.

In the Mediterranean, the UNEP/MAP MED POL Monitoring programme included from its inception the study of eutrophication as part of its seven pilot projects approved by the Contracting Parties at the Barcelona meeting in 1975 (UNEP MAP, 1990a, b). The issue of a monitoring strategy and assessment of eutrophication was first raised at the UNEP/MAP MED POL National Coordinators Meeting in 2001 (Venice, Italy) which recommended to the Secretariat to elaborate a draft programme for monitoring of eutrophication in the Mediterranean coastal waters. In spite of a series of assessments reviewing the concept and state of eutrophication, there are important gaps in the capacity to assess the intensity of this phenomenon, even more to compare or grade the various sites. Efforts have been devoted to define the concepts to assess the intensity and to extend experience beyond the initial sites in the Adriatic Sea admittedly the most eutrophic area in the entire Mediterranean Sea. The 19th Meeting of the Contracting Parties (COP 19), held in February 2016, in Athens, Greece, adopted the Integrated Monitoring and Assessment Programme (IMAP) of the Mediterranean Sea and Coast and Related Assessment Criteria (Decision IG. 22/7), with a list of regionally agreed good environmental status descriptions, common indicators and targets, with pollution and litter related assessment criteria.

To overcame gaps in the assessment of Ecological Objective 5 - Eutrophication between the Mediterranean countries and to show step by step the development of criteria, reference conditions and boundaries between classes that will be used for the assessment of GES, as the final goal of the IMAP, the concept of this Guidelines was developed. As this process is not a simple one, it implies the use of sophisticated statistical methods, the rationale of different approaches developed by some Mediterranean countries have to be explained into details as to help the remaining countries to implement fully the IMAP itself.

At the moment the main gap in the assessment of eutrophication, as identified in the MED QSR 2017 (Decision IG. 23/6), is related to the nutrient concentrations (CI 13) for which commonly agreed thresholds have not been determined, negotiated and agreed at the sub-regional or regional level. In this Guidelines, only the approach developed for concentration of chlorophyll *a* (CI 14) in the water column will be explained.

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List of acronyms / abbreviations

BQE	Biological Quality Element
GES	Good Environmental Status
BC	Background Concentration
MED GIG¸	Mediterranean Geographical Intercalibration Group
MSFD	EU Marine Strategy Framework Directive
Nm	Nautical Mile
QSR	Quality Status Report
TP	Total Phosphorous
WFD	EU Water Framework Directive

1 INTRODUCTION

Eutrophication is a process driven by enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, leading to: increased growth, primary production and biomass of algae; changes in the balance of nutrients causing changes to the balance of organisms; and water quality degradation (IMAP, 2017). Seawaters depending on nutrient loading and phytoplankton growth are classified according to their level of eutrophication. Low nutrient/ phytoplankton levels characterize oligotrophic areas, water enriched in nutrients is characterized as mesotrophic, whereas water rich in nutrients and algal biomass is characterized as eutrophic.

GES with regard to eutrophication is achieved when the biological community remains well-balanced and retains all necessary functions in the absence of undesirable disturbance associated with eutrophication (e.g. excessive algal blooms, low dissolved oxygen, declines in sea-grasses, kills of benthic organisms and/or fish) and/or where there are no nutrient-related impacts on sustainable use of ecosystem goods and services.

1.1 Development of assessment thresholds and identifying reference conditions for eutrophication in order to be able to monitor the achievement of GES

Three approaches may be used for GES determination:

a. In order to assess quantitatively the achievement of GES in relation to eutrophication, a measurable assessment threshold may be set, including the definition of reference conditions. GES assessment thresholds and reference conditions (background concentrations) may not be identical for all areas, especially where the marine environment is already disturbed by human presence for many years. In these cases, a decision has to be made whether to set the threshold value for GES achievement independently to the setting of the reference conditions. The approach is based on the recognition that area-specific environmental conditions must define threshold values. A threshold value could include provisions to allow for statistical fluctuations (example: no nutrients and chlorophyll aa values exceeding the 90th percentile are present in a frequency more than statistically expected for the entire time series). GES could be defined on a sub- regional level, or on a sub-division of the sub-region (such as the northern Adriatic), due to local specificities in relation to the trophic level and the morphology of the area.
b. A second approach to determine GES for eutrophication is to use trends for nutrients contents, and direct and indirect effects of eutrophication. When using the trend approach, a reference value representing the actual situation is needed, for comparison. In the case of nutrients and chl-a, such reference values exist due to data availability in most areas. Therefore, GES could be defined as no increasing trends in nutrient and/or chlorophyll-a concentrations over a defined period of time in the past (ex. 6 years), which are not explained by hydrological variability. For indirect effects, GES could ask for no decreasing trend in oxygen saturation beyond what would be statistically expected.
c. GES thresholds and trends are recommended to be used in a combined way, according to data availability and agreement on GES threshold levels. In the framework of UNEP/MAP, MED POL there is experience with regard to using quantitative thresholds. It is proposed that for the Mediterranean region, quantitative thresholds between “good” (GES) and “moderate” (non-GES) conditions for coastal waters could be based as appropriate on the work that is being carried out in the framework of the MED GIG intercalibration process of the WFD, a project closely followed by the UNEP/MAP MED POL programme.

