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Annex

Mediterranean Sea GIG: Transitional Waters - Macroalgae and angiosperms

Contributors

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1. Introduction

- Three Member States (France, Greece, and Italy) compared and harmonised their national macroalgae and angiosperms assessment systems. Spain participated in some meetings and submitted a method (CYMOX-Multivariate index based on *Cymodocea nodosa*) and data from heavily modified transitional water bodies. Since the HMWBs were not in priority of the 2nd MEDGIG phase Spain decided to be excluded from the IC exercise after the MEDGIG meeting in Rome (February 21-22, 2011).
- Although Macroalgae and Angiosperms are separate BQEs in transitional waters all three Member States use one method to assess both. The GIG provided a justification for assessing Macroalgae and Angiosperms as one BQE and demonstrated that there is no difference if the one-out-all-out principle is applied or not.
- All methods follow a similar assessment concept.
- Intercalibration "Option 3" was used - direct comparison of assessment methods using a common dataset via application of all assessment methods to all data available.
- The comparability analysis show that national methods from France and Italy give a closely similar assessment (in agreement to comparability criteria defined in the IC Guidance), so no boundary adjustment was needed; In the case of Greece it was necessary boundaries adjustment.
- The final results include EQRs of Italy, France, and Greece macroalgae and angiosperms assessment systems for the common intercalibration IC TW type 1.

2. Description of national assessment methods

Three countries participated in the intercalibration with finalised macroalgae and angiosperms assessment methods (Table 2.1).

Table 2.1 Overview of the national assessment methods

Member State	Method	Status
France	Exclame (Macrophytes quality of transitional waters bodies)	Finalized formally agreed national method
Greece	EEI-c (Ecological Evaluation Index – continuous for transitional waters)	Finalized formally agreed national method
Italy	R-MaQI (Macrophyte Quality Index- Rapid version)	Finalized formally agreed national method

All methods classify species in two or more sensitivity groups and assess the coverage of these groups compared to the area studied. The French method takes also into account total macrophytes coverage and, for the worst classes, species richness. Despite their similarities these three methods differ in the number of sensitivity groups and the combination rule to obtain the global EQR.

France - Exclame (Macrophytes quality of transitional waters bodies; Qualité des macrophytes des masses d'eau de transition).

Two separate EQRs are calculated:

- EQRc (composition) assesses the relative coverage of reference species (characteristic of a high status). It also takes into account species richness only to discriminate between poor and bad status. This EQRc is not a “real” ratio in the sense that it is not the result of a division but the result of a formula (a different one for different ranges of relative coverage). EQRc can range continuously from 0.2 to 1. If it is lower than 0.2 it can only be 0.1. EQRc can only be calculated for sites with total macrophytes coverage of 5% or more.
- EQRa (abundance) assesses the percentage of the study area that is covered by vegetation. It can take continuous values from 0 to 1. It is not a “real” EQR but the result of a formula (different one for different ranges of total macrophytes coverage).

The global EQR (EQRmac) is more influenced by composition (EQRc) than abundance (EQRa) and results from the following combination rule: When EQRa is at least equal to 0.6 then EQRmac=EQRc. When EQRa is less than 0.6 then EQRmac is less than EQRc (different formulas apply for different EQRc and EQRa combinations).

In a nutshell, the global EQR is the EQR of composition except if the EQR of abundance is less than 0.6. In this last case the global EQR is less than EQR of composition. The global EQR is also not a “real” ratio but a result of a formula. It can range continuously from 0 to 1.

Greece - EEI-c (Ecological Evaluation Index – continuous form) classifies macrophytes species in five functional groups that fall in two ecological status groups (ESG I & II). ESG I includes late successional species of two functional groups while ESG II includes opportunistic species of three functional groups. A score for each ESG is calculated according to the relative coverage of the different functional groups. The EEI-c is calculated by a hyperbolic equation and becomes an EQR by comparing with reference conditions value after adjustment in order that the EQR ranges from 0 to 1 (Figure 2.1).

The combination rule to calculate EEI-c method – EQR is given by the formulas below:

EEI-c calculation formula

$p(x,y) = a + b*(x/100) + c*(x/100)^2 + d*(y/100) + e*(y/100)^2$

$+ f*(x/100) *(y/100)$

where x is the score in ESG I, y is the score in ESG II and a, …, f are the coefficients of the hyperbola:

a = 0.4680 b = 1.2088 c = - 0.3583

d = - 1.1289 e = 0.5129 f = - 0.1869

EQR calculation formula

EEI_{EQR}=1.25*(EEIvalue/RCvalue)-0.25, where RC=10

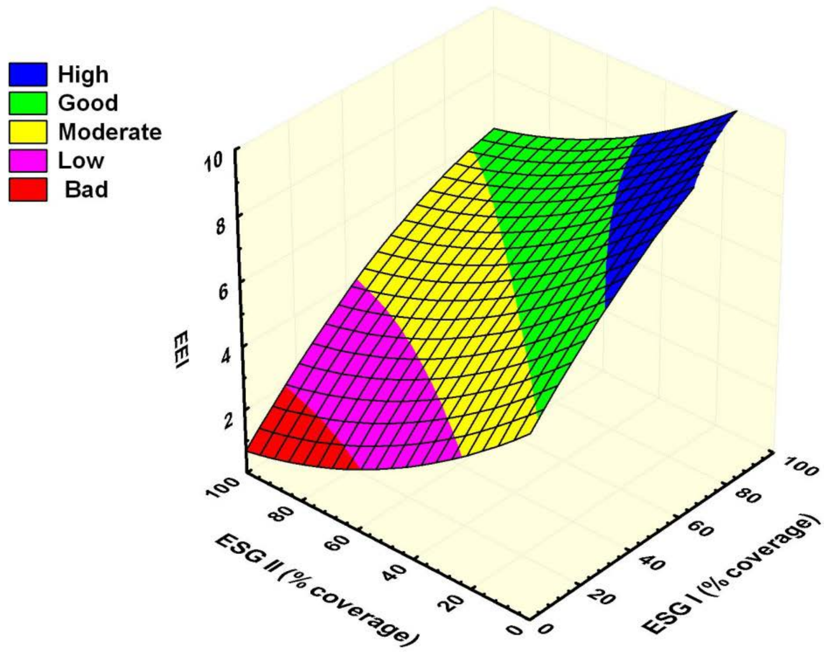


Figure 2.1 An illustration of EEI-c hyperbolic function as a function of ESG I, II (% coverage)

Italy - R-MaQI (Macrophyte Quality Index- Rapid version) classifies macroalgae in three sensitivity groups and Angiosperms in four sensitivity groups. According to the relative coverage of the different groups and based on a key (see below) a number ranging from 0 to 1 is attributed corresponding to a quality class. This number is called "EQR" but is not a ratio and not continuous (it can only be 0, 0.15, 0.25, 0.35, 0.45, 0.55, 0.65, 0.75, 0.85 &1). The R-MaQI is based on the E-MaQI (expert version) that gives EQRs that are continuous and real ratios (resulting from a division with the reference conditions values).

The R-MaQI index was developed as a categorical index (Figure 2.2), in order to overcome the limitation related to the minimum number of species (20) required by the previous continuous index (E-MaQI: Sfriso et al., 2009) and therefore to allow the classification of all Italian transitional water bodies. The choice of using a non-continuous index is therefore justified by the need to increase the accuracy of classification by an ecological point of view also where sites are characterized by a low number of species and low coverage.

The index is composed by continuous metrics (e.g. percentage of sensitive species, macroalgal relative abundance and seagrass and macroalgal total coverage). Therefore adjustments required by the IC exercise to reach comparability had been performed changing the boundaries at metric level (*Zostera noltii* and *Ruppia* coverage).

Finally, the development of a categorical index was supposed to be fully compliant with WFD requirements on the basis of the Commission Decision of 30 October 2008 (2008/915/EC), where categorical indices were included.

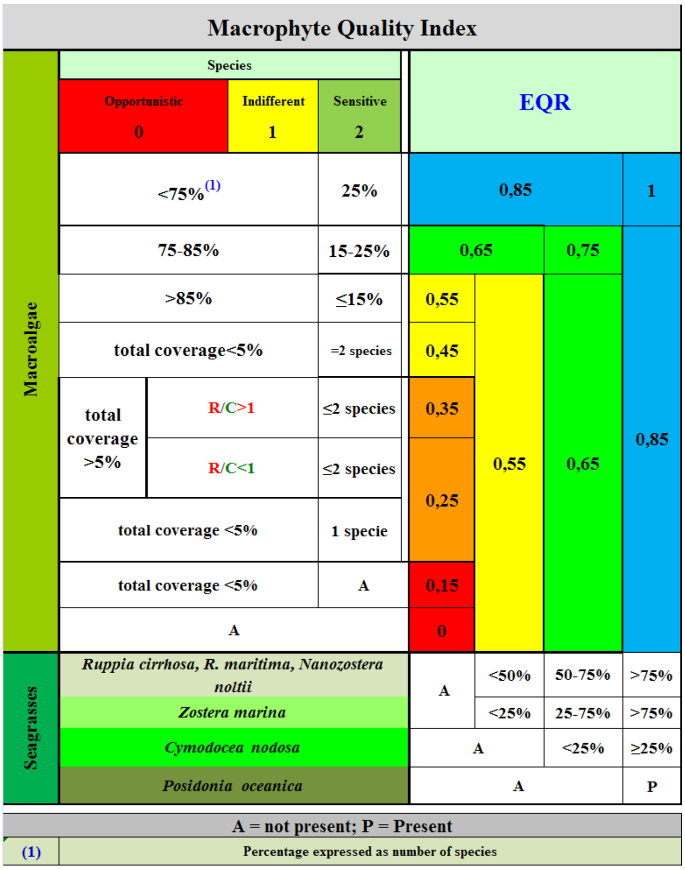


Figure 2.2 The key to calculate MAQI method – EQR

2.1. Methods and required BQE parameters

All assessment systems include:

- abundance metrics, mostly expressed as relative coverage of species belonging to groups of different sensitivity,
- disturbance sensitive taxa metrics expressed as coverage of species belonging to groups of different sensitivity.

Table 2.2 Overview of the metrics included in the national assessment methods

Member State	Full BQE method	Abundance	Disturbance sensitive taxa	Combination rule of metrics
FR	Yes*	Total coverage of macrophytes and relative coverage of sensitive species	Communities sorted into 2 sensitivity groups (reference and non-reference species)	Please, see above the description of the national methods
GR	Yes*	Coverage of groups of different sensitivity relative to the whole area sampled	Species sorted into 5 sensitivity groups	
IT	Yes*	Coverage of species or groups of different sensitivity relative to the whole area sampled	Macroalgae species sorted in 3 sensitive groups. Different levels of sensitiveness are taken into account also for seagrass species (4 groups).	

*Although Macroalgae and Angiosperms are separate BQEs in transitional waters all three Member States use one method to assess both. The GIG was asked to provide a justification for assessing Macroalgae and Angiosperms as one BQE and to demonstrate that there is no difference if the one-out-all-out principle is applied or not. In a nutshell, the GIG argued that Macroalgae and Angiosperms share similar resources (light and nutrients) and substrate (soft bottom). Angiosperms are more indicative of unimpacted conditions while macroalgae of the degraded conditions. Macroalgae and angiosperms alone cannot indicate the whole ecological status gradient from high to bad. The detailed provided justification follows below:

The principle of one-out-all-out can only be applied between benthic macrophytes (macroalgae and angiosperms) and phytoplankton quality elements in coastal lagoons soft bottom communities, which represent the most characteristic habitat-type of coastal lagoons. According to Viaroli *et al.* (2008) pristine coastal lagoons are considered as dominated by extensive meadows of perennial seagrass species, since in oligotrophic waters rhizophytes take advantage of nutrient supply from the sediment. An increasing nutrient input is thought to favour in a first phase phytoplankton and fast growing epiphytic microalgae, and later on floating ephemeral macroalgae which alternate with phytoplankton communities. Finally, the increased water turbidity is assumed to depress macroalgal growth leading to a dominance of phytoplankton species (Figure 2.3).

The functional differences of benthic vegetation components related to life-cycle strategy (r-, *K*-selected species) have been also used to describe benthic vegetation succession along a nutrient gradient (Figure 2.4). Namely, the oligotrophic-pristine and the eutrophic-degrated conditions have been assumed to represent two alternative stable states or attractors. Under low nutrient and clear water conditions of the pristine-oligotrophic state, the late-successional angiosperms *Ruppia* and *Zostera* become dominant. By contrast, opportunistic seaweeds as *Gracilaria, Ulva* and *Cladophora* along with cyanobacteria indicate the degraded-eutrophic state, which is characterized by high nutrients conditions. Nutrient excess is considered to induce the shift between the two alternative states by favouring the rapid growth and/or the colonization ability of seaweeds to exclude angiosperms. The coexistence of macroalgae and angiosperms is a symptom of intermediate conditions. Both states are hypothesized to be resilient through feedback mechanisms. For example, rooted plants tend to sustain clear water state through canopy and rhizomes by moderating water turbulence and stabilizing sediment.