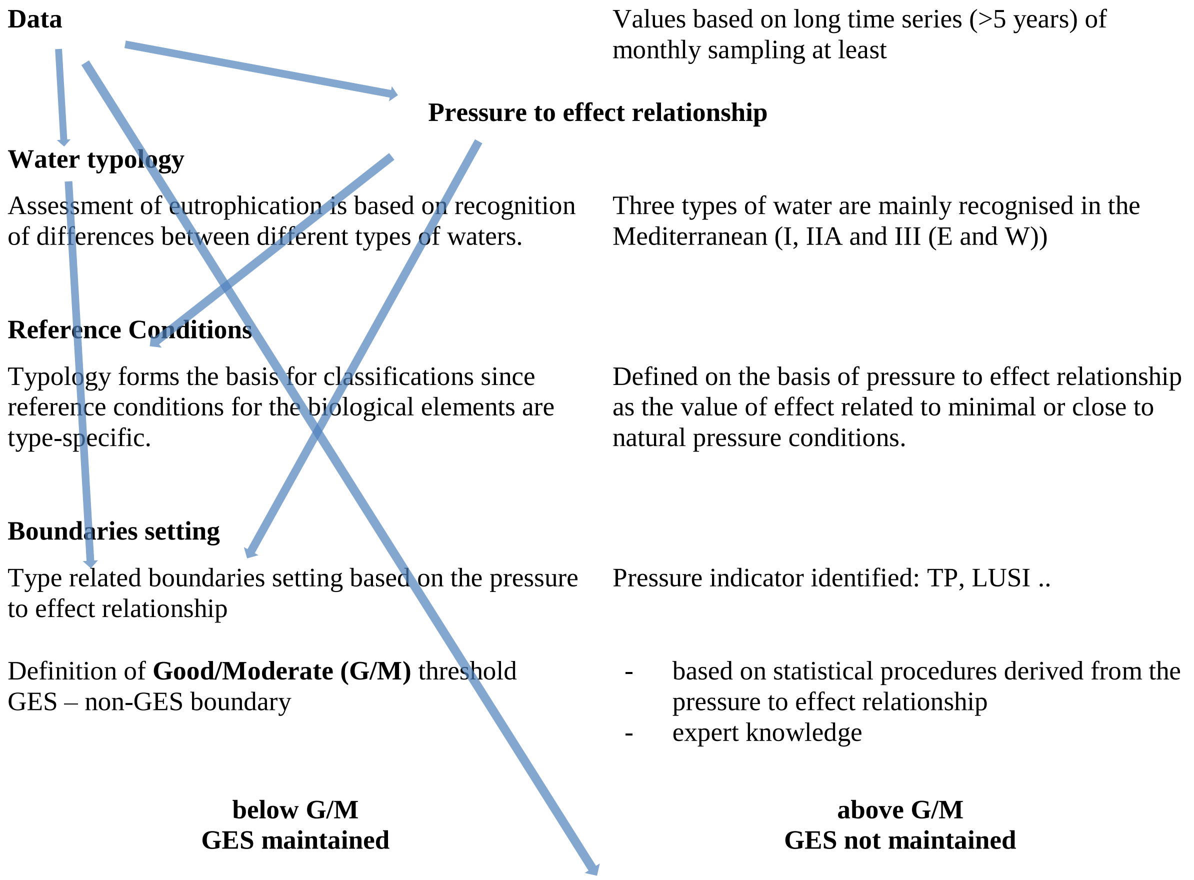
1.2 Toward GES assessment

As suggested by the on-line expert group on eutrophication established by the Contracting Parties (UNEP(DEPI)/MED WG.420/Inf.10) and later enforced in the Decision IG. 22/7 it is recommended that with regard to nutrient concentrations, until commonly agreed thresholds have been determined, negotiated and agreed upon at a sub-regional or regional level, GES may be determined on a trend monitoring basis. With regards to chlorophyll *a*, the on-line Mediterranean eutrophication group recommend the reference and threshold values of the MEDGIG approach to be used for assessing eutrophication status as presented in Table 1 (results of the 2nd phase of MEDGIG exercise). Reference and threshold (Good/Moderate status) derived values (G-mean annual values based on long time series (>5 years) of monthly sampling at least) differ from type to type on a sub-regional scale and were built with different strategies. Summaries values are given in Table 1.

Table 1. Reference and threshold values of chlorophyll *a* (Chl*a*) in Mediterranean coastal water types (according to Commission Decision of 20 September 2013 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 and repealing Decision 2008/915/EC). Enforced in Decision IG. 22/7.

Coastal Water Typology	Reference conditions of Chl*a* (µg L^{-1})		Boundaries of Chl*a* (µg L^{-1}) for G/M status	
	G_mean	90th percentile	G_mean	90th percentile
Type I	1.40	3.33[*] - 3.93[**]	6.30	10.00[*] - 17.70[**]
Type II-FR-SP		1.90		3.58
Type II-A Adriatic	0.33	0.80	1.50	4.00
Type II-B Tyrrhenian	0.32	0.77	1.20	2.90
Type III-W Adriatic			0,64	1.70
Type III-W Tyrrhenian			0,48	1.17
Type III-W FR-SP		0.90		1.80
Type III-E		0.10		0.40
Type Island-w		0.60		1.20 – 1,22

[*] applicable to Gulf of Lion
[**] applicable to Adriatic



Data

Values based on long time series (>5 years) of monthly sampling at least

Pressure to effect relationship

Water typology

Assessment of eutrophication is based on recognition of differences between different types of waters.

Three types of water are mainly recognised in the Mediterranean (I, IIA and III (E and W))

Reference Conditions

Typology forms the basis for classifications since reference conditions for the biological elements are type-specific.

Defined on the basis of pressure to effect relationship as the value of effect related to minimal or close to natural pressure conditions.

Boundaries setting

Type related boundaries setting based on the pressure to effect relationship

Pressure indicator identified: TP, LUSI ..

Definition of **Good/Moderate (G/M)** threshold
GES – non-GES boundary

- based on statistical procedures derived from the pressure to effect relationship
- expert knowledge

below G/M
GES maintained

above G/M
GES not maintained

Figure 1. Simplified scheme of GES assessment

2 WATER TYPOLOGY

Typology is very important for further development of classification schemes of a certain area. In the Mediterranean a considerable number of eutrophication experts have built a typology scheme for the Mediterranean coastal waters during the first inter-calibration phase for the EU Water Framework Directive implementation, which is still in use after their update according to Commission Decision 2013/480/UE and represents a very simple typology approach that could be easily applied Mediterranean wide for coastal waters (*sensu* WFD, i.e. 1 Nm), since these coastal waters have been intercalibrated. The typology is mainly focused on hydrological parameters, characterizing water bodies' dynamics and circulation, and is based on the introduction of the static stability parameter (derived from temperature and salinity values in the water column). Such a parameter, having a robust numerical basis, can describe the dynamic behaviour of a coastal system. Surface density is adopted as a proxy indicator for static stability as both Temperature and Salinity are relevant in the dynamic behaviour of a coastal marine system: both are involved in circulation and mixing dynamics and all information is then nested in the surface density parameter (Giovanardi *et al.*, 2006).
On the basis of surface density (σ_t) values three major water types with subdivisions have been defined:

Type I	coastal sites highly influenced by freshwater inputs,
Type IIA	coastal sites moderately influenced not directly affected by freshwater inputs (Continent influence),
Type IIIW	continental coast, coastal sites not influenced/affected by freshwater inputs (western Basin),
Type IIIE	not influenced by freshwater input (eastern Basin),
Type Island	coast (western Basin).

As it can be seen, the Coastal water type III was split in two different sub basins, the western and the Eastern Mediterranean, according to the different trophic conditions and is well documented in literature. Thus, it is recommended to define the major coastal water types in the Mediterranean Sea to assess eutrophication (Table 1). This type subdivision based only on salinity, is perfectly comparable with the previous ones, based on density.

Table 2. Major coastal water types in the Mediterranean

	Type I	Type IIA, IIA Adriatic	Type IIIW	Type IIIE	Type Island-W
σ_t (density)	<25	25<d<27	>27	>27	All range
S (salinity)	<34.5	34.5<S<37.5	>37.5	>37.5	All range

In order to better understand the differences between types, the issues presented bellow need to be considered. 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). Furthermore, it was proposed the subdivision of type II, which includes marine waters with intermediate salinities in two subtypes: type II-A and type II-B. The South of Spain (the main part of Andalusian coast) is clearly affected by the influence of the Atlantic waters, so the natural salinity, nutrients and Chl-a concentrations do not correspond with type III. Moreover, the lower salinities of before defined type II were explained by freshwater inputs, coming mainly from the continent. It

should be emphasized that in the vicinities of Gibraltar Strait there are also lower salinities that come from the Atlantic, and that is why this subdivision in Type II-A (the original one) and Type II-B (affected by Atlantic influence) was proposed.
Regarding water typology definition it is recommended (Decission 22/7) to use the typology of coastal waters as presented in Table 1 and to apply the above criteria and define the coastal water types.

3 REFERENCE CONDITION

As established by the WFD CIS Guidance Document No. 5 (2003), Reference Conditions (RC) represent “a description of the biological quality elements that exist, or would exist, at high status. That is, with no, or very minor disturbance from human activities. The objective of setting reference condition standards is to enable the assessment of ecological quality against these standards”.
In developing the RC, for better understanding of the process, two examples of Mediterranean countries will be presented:

Setting the RC for Adriatic and Tyrrhenian Sea
An acceptable approach is to use a comprehensive pressure indicator that is able to address the potential transport of nutrients (natural loads plus anthropogenic loads) from the mainland to the sea, and that also measure, albeit roughly, this transport verifying the eventual absence of pressures of some importance exerted by human activities. For this purpose, consider using the dilution factor as was the case when the RC for the Adriatic and Tyrrhenian Sea were developed (Giovanardi *et al.*, 2018)

The dilution factor is formulated as: F_dil=[(S-s)/S]*100, where S = open sea salinity, s = measured salinity at a given coastal sampling point (Giovanardi and Vollenweider, 2004). According to this definition, F_dil does not represent a true pressure indicator; however, it is indisputable that the input of nutrients in a coastal area should be strictly related to the fresh waters of continental origin.
The role of the F_dil indicator in assigning the chlorophyll *a* RC is depicted in Figure 1. The data points refer to coastal areas belonging to all typologies of water bodies in the Adriatic and Tyrrhenian seas, in order to ensure maximum variation range for the related water quality parameters. As suggested in the RC development drawn a boundary line between the area with data points and the area with no data points. For each fixed value of the F_dil indicator, corresponding chlorophyll *a* values (as annual G_means) can range from a minimum identified by the separation line to a maximum, which will depend on the weight of the nutrient loads on the coastal systems.

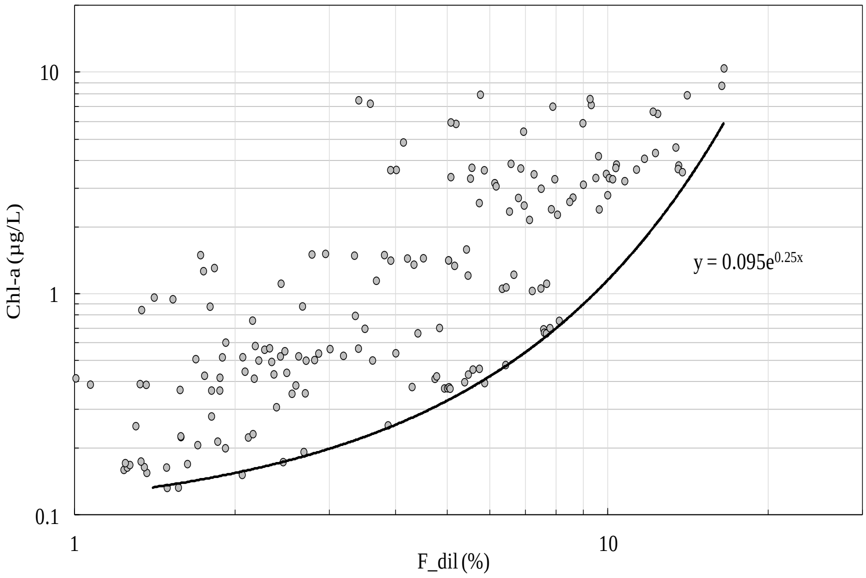


Figure 1. Scatter plot of annual *G_means* of chlorophyll *a* (Chl-a) against the dilution factor (F_dil)

for Types I and II A. The curve marks the boundary of the lower limit of chlorophyll *a* values (RC). Original Figure from Giovanardi *et al,* 2018.

This separation line can be interpreted as the threshold between natural and anthropogenic pressures. Assume that the nutrient loads, either natural or generated by minor human activities, determine a response of the coastal systems that is well-represented by concentrations of chlorophyll *a* lying on the curve (Figure 1). Thus, the assessment of RC does not derive from theoretical considerations or expert judgments, but refers to real situations occurring along the Adriatic and Tyrrhenian coasts. According to this approach, chlorophyll *a* RC represent a continuous variable functionally related to a wide spectrum of salinities that characterize different typologies of seawater. In order to define more accurately chlorophyll *a* RC for each type, the data corresponding to individual Adriatic and Tyrrhenian Types were considered separately. Then it is possible to plot the curves separately for all types (Figure 2), which now represent the RC for each type.