In summary, seaweeds and angiosperms are sharing similar resources, e.g. light and nutrients, in coastal lagoons and constitute components of the similar habitat type: soft bottom communities. Since the angiosperms are more indicative of the pristine conditions and the seaweeds of the degraded conditions the seaweeds or the angiosperms alone cannot indicate the whole of ecological status classes' gradient from high to bad.

In contrast to coastal lagoons the principle of one-out-all-out can be applied between macroalgae and angiosperms in coastal waters because they form typical communities of separate habitat types, i.e. rocky and sedimentary coasts, respectively.

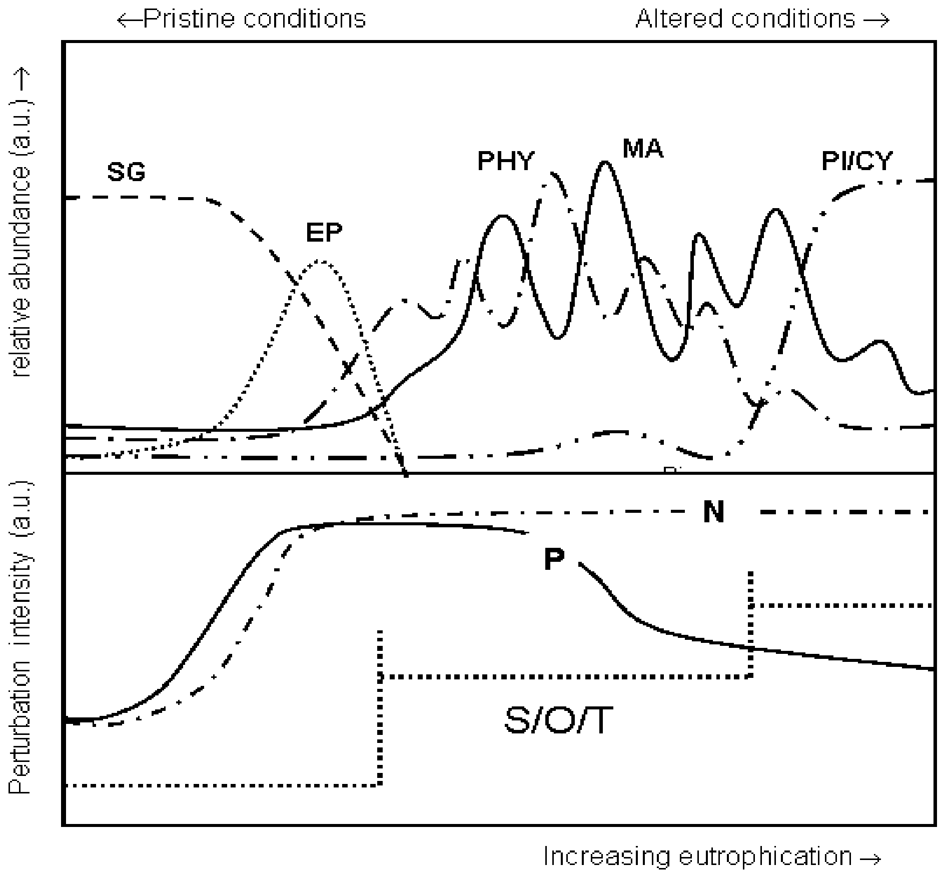


Figure 2.3 Conceptual representation of the succession of aquatic vegetation along an increasing eutrophication gradient [from Viaroli et al. 2008, modified from Schramm (1999) and Nilssen (1978)]. Community shifts are accompanied by high-intensity perturbations. Smaller oscillations do not correspond to changes in the community structure. Legend - SG: seagrass; EP: epiphytes, PHY: phytoplankton, MA: macro-algae; PI/CY: picofitoplankton /cyanobacteria; P: phosphorus concentration, N: Nitrogen concentration, S: sulphide level, O: oxygen deficit, T: water turbidity.

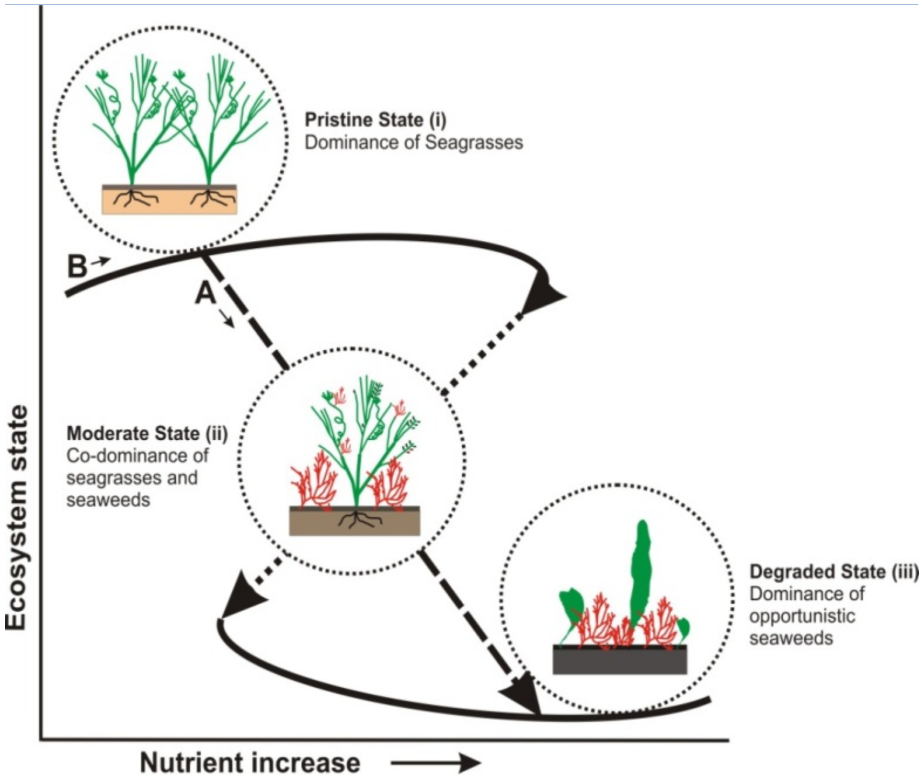


Figure 2.4 Conceptual model of two alternative stable states of marine benthic vegetation across a eutrophication (ecological status) gradient in lagoons. A conventional (A) and dynamic (B) view of vegetation changes in coastal lagoons is indicated (Viaroli et al. 2008).