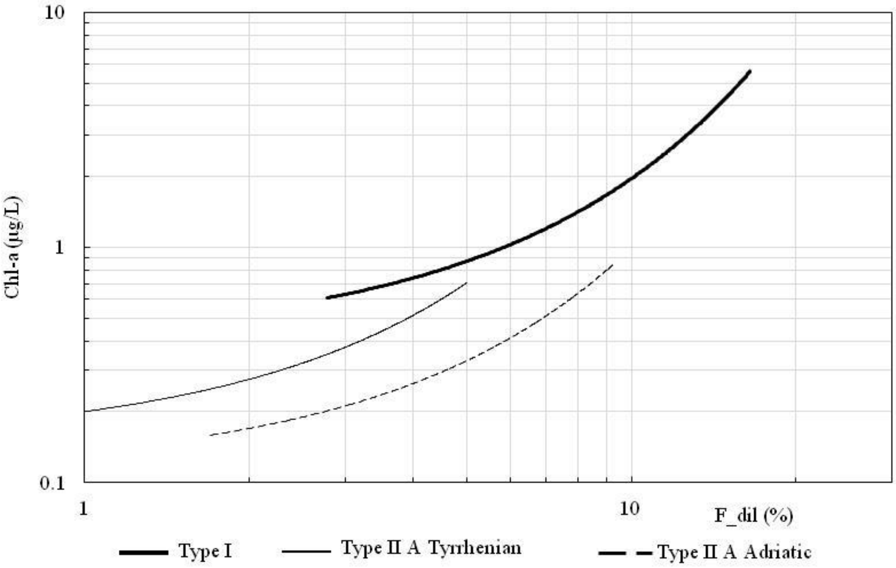


Figure 2. Reference conditions for chlorophyll *a* (Chl a) corresponding to different Types, depending on the gradient of the dilution factor (F_dil). Original Figure from Giovanardi *et al,* 2018.

The best functional relationships between chlorophyll *a* RC and F_dil were always exponential. The equations describing these relationships have been used to derive a unique chlorophyll *a* RC per type corresponding to the mean value of F_dil. Table 3 summarizes the results.

Table 3. Summary table for BQE phytoplankton reference conditions (RC) based on chlorophyll *a*.

Type	Functional relationships	F_dil (%) *Mean value*	RC - Chl-a (µg/L) as *G_Mean*	RC - Chl-a (µg/L) as *90th percentile**
Type I	$y = 0.388\ e^{0.162x}$	7.9	1.40	3.9
Type II A Adriatic	$y = 0.109\ e^{0.221x}$	4.96	0.33	0.87
Type II A Tyrrhenian	$y = 0.146\ e^{0.315x}$	2.47	0.32	0.77

* based on a theoretical *sd* value of the Log-transformed Chl-a data distributions. The recommended calculation procedure is explained in Giovanardi *et al.*, 2018.

Setting the RC for Catalan cost

This approach was developed considering the use of Land Use Simplified Index (LUSI) as the measure of pressure from land[1].

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[1] To be further developed.

4 PRESSURE TO EFFECT RELATIONSHIP

The definition of pressure to effect relationship is critical for the approach in its whole and having a complete understanding of the functional relations which link pressures to ecological effects are at the end a direct connection to programmes and measure as the final goal of the assessment process.
As the statistical approach to the whole problem is demanding the help of a statistician will be welcome.

Pressure to effect relationship for Adriatic and Tyrrhenian Seas
The bellow presented texts provides available scientific background for degerming relationship for Adriatic and Tyrrhenian Seas (Giovanardi et al, 2018).

To test the sensitivity of the selected metrics to different pressure indicators, multiple regression analysis with linear models (LMs) has to be performed first of all. By means of this stepwise regression technique, the chlorophyll *a* concentration variation were tested against the pressure indicators provided by the Adriatic and Tyrrhenian data sets (nutrient concentrations, oxygen saturation (as *aD_O*), dilution factor and Secchi depth). Annual geometric means of the parameters were used in the analysis.

Statistical analyses were performed using statistical packages offered by the program R. Data processing involved the use of techniques of regression analysis, provided by the package *stats*. The function *lm* was used to fit linear models and to carry out regressions. The function *predict* allowed to calculate confidence intervals (with confidence levels $P = 0.95$ and $P = 0.99$) for the estimated values of the dependent variable. The command *step* was used to perform stepwise regression analysis. The mode of stepwise search was chosen as *direction* "backward". The following diagnostic tests were used: i) Shapiro-Wilks test (command *shapiro.test(residuals),* from package *stats*), which assures us that the errors (*i.e.* residuals) distribution approaches normality, ii) Breusch-Pagan test (command *bptest* from package *lmtest*) against heteroskedasticity of residuals variances, and iii) Durbin-Watson test (command *dwtest* from package *lmtest*) on absence of serial correlations among the residuals. For more details on these topics consult Ricci (2006). Finally, in the case of stepwise regression, the risk of multicollinearity was controlled using the *vif* (Variance Inflation Factor) function, taken from the package *faraway*.

For Type I among all the possible combinations, the stepwise regression technique provided the following linear model:

```
lm (formula = Chl-a ~ F_dil + aD_O + TP + DIN, data = Type_I)
```

the fitted linear model explains 89% of the total chlorophyll *a* variability. The maximum weight in determining this variability accounts to TP. Summary statistic is provided in Table 4.

Table 4. Results of the stepwise regression applied to Type I coastal waters data. For each regression coefficient (Estimate), the value of Student's test (under hypothesis □ = 0), the relative *P*-value and the degree of significance expressed by the number of asterisks, are provided. *Multiple R-squared*: 0.8886, *F-statistic*: 27.93 on 4 and 14 DF, *P-value*: 1.533E-06

	Estimate (□)	*t* value	Pr(>|*t*|)	
(Intercept)	-2.4536	-4.705	3.380E-04	***
F_dil	0.1598	4.296	7.390E-04	***
aD_O	0.3212	5.241	1.250E-04	***
TP	3.6530	8.021	1.330E-06	***
DIN	-0.1100	-5.646	6.040E-05	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

For Type II A coastal waters (Adriatic and Tyrrhenian data combined) the linear model provided by the stepwise regression technique was:

lm (formula = Chl-a ~ F_dil + TP, data = Type_II A)

The linear model is quite simple, only two regressors were chosen with a largely dominant weight of TP over the weight of F_dil (Table 5). Moreover, *multiple R_squared* shows that the amount of chlorophyll *a* variability explained by this model is 78%.

Table 5. Results of the stepwise regression applied to Type II A data. Multiple R-squared: 0.7758, F-statistic: 36.33 on 2 and 21 DF, P-value: 1.521E-07.