2.2. Sampling and data processing

Table 2.3 Overview of the sampling and data processing of the national assessment methods

Member State	Sampling device	Surveyed compartment/ habitat/ecotope	Sample processing	How is abundance measured
FR	Scuba diving - visual observation	All available habitats in subtidal zone. The habitat is soft bottom sediments	The vegetation of the entire transect is mapped. Angiosperms are identified to species level and macroalgae to the lowest possible taxonomic level and enough to classify them in the relevant sensitivity group. The relative coverage of each species/taxa/group is calculated. Single plants are taken to validate correct identification.	Percent coverage
GR	Metal hand-held box corer (17 cm long x 17 cm wide x 15 cm high), which is vertically pushed through the benthic vegetation and sediment	Soft bottom in subtidal zone	Organisms of the complete sample are identified at species level. The surface covered by each species <u>in vertical projection</u> is quantified as % of coverage.	Percent coverage
IT	Rake	Soft bottom in subtidal zone	Organisms of the complete sample are classified in the relevant sensitivity group. This might be equal or higher than species level.	Percent coverage

2.3. National reference conditions

Table 2.4 Overview of the methodologies used to derive the reference conditions for the national assessment methods

Member State	Type and period of reference conditions	Number of reference sites	Location of reference sites	Reference criteria used for selection
FR	La Palme: June 2007 Palo: June 2009	2	French Mediterranean coast including Corsica Etang de La Palme: 42°57'930"N, 3°00'184"E Etang de Palo : 41°57'165"N, 9°45'289"E	Existing near-natural reference sites, Expert knowledge
GR	Klisova: July 2010 Fanari: July 2009	2	North & Western Greece (where the main coastal lagoons are located). Klisova: 38°20'089"N, 21°25'424"E Fanari: 40°57'476"N, 25°08'489"E	Least disturbed conditions, Expert knowledge
IT	Portosecco: June 2011 Santa Maria del Mare: June 2011	2	North Eastern Italy (Venice). Portosecco: 45°19'142"N, 12°18'465"E Santa Maria del Mare: 45°19'568"N, 12°18'478"E	Least Disturbed Conditions

2.4. National boundary setting

Table 2.5 Explanations for national boundary setting

Member State	Type of boundary setting: Expert judgment – statistical – ecological discontinuity – or mixed for different boundaries?	Specific approach for H/G boundary	Specific approach for G/M boundary	Boundary set-ting procedure: method tested against pressure
FR	The boundary setting was not done in relation to pressures but with a, more or less, arbitrary division of the EQR continuum	Calibrated against pre-classified sampling sites and a posteriori equidistant division of the EQR gradient	Calibrated against pre-classified sampling sites and a posteriori equidistant division of the EQR gradient	YES, it has been tested against Eutrophication, General degradation, Habitat destruction
GR	The boundary setting was not done in relation to pressures but with a, more or less, arbitrary division of the EQR continuum	Calibration against reference sites, equidistant division of the EQR gradient and adjustment by an hyperbolic model *	Calibration against reference sites, equidistant division of the EQR gradient and adjustment by an hyperbolic model *	YES, Eutrophication, General degradation, Habitat destruction, Pollution by organic matter
IT	The boundary setting was not done in relation to pressures but with a, more or less, arbitrary division of the EQR continuum	Equidistant division of the EQR gradient	Calibration against reference sites and equidistant division of the EQR gradient	YES, Eutrophication, Habitat destruction, Hydromorphological degradation, Pollution by organic matter

*The Greek method (EEI) was first developed as a non-continuous index (Orfanidis et al., 2001). In this first version boundaries were equidistant. In a later version (Orfanidis et al., 2011) it became a continuous index, calculated by a hyperbolic model combining the scores obtained for the two main ecological groups. Using 2000 generated values and applying the model the boundaries were recalculated and adjusted in a way that they are not equidistant anymore.

2.5. Results of WFD compliance checking

Table 2.6 List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by one of five classes (high, good, moderate, poor and bad).	All MS: yes
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions (Boundary setting procedure)**	All MS: yes
• Scope of detected pressures	Yes, see section on Pressures addressed
• Has the pressure-impact relationship of the assessment method been tested?	Yes, see section on Pressures addressed
• Setting of ecological status boundaries: methodology and reasoning to derive and set boundaries	Equidistant division of the ecological gradient (with adjustment by an hyperbolic model for GR) see section on boundaries setting.
• Boundary setting procedure in relation to the pressure: Which amount of data/pressure indicators have been related to the method and what was the outcome of the relation?	See section on Pressures addressed
• Reference and Good status community description: Is a description of the communities of reference/high – good – moderate status provided? Not only a formula or an EQR value, but the range of values for the different parameters included in the method that result in high – good – moderate status	See section on Ecological Characteristics.
3. **All relevant parameters** indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	All methods consider abundance and disturbance sensitive taxa.
• Complete list of biological metric(s) used in assessment	Only the FR method explicitly includes an explicit metric on total abundance (percentage of study area covered by vegetation). In the GR and IT method there is no explicit metric on total abundance but all methods consider percentage abundance of groups of different sensitivity in relation to the total area or the total cover.
• Data basis for metric calculation: single sample in space or time for metric calculation or aggregated data in space or time?	Aggregated data
• Combination rule for multimetrics	All MS: Yes, see section on required BQE parameters
4. Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by	All MS: Yes. All methods apply to the one IC type defined.