	Estimate (□)	*t* value	Pr(>|*t*|)	
(Intercept)	-0.0097	-0.167	0.8692	*n.s.*
F_dil	0.0414	3.323	3.231E-03	**
TP	1.6219	4.089	5.250E-04	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

As TP accounts for the maximum weight in determining the variability of chlorophyll *a*, for both Type I and Type II A Adriatic and Tyrrhenian coastal waters, this parameter can be considered as the most eligible indicator of the pressure gradient. Since the phosphorus pool in the water column (TP) can be considered as an internal measure of external phosphorus enrichment.
After all the calculated above relationships showed that chlorophyll *a* sensitivity, considered as the response of coastal systems to the availability of nutrients in terms of phytoplankton biomass production, is largely controlled by total phosphorus, which can therefore assume the role of the main pressure indicator.

The important regression equations used subsequently for the construction of the ecological classification criteria are summarized in Table 6.

Table 6. List of functional relationships of interest per Type. For each regression equation, the sample size N and the R-squared values are provided.

Functional link	**Type II A Tyrrhenian**	**Type I**	**Type II A Adriatic**
1.TP vs TRIX	[TP] = exp [(TRIX – 5.363)/1.305] N = 19	[TP] = exp [(TRIX – 6.064)/1.349] N = 15	[TP] = exp [(TRIX – 6.148)/1.583] N = 52
2. Chl-a *vs* TP	$[\text{Chl-a}] = 1.656\ [\text{TP}]^{1.178}$ N = 19; $R^2 = 0.845$; $P = 3.29\ 10^{-8}$	$[\text{Chl-a}] = 10.591\ [\text{TP}]^{1.237}$ N = 15; $R^2 = 0.835$; $P = 4.45\ 10^{-6}$	$[\text{Chl-a}] = 3.978\ [\text{TP}]^{1.347}$ N = 52; $R^2 = 0.896$; $P = 2.2\ 10^{-16}$

The nature of these relationships is almost always *log-log* type, which provides the highest degree of correlation. The equations in row 1 were obtained from the inverse relationship between the TRIX index and its component TP. For Type I and II A Adriatic these equations were prepared separately per Type, using the same data as those used to assess the next functional relationships between TP and chlorophyll *a*. Finally, equations in row 2 exploit the relationship between TP and chlorophyll *a*, with the aim of fixing the limits among the ecological quality classes of the classification criterion.

Pressure to effect relationship addressed with Land Use Simplified Index (LUSI)
The Land Use Simplified Index (LUSI) was developed to asses costal pressures related to

eutrophication in 2011 by Flo *et al* (2011). LUSI serves as a proxy enabling the indirect assessment of continental nutrient loads and concentrations, and their dilution in coastal waters. Therefore, it estimates the eutrophication risk of coastal waters. It is based on systematic information describing both the anthropogenic land uses that influence coastal waters (urban, industrial, agricultural, and riverine) and coastline morphology. In a recent publication (Flo *et al*, 2019) his rationale is explained with a detailed use to asses costal pressures related to eutrophication. In Annex II the LUSI calculation methodology is presented as provided in Flo *et al*, 2019.[2]

5 BOUNDARIES SETTING

Based on the case of Adriatic (TP as pressure indicator)
With the definition of RC for Type I and Type II A coastal waters and the unveiling of their pressure/impact relationships, we have provided all the necessary tools for defining the Classification criteria for BQE phytoplankton in Adriatic and Tyrrhenian coastal waters. As the Trophic Index (TRIX, more details in Annex I) was developed first for the northern Adriatic and it ecological use is well known it was decided to use it as an internal scale for the setting boundaries.
The first step in setting the boundaries was the definition of the most important boundary: Good/Moderate (G/M) boundary, which delimits the need for taking measures in case of good ecological status failure. Firstly, the boundary was set for TP, as it appeared to be the best pressure indicator for phytoplankton in this study. The G/M boundary for TP was calculated using the equations in row 1 of Table 6, at the corresponding TRIX boundary between Good and Mediocre Trophic Status (TRIX = 5; see Table 2), which matches the transition from mesotrophic to eutrophic conditions in the coastal ecosystem. This boundary was used for both Type II A Adriatic and Tyrrhenian Seas, giving the values of 0.48 and 0.76 µmol/L, respectively. For Type I, the value of TRIX for deriving the G/M boundary was increased to 5.25, in order to take into account, the nutrient loads originating from natural sources carried by the Po River into the Adriatic Sea, presumably in not negligible amounts. In this way, the G/M boundary for TP was set at 0.55 µmol/L for Type I. In such a way, the resulting boundaries allow for more realistic and effective management policies.

Having set the boundary between G/M for the pressure parameter TP, it was possible to calculate the corresponding G/M boundary for the impact parameter chlorophyll *a*, using the equations in row 2 of Table 5, for the corresponding Types. The functional relationship between TP and chlorophyll *a* gives the following mandatory G/M boundaries: 1.50 µg/L and 1.20 µg/L for Types II A Adriatic and Tyrrhenian, respectively, and 5.00 µg/L for Type I.

In the same way, the other boundaries for Types II A Adriatic and Tyrrhenian were also set at TRIX values delimiting the trophic scale in Table 2: 4 for High/Good (H/G) boundary, 6 for Moderate/Poor (M/P) boundary and 7 for Poor/Bad (P/B) boundary. For Type I, the TRIX values used to derive the H/G and M/P boundaries were increased by a quarter of a point, likewise for the G/M boundary, while for the P/B boundary TRIX=7 was kept. The corresponding TP and chlorophyll *a* boundaries were calculated using the equations in rows 1) and 2) of Table 5 and are presented in Tables 6 – 7 for the different Types.

The identified P/B boundaries refer to "virtual" conditions, since it was not possible to detect real situations relating to ecological class “Bad” in any of the datasets analysed in this work. TP concentrations characterizing “Bad” ecological class have been extrapolated from the functional relationships extended to the area of the diagrams not actually covered by observations. It is impossible to predict how coastal systems would behave with such high concentrations of phosphorus, especially since we are talking about annual averages. We therefore consider this class as indicative, but not strictly necessary for proper ecological classification of the BQE phytoplankton based on chlorophyll *a* concentration.

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[2] To be further developed.

Table 7. Reference conditions and boundaries of ecological quality classes for BQE phytoplankton under WFD expressed by different parameters for Type I coastal waters.

Boundaries	TRIX	Chl-a annual *G_Mean* µg/L	Chl-a *90th percentile** µg/L	TP annual *G_Mean* µmol/L
Reference Conditions	-	1.40	3.93	-
H/G	4.25	2.0	5.6	0.26
G/M	5.25	5.0	14.0	0.55
M/P	6.25	12.6	35.2	1.15
P/B	7	25.0	70.1	2.00

* based on a theoretical sd value of the Log-transformed Chl-a data distributions equal to 0.35. The recommended calculation procedure is better explained in Giovanardi et al., 2018.

Table 8. Reference conditions and boundaries of ecological quality classes for BQE phytoplankton under WFD expressed by different parameters for Type II A Adriatic coastal waters.

Boundaries	TRIX	Chl-a annual *G_Mean* µg/L	Chl-a *90th percentile** µg/L	TP annual *G_Mean* µmol/L
Reference Conditions	-	0.33	0.87	-
H/G	4	0.64	1.7	0.26
G/M	5	1.5	4.0	0.48
M/P	6	3.5	9.3	0.91
P/B	7	8.2	21.7	1.71

* based on a theoretical *sd* value of the Log-transformed Chl-a data distributions equal to 0.33. The recommended calculation procedure is better explained in Giovanardi *et al.*, 2018.

Based on the case of Catalan coast (LUSI as pressure indicator)[3]

6 SIMPLIFIED ASSESMENT

The final version of this chapter will contain:

- A simplified assessment flow figure;
- The code to be used in R software package to build the figures for the historical profile;
- The annual profile was built with the software package Ocean Data View, whilst the minimum requirements will be explained.

To allow the assessment of the state of eutrophication on the basis of suggested indicators it is conceptually acceptable to use a system that integrate if possible historical data, together with collected annual data. It consists of a station eutrophication profile that displays historical eutrophication data at a station or area. Values for all parameters for CI13 and CI14 have to be presented as annual Box and Whisker graph. Example is provided on figure “X”. The boundaries between H/G and G/M are also presented on the graph “Y” and allow an easy evaluation of the state.

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[3] To be further developed.

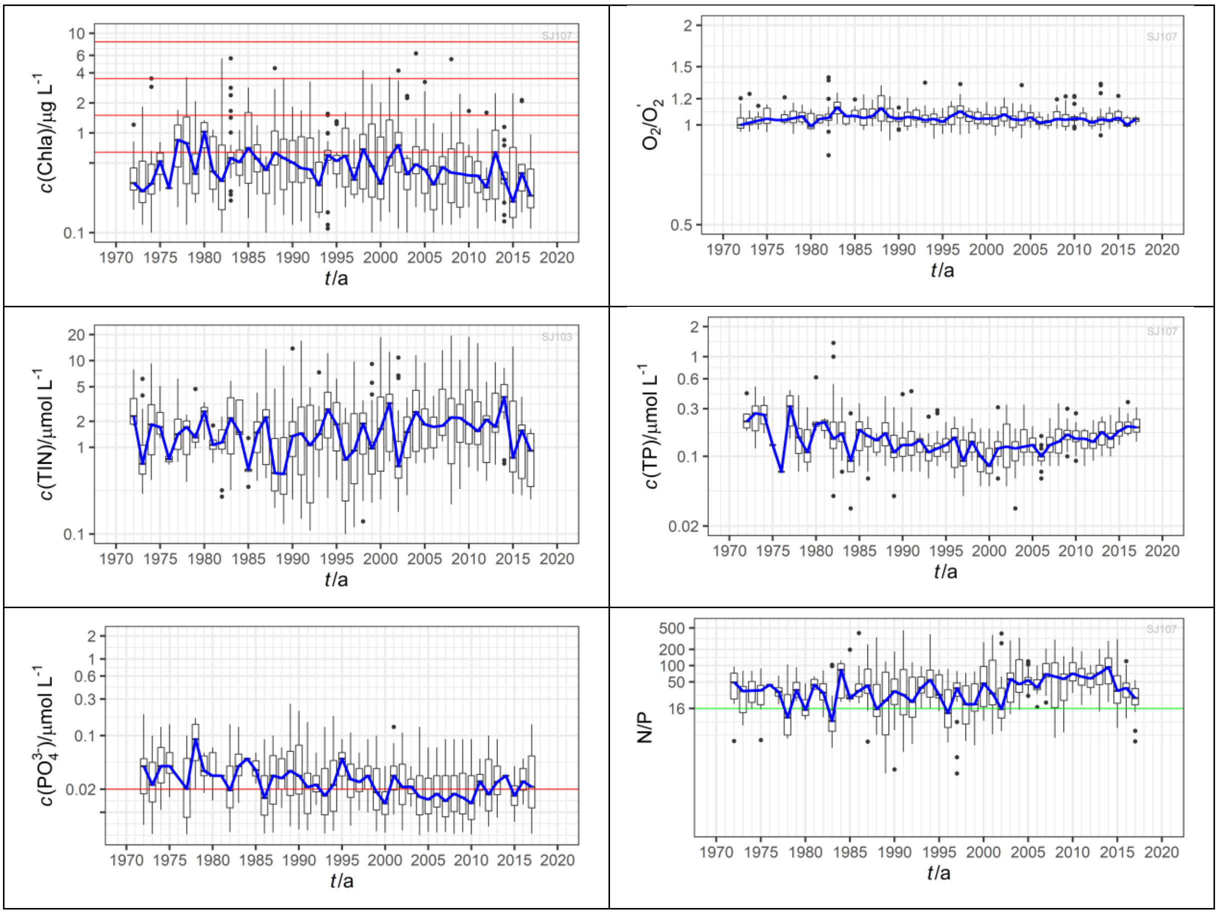

Figure X Box and Whisker representation of concentration (c) of chlorophyll *a*, total dissolved inorganic nitrogen (TIN), total phosphorus (TP), orthophospate (PO_4^{3-}), oxygen saturation (O2/O2') and the ratio between total dissolved inorganic nitrogen and orthophosphate (N/P) for the period 1970-2017 at station A20 (13 Nm W off Rovinj). Limits of boundaries are from the Table 8. Figures are the courtesy of the Centre for Marine Research in Rovinj.

Additionally, an annual eutrophication profile has also to be prepared that indicates the processes that drive the eutrophication state during the sampled year. It consists of the annual distribution of relevant parameters (e.g. concentration of chlorophyll a, orthophosphate, orthosilicate and inorganic nitrogen, and oxygen saturation and N/P ratio) at a station or area (example in Figure "Y"). To build this type of graphs the best available software is Ocean Data View.