Compliance criteria	Compliance checking conclusions
WG ECOSTAT	
• Is the assessment method applied to water bodies in the whole country?	Yes
• Specify common intercalibration types	See section on typology
• Does the selection of metrics differ between types of water bodies?	All MS: No
5. The water body is assessed against **type-specific near-natural reference conditions**	All MS: Yes.
• Scope of reference conditions	See section on national reference conditions
• Key source(s) to derive reference conditions	See section on national reference conditions
• Number of sites, location and geographical coverage of sites used to derive reference conditions	See section on national reference conditions
• Time period (months+years) of data of sites used to derive reference conditions	See section on national reference conditions
• Reference site characterisation: criteria to select them	No info provided.
• Is a true reference used for the definition of High status or an alternative benchmark estimation?	Yes, a true reference is defined to calibrate high status sites against.
6. Assessment results are expressed as **EQRs**: • Are the assessment results expressed as Ecological Quality Ratios (EQR)?	All MS: Yes.
7. Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	All MS: Yes.
• Has the uncertainty of the method been quantified and is it regarded in the assessment ?	No info provided
• Specify how the uncertainty has been quantified and regarded	No info provided
8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	All MS: Yes, see section on sampling and data processing
9. Selected taxonomic level achieves adequate **confidence and precision** in classification	All MS: Yes
• Minimum size of organisms sampled and processed	See section on data acceptance criteria
• Record of biological data: level of taxonomical identification – what groups to which level	See section on data acceptance criteria

Conclusions of the compliance checking: There are some derogations from the letter of the Directive concerning assessing macroalgae and seagrasses separately in transitional waters for which the GIG has provided a relevant justification.

3. Results IC feasibility checking

3.1. Typology

Table 3.1 Description of common intercalibration water body types and the MS sharing each type

<table>
<tr><th>Common IC type</th><th>Type characteristics</th><th>MS sharing IC common type</th></tr>
<tr><td>IC type 1</td><td>Meso-, poly- and euhaline coastal lagoons (>5‰) either confined or not confined</td><td>France: yes
Greece: yes
Italy: yes</td></tr>
<tr><td colspan="3">No other common types</td></tr>
</table>

Intercalibration is feasible in terms of typology.

3.2. Pressures addressed

Intercalibration is feasible in terms of **pressures** addressed by the methods.

All methods have a significant response to the same pressures.

<table>
<tr><th>Member State</th><th>Pressure or combination of pressures</th><th>Strength of relationship Methods vs Pressure index</th></tr>
<tr><td>France</td><td rowspan="3">A common pressure index was calculated taking into account the following pressures :
Non-point pollution sources (Agricultural diffuse inputs, Freshwater input)
Point pollution sources (Domestic discharges, Industrial discharges)
Morphological alternations- habitat loss (Urban, Industry)
Alternations of hydrological regime (Port activity, Navigation),
Resource exploitation (Fin-Fisheries, Shell-fisheries),
A score for each pressure was given with expert judgment</td><td>$r^2 = 0.7519$</td></tr>
<tr><td>Greece</td><td>$r^2 = 0.8506$</td></tr>
<tr><td>Italy</td><td>$r^2 = 0.8268$</td></tr>
</table>

The regression of each method in relation to pressures is presented below (Figure 3.1 to Figure 3.3):

France

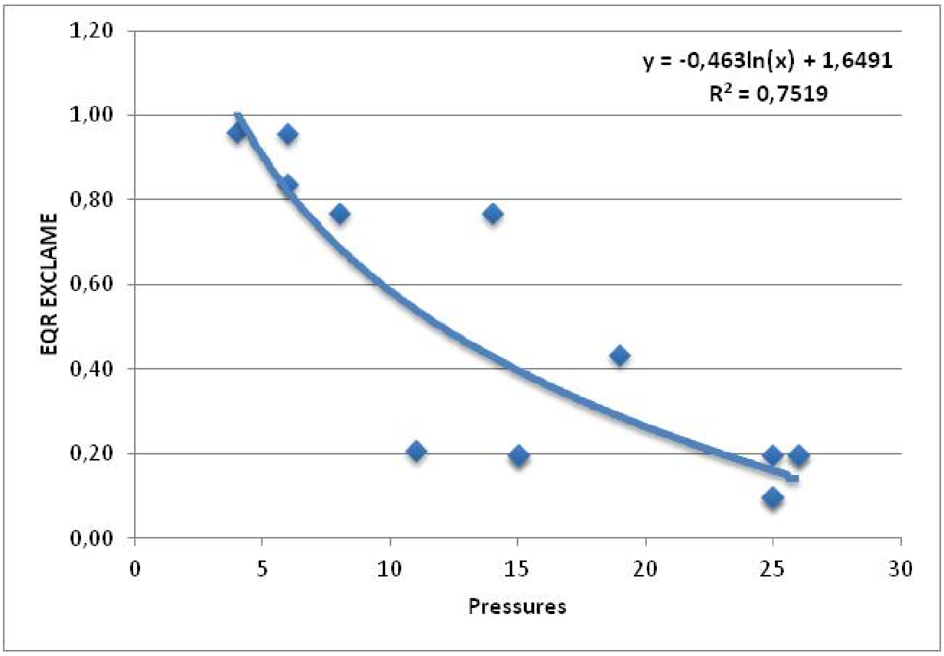


Figure 3.1 Relation between Exclame and total pressures in the French coastal lagoons

Greece

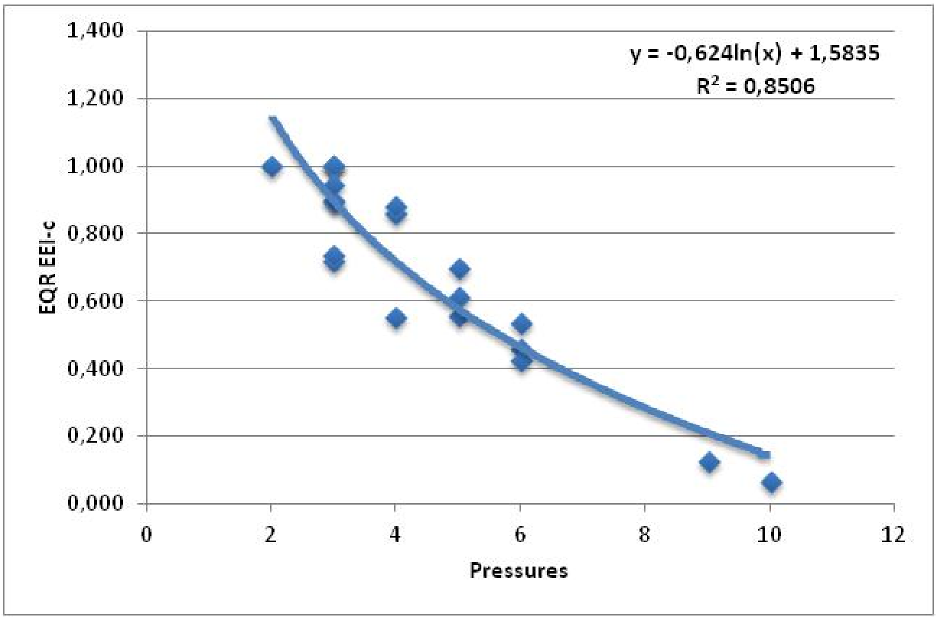


Figure 3.2 Relation between EEI-c and total pressures in the Greek coastal lagoons

Italy

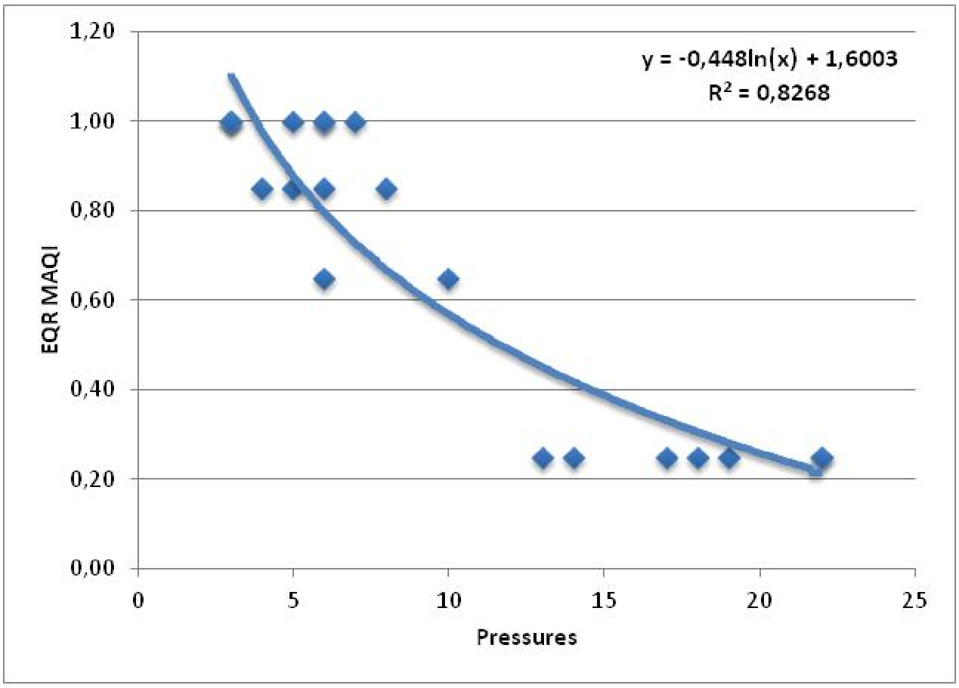


Figure 3.3 Relation between R-MAQI and total pressures in the Italian coastal lagoons

3.3. Assessment concept

All national methods follow a similar assessment concept as they all classify macrophytes in sensitivity groups and calculate the relative coverage[1] of each group. The methods of IT & GR focus on soft bottom while the one of FR includes all available habitats but is not an important difference as soft sediment is the dominant habitat in coastal lagoons.

Method	Assessment concept	Remarks
France	All available habitats per site	The main habitat is "soft bottom sediments"
Greece	Subtidal zone single habitat	Soft bottom sediments
Italy		

[1] The coverage is calculated by two different methods. For more details, see Annex A.

4. Collection of IC dataset and benchmarking

4.1. Dataset description

In Greece, the macroalgal community (species diversity and abundance) of twenty (20) sampling sites was studied at ca. 1 m depth by means of a box corer of 17 cm x 17 cm in size. One hundred (100) destructive samples in total were sorted carefully in the laboratory and the surface covered by each species in vertical projection was quantified as % of coverage. The final data were submitted as mean values (5 samples per site) at the site scale.

In Italy, the macroalgal community (species diversity and abundance) of twenty (20) sampling sites was studied at ca. 1 m depth by means of a metallic frame 70 cm x 70 cm in size. Sixty (60) destructive samples in total were sorted carefully in the laboratory and the surface covered by each species in vertical projection was quantified as % of coverage. The final data were submitted as mean values (3 samples per site) at the site scale.

In France, the macroalgal community (species diversity and abundance) of fourteen (14) sampling sites was studied at ca. 1-9 m depth by means of visual sensing by two independent divers at the site scale. Representative material was selected and studied carefully in the laboratory to identify taxonomical difficult species. While the total coverage of the Greek and Italian sites usually exceeded 100% due to the estimation of all different vegetation layers (canopy, bushy layer, crusts and epiphytes), the maximal total coverage of the France data was 100% (all in one vegetation layer). However, since all data have been submitted at the site scale the difference in the sampling design strategy between Greece and Italy at one side and France at the other side was regarded as non-considerable to introduce bias in the analysis for the Common Metric as well as in the Indices estimation.

Table 4.1 Description of data collection within the GIG

Size of common dataset: total number of sites	54 datapoints
Number of Member States	3
Repackage/disaggregation of samples/WB results?	Data points represent the result of different samples taken at one site at one point in time
Gradient of ecological quality	Fully covered
Coverage per ecological quality class	High: GR: 9, IT: 11, FR: 3 sites Good: GR: 6, IT: 2, FR: 2 sites Moderate GR: 3, IT: 7, FR: 1 sites Poor GR: 1, IT: 0, FR: 6 sites Bad GR: 1, IT: 0, FR: 2 sites

Table 4.2 Description of the data set

Member State	Number of sites		
	Biological data	Physico- chemical data	Pressure data
France	14 sites	14 sites	14 sites
Greece	20 sites	20 sites	20 sites
Italy	20 sites	20 sites	20 sites

4.2. Data acceptance criteria

Table 4.3 Overview of the data acceptance criteria used for the data quality control

Data acceptance criteria	Data acceptance checking
Data requirements	Total coverage should be >10%. Thus, hypertrophic or strongly disturbed sites where benthic macrophytes start to disappear were not included in IC dataset.
The sampling and analytical methodology	All samples were taken in late spring-summer.
Level of taxonomic precision required	Level of taxonomic resolution was at least at genus level and enough to classify the taxon to the relevant sensitivity group.
The minimum number of sites/samples per intercalibration type	No specifications were set for the minimum number of sites. The whole process indicated that the sites used for the one common type intercalibrated were adequate.
Sufficient covering of all relevant quality classes per type	-

4.3. Common benchmark

Initially, alternative benchmark sites were defined as the ones having total pressure ≤ 6. This selections was validated by the presence of marine angiosperms communities. Additionally, Simper analysis indicated that the benchmark sites selected are characterized by the dominance of three angiosperm species: *Cymodocea nodosa* (49.9%), *Ruppia cirrhosa* (35.67%) and *Zostera noltii* (10%). However, due to high natural variability of coastal lagoon ecosystems across the Mediterranean Sea and to relative low number (2) of benchmark sites provided by each country it was decided not to use the benchmark values in the IC exercise. Instead, all data points (summarised by individual regression curves) are used to determine the differences between the countries (**continuous benchmarking**).