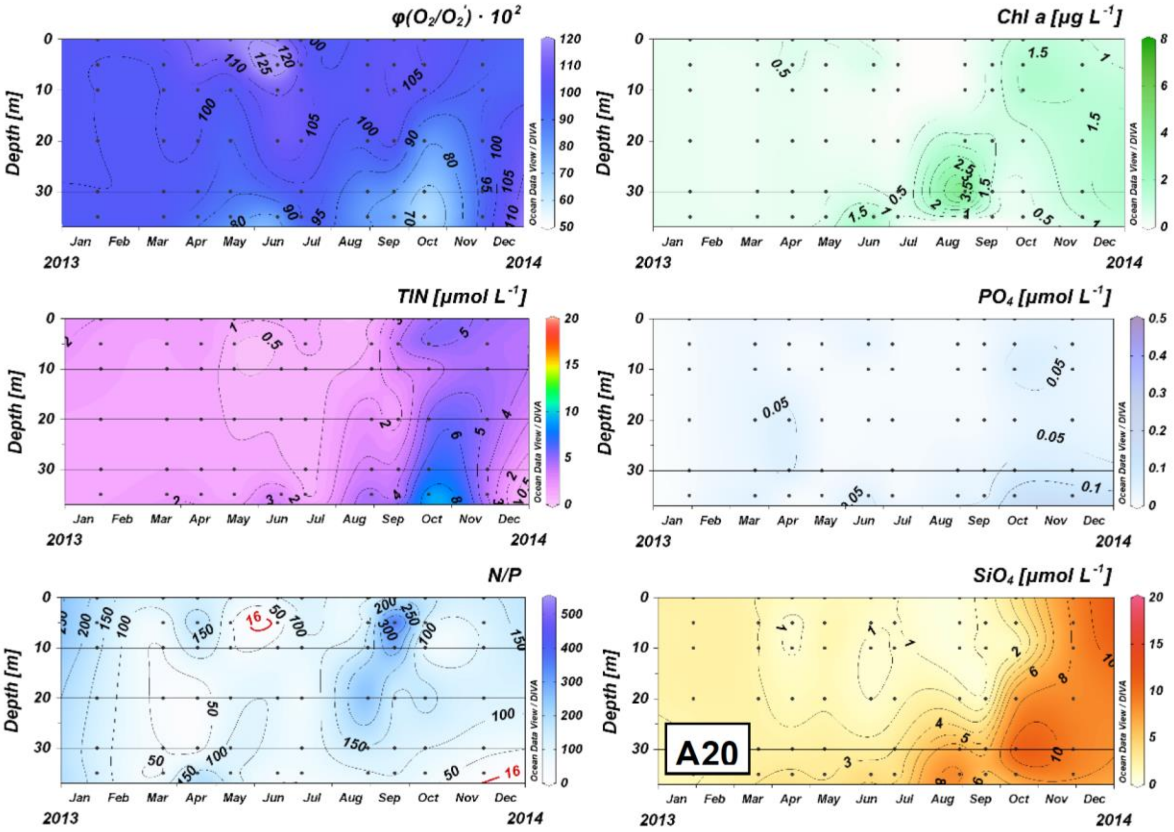

Figure Y The distribution of oxygen saturation (φ(O2/O2'), concentration of chlorophyll a (Chl *a*), total dissolved inorganic nitrogen (TIN), orthophosphate (PO4) and orthosilicate (SiO4), and the ratio of N/P with depth at station A20 during 2013. Figures are the courtesy of the Centre for Marine Research in Rovinj.

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Annex I
Trophic state classification criterion based on TRIX Index

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:

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

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.

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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)

Annex II:
Land Use Simplified Index (LUSI)

Land Use Simplified Index (LUSI)

Methodology reprinted from Flo *et al.*, 2019

The main objective of LUSI is to assess coastal pressures related to eutrophication. LUSI serves as a proxy enabling the indirect assessment of continental nutrient loads and concentrations, and their dilution in coastal waters. Therefore, it estimates the eutrophication risk of coastal waters. It is based on systematic information describing both the anthropogenic land uses that influence coastal waters (urban, industrial, agricultural, and riverine) and coastline morphology. The latter determines the degree of coastal water confinement and therefore the likelihood that continental freshwater inflows and the nutrients they contain will be diluted. LUSI not only fulfils the methodological gap, by providing a simple method to assess coastal pressures when there is a lack of information, but also the requirements of the WFD, by yielding a true pressure assessment (i.e., not confounded with impact) and allowing the establishment of pressure-impact relationships with impact indicators, such as those related to the Biological Quality Elements of the WFD.

Rationale

Ketchum (1972) defined the coastal area as the band of dry land and adjacent ocean space (water and submerged land) in which terrestrial processes and land uses directly affect oceanic processes and uses, and vice versa. One of the most important coastal process boosted by continental pressures is eutrophication. Eutrophication is driven by nutrients and it has been greatly enhanced by human activities on land, which result in the high-level production of nutrients that reach coastal waters (Chislock et al., 2013). Accordingly, the rationale for LUSI is based on the following assumptions:

I Coastal land uses determine nutrient loads and concentrations of continental freshwater inflows reaching coastal waters. The nutrient richness of these inflows forms a gradient that ranges from freshwaters with minimum nutrient values, such as those within areas where anthropogenic activities are minimal, to the maximum values generated in areas where intense anthropogenic activities pose a high risk of inducing eutrophication (Table 1). Based on this gradient, continental freshwater inflows can be classified as: (i) natural and non-irrigated (rain-watered) agricultural sources, (ii) irrigated agricultural sources, (iii) industrial sources, and (iv) urban sources. The source indicates the pressure of a particular inflow on coastal waters. Coastal areas affected by riverine inflows are considered separately.

II Coastal waters with riverine inflows are influenced by the sum of watershed land uses. Therefore, in these cases the influence of the river should be added to the influence of the land uses performed in the neighbouring continent(I). Riverine pressure is mainly a mixture of influences of natural and anthropic origin with different nutrient richness. However, for simplicity reasons LUSI does not include the discerning of the riverine pressure origin and assumes a standard nutrient concentration for riverine inflows. Accordingly, riverine pressure can be assessed using the mean coastal water salinity, as an inverse measure of freshwater content. A lower salinity implies the arrival of greater freshwater inflows from the continent and higher nutrient loads into coastal waters and, thus, a higher pressure. Maximum salinity occurs in the complete absence of freshwater inflows.

III Coastline morphology can modify the influence of continental pressures on coastal waters. It determines the degree of coastal water confinement and therefore the likelihood that continental freshwater inflows and the nutrients they contain will be diluted. In concave areas, such as bays, water is confined, residence times are long and water circulation is reduced. Consequently, continental freshwater inflows are diluted at a low rate, potentially leading to rising nutrient concentrations and an enhanced risk of eutrophication. By contrast, in convex areas, such as headlands, inflows are easily diluted, and the risk of eutrophication is attenuated. Straight coastlines do not modify the influence of continental pressures reaching coastal waters.

Requirements

LUSI requires information on the anthropogenic land uses that influence coastal waters and on coastline morphology. The pressures taken into account by LUSI include agricultural (irrigated land only), industrial, and urban land uses as well as riverine effects. Information on land uses and coastline morphology is available from different sources, including governmental sources, such as census data, satellite maps, such as those from Landsat or Google Earth, airplane, and drone survey images or combinations of them, as suggested by Lautenbach et al. (2011). However, land cover maps are the most useful for the calculation of LUSI, as they provide information on land use, the area occupied by the various types of land use, and the morphology of the coastline. There are several publicly available land cover maps with different degrees of coverage (continent, country, and region). Their appropriateness with respect to LUSI depends on the area of interest or whether distinct areas will be compared. For example, for Catalonia, in the NW Mediterranean, three maps are available: the Coordination of Information of the Environment land cover map [CORINE land cover map or CLCM; European Enviromental Agency (2012)], which covers Europe; the Sistema de Información sobre Ocupación del Suelo de España [SIOSE; Instituto Geográfico Nacional. (2011)], which covers Spain; and the Mapa de Cobertes del Sòl de Catalunya [MCSC; Centre de Recerca Ecològica i Aplicacions Forestals (2009)], which covers Catalonia. To determine riverine influences, the mean salinity value of the coastal water area of interest must be obtained. A truly representative value implies the need for a raw dataset acquired by a sampling frequency sufficient to capture the variability in the salinity. Such information is sometimes available from water management or environmental agencies. Ideally, for the calculation of LUSI, the land cover map and salinity dataset should cover the same time period.

Protocol

To calculate LUSI for a coastal water area, its quantitative information on pressures is classified into categories and assigned a score; then, all the scores are summed and multiplied by a correction factor related to coastline morphology. The protocol to calculate the LUSI is as follows:

I Pressure categories and their corresponding scores are assigned to describe urban, agricultural (irrigated land) and industrial pressures. The assignment depends on the percentage of land coverage accounted for by the respective activities in the continental area of study, which by definition extends from the coastline to 1.5 km inland. The percentage of land coverage is calculated using GIS software and a land cover map. Urban and agricultural pressures are divided into three categories and industrial pressure into two, and each category is associated with a score (Table AII.1). The three categories of urban and agricultural pressures were established considering low, moderate, and high percentage of land coverage and the two categories of industrial pressures considering low and high coverage, as a simple way to divide these gradients. Besides, the extension of the continental area of study was established as the land area that influences most directly coastal waters.

II A pressure category and its corresponding score are assigned to describe riverine pressure. In this case, the assignment depends on the mean salinity of the studied coastal water area. This gradient ranges from 0 to the maximum salinity occurring in the complete absence of freshwater inflows. For example, for Catalonia, the maximum salinity was established at 38.4. Riverine pressure is divided into three categories and each one is associated with a score (Table AII.1). These three categories were established following those of the Water Framework Directive Intercalibration Process for the Mediterranean Sea regarding the specific typology for Biological Quality Element Phytoplankton (Camp et al., 2016).

III The four pressure scores are summed. The resulting value summarizes the continental pressures reaching the studied coastal waters. Each score was established to have the same weight. However, similar categories of different pressures have different scores, following the gradient stated in the rationale regarding the nutrient richness of freshwater inflows affected by different land uses (I). For example, urban pressure was given higher scores than the other pressures.

IV The summed scores are multiplied by a coastline correction factor to obtain the LUSI value. This factor is used to consider the effect of coastline morphology on the influence of continental pressures on coastal waters and it is based on the shape of the coastline of the study area. For a concave coastline, where the confinement of coastal waters enhances the influence of continental pressures, the correction factor is 1.25; for a convex coastline, where the influence is diminished by the high dilution rates of freshwater inflows, the correction factor is 0.75; for a straight coastline, the influence is unchanged and the correction factor is 1.00 (Table AII.2). These values were chosen because they modify the influence of continental pressures on coastal waters but they cannot alter it in a considerable way. For example, coastal waters that receive large amounts of continental pressures will be highly influenced by them even if their dilution capacity is high. Thus, LUSI is calculated following Equation (1):

LUSI = (urban score + agricultural score + industrial score + riverine score)× coastline correction factor

LUSI values provide a semi-quantitative assessment of continental pressures on the coastal waters of the studied area. They have no units and range from 0.75 to 8.75. A lowLUSI value indicates that coastal waters are not or only slightly influenced by continental pressures and/or that these pressures are diluted. On the contrary, a high LUSI value indicates that coastal waters are strongly influenced by continental pressures and/or that these pressures are not diluted. LUSI is to be applied where there is no previous information on continental pressures on coastal waters. However, if information from the area of study is available, such as punctual or diffuse continental nutrients loads reaching coastal waters or coastal waters residence times, the LUSI can be modified to include it. For example, for concave coastal areas with long residence time it can be assumed that the continental inflows would be diluted at a lower rate than in concave areas with short residence time. In the former case, the confinement correction factor for concave coastal morphology can be increased accordingly to the residence time to obtain a more accurate value of the influence of land pressures on the studied coastal area.

Table AII.1 Pressures categories and their scores used to calculate LUSI.

Land use pressures			Riverine pressure	Pressure score
Urban (%LC)	Agricultural (irrigated) (%LC)	Industrial (%LC)	Salinity (Mean)	
	≤10%	≤10%	≥37.5	0
≤33%	10–40%	>10%	34.5–37.5	1
33–66%	>40%		<34.5	2
>66%				3

Land use pressures [urban, agricultural (irrigated), and industrial] are classified based on the percentage of land coverage (%LC) used for the respective activities, and riverine pressure based on the mean salinity of coastal water.

Table AII.2 Coastline morphology and the corresponding correction factors used to calculate LUSI.

Coastline morphology	Correction factor
Concave	1.25
Convex	0.75
Straight	1.00

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