4.4. Benchmark standardization

For standardization of national EQRs the correction values (offset subtraction) have been estimated using the GLM model.

5. Comparison of methods and boundaries

5.1. IC option and common metrics

IC Options 3 was used as all participating countries (Greece, Italy, France) have applied different methods, and have similar data acquisition.

5.2. Results of the regression comparison

Linear regression was used to relate the pseudo common metric with the EQR of each national method. All methods have significant correlations with the pseudo-common metric (Table 5.1), so, no method were excluded.

Table 5.1 Results of the regression analysis (National EQRs vs PCM)

Member State/Method	r	p	slope
France	0.888	< 0.001	0.932
Greece	0.836	< 0.001	0.779
Italy	0.727	< 0.001	0.920

- The **Pearson correlation coefficient** ranges from 0.888 to 0.727. The requirement that r ≥ 0.5 is fulfilled.
- The slope of the regression range from 0.779 to 0.927. The requirement that the slope should lie between 0.5 and 1.5 is fulfilled.
- Checking of methods comparability: No parameter fee statistical test have been performed in addition to the regression analysis,

5.3. Comparability criteria

Assessing level of boundary bias

Table 5.2 Overview of the IC comparability criteria

Member State	H/G bias	G/M bias	Absolute class difference (3 classes, 108 comparisons)
Requirement	>-0.250	>-0.250	<1.0
France	-0.162	-0.147	0.287 (0.2870**)
Greece	0.360 (0.203**)	0.493 (0.401**)	0.259 (0.28**)
Italy	-0.175	-0.230	0.379 (0.3981**)
Avg	0.23 * (0.18**)	0.21* (0.21**)	0.308 (0.322**)

*calculated from absolute values

** after adjustment

For the Greek method, both H/G and G/M boundaries are too precautious, so boundary adjustment was necessary (H/G and G/M boundaries were lowered to 0.7 and 0.4 respectively).

Assessing class agreement

The average absolute class difference for each national method was calculated. This measure quantifies how far on average a national classification deviates from all other classifications when assessing a sample. The average absolute class difference must be smaller than 1.0 (Sebastian Birk, Nigel Willby and Dirk Nemitz, 2011. User's Manual of the Intercalibration Spreadsheets).

To calculate class agreement the ordinal-scaled has been used (1=high, 2=good, 3=moderate or worse - classes *moderate*, *poor* and *bad* lumped together) as indicated in User's Manual of the Intercalibration Spreadsheets.

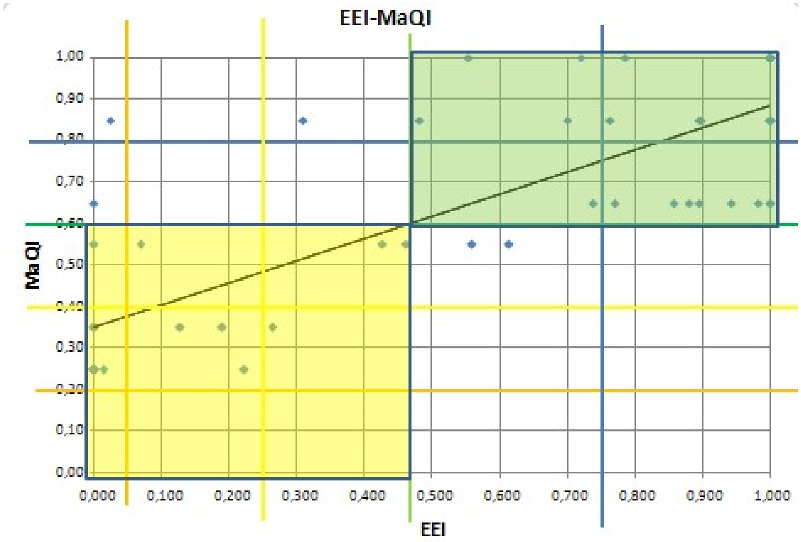
EEI-MaQI	EEI-Exclame	MaQI-Exclame
0.35	0.16	0.4

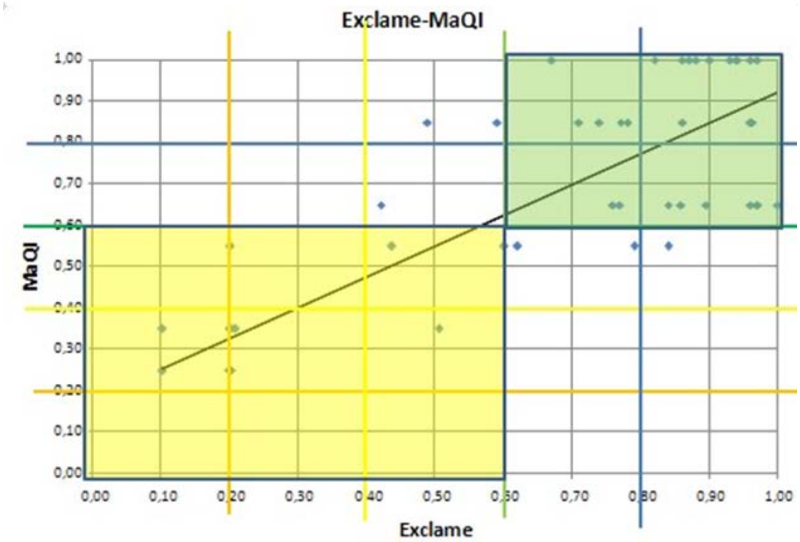
Average absolute class difference

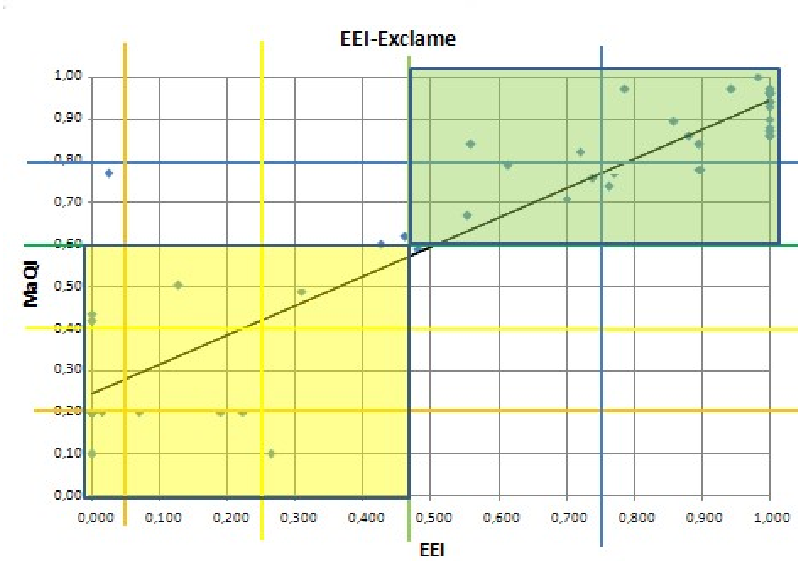
Also using an ordinal-scaled with 5 values (considering the differences between moderate-poor and poor-bad) the average absolute class differences are smaller than 1.

EEI-MaQI	EEI-Exclame	MaQI-Exclame
0.72	0.43	0.69

The following graphics show the agreement between indices in relation to the critical boundary (better or worse than good).


EEI-MaQI
MaQI
EEI


Exclame-MaQI
MaQI
Exclame


EEI-Exclame
MaQI
EEI

6. Final results to be included in the EC

6.1. Table with EQRs

Final results of the IC - EQRs methods are given in Table 6.1.

Table 6.1 Overview of the IC results for the national methods

Biological Quality Element			Macroalgae and seagrasses	
Results transitional waters: Ecological quality ratios of national classification systems				
Member State	Type	National classification systems intercalibrated	Ecological Quality Ratios	
			High-Good boundary	Good-Moderate boundary
France	IC type 1	Exclame	0.80	0.60
Greece	IC type 1	EEI-c - Ecological Evaluation Index	0.70	0.40
Italy	IC type 1	MaQI – Macrophyte Quality Index	0.80	0.60

6.2. Correspondence common types versus national types

The results are directly applicable to the national types that belong to the common type.

7. Ecological characteristics

7.1. Description of reference or alternative benchmark communities

For benchmark sites with total pressure equal or lower than 6 the simper analysis indicated that the benchmark sites selected are characterized by the dominance of three angiosperm species: *Cymodocea nodosa* (49.9%), *Ruppia cirrhosa* (35.67%) and *Zostera noltii* (10%).

7.2. Description of good status communities

Simper analysis indicated that the macrophyte communities representing the "borderline" conditions between good and moderate ecological status in the Mediterranean coastal lagoons are communities where the macroalgae-cyanobacteria coexist with the angiosperms (see Table 7.1).

Table 7.1 Simper analysis of Good-Moderate ESC of France, Greek and Italian coastal lagoons using non transformed coverage (%) data.

Groups Moderate & Good ESC					
Average dissimilarity = 89,53					
	Moderate ESC	**Good ESC**			
Species	**Av. Abundance**	**Av. Abundance**	**Av.Diss**	**Contribution (%)**	**Cumulative (%)**
Ruppia cirrhosa	15,1	18,48	14,17	15,83	15,83
Cyanobacteria	14,5	1,85	10,69	11,94	27,77
Gracilaria dura	14,25	0,5	8,81	9,85	37,62
Cymodocea nodosa	0	12,7	5,9	6,59	44,21
Zostera noltii	0	10	4,6	5,14	49,35
Polysiphonia elongata	9,52	1	4,56	5,1	54,45
Cystoseira barbata	15,87	0,04	4,51	5,04	59,49
Ulva sp.	15,75	0	4,46	4,98	64,47
Vaucheria submarina	0	9	4,38	4,9	69,37
Nanozostera noltii	0	6	4,11	4,59	73,95
Gracilaria bursa-pastoris	6,67	5,04	3,8	4,24	78,19
Alsidium corrallinum	0	3,94	2,42	2,7	80,89
Halopithys incurvus	0	5,72	2,38	2,66	83,55
Lophosiphonia obscura	4,27	0,19	1,29	1,44	84,99
Hypnea musciformis	4,27	0,12	1,25	1,4	86,39
Acanthophora najadiphormis	4,27	0	1,21	1,35	87,74
Cladophora liniformis	4,27	0	1,21	1,35	89,09
Gracilaria armata	4,27	0	1,21	1,35	90,44

8. References

Nilssen J. P., 1978. Eutrophication, minute algae and inefficient grazers. *Memorie Istituto Italiano di Idrobiologia,* 36: 121–138.

Schramm W., 1999. Factors influencing seaweed responses to eutrophication: some results from EU-project EUMAC. *Journal of Applied Phycology,* 11: 69–78.

Sfriso, A., Facca C., Ghetti, P. F., 2009. Validation of the Macrophyte Quality Index (MaQI) set up to assess the ecological status of Italian marine transitional environments. *Hydrobiologia*, 617, 117–141.

Orfanidis, S., Panayotidis, P., Stamatis, N., 2001. Ecological evaluation of transitional and coastal waters: a marine benthic macrophytes based model. *Mediterranean Marine Science*, 2 (2): 45-65.

Orfanidis, S., Panayotidis, P., Ugland K., 2011. Ecological Evaluation Index continuous formula (EEI-c) application: a step forward for functional groups, the formula and reference condition values. *Mediterranean Marine Science*, 12 (1): 199-231.

Viaroli P., Bartoli M., Giordani G., Naldi M., Orfanidis S., Zaldivar J. M., 2008. Community shifts, alternative stable states, biogeochemical controls and feedbacks and in eutrophic coastal lagoons: a brief review. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18: S105-S117.

Annex

A. Methods of calculating coverage

Two different methods are applied to calculate the coverage:

Method A: Cover (%): The area of ground (sediment) covered by vegetation of a particular plant species expressed as a percentage. <u>The maximum total cover (%) cannot exceed 100%.</u>

Method B: Coverage (%): That part of a sampled area covered by a particular plant species or individual plant canopy; typically expressed as a percentage. <u>The maximum total coverage can exceed 100%.</u>

A theoretical example of plant species abundance estimation can be seen in Figure A.1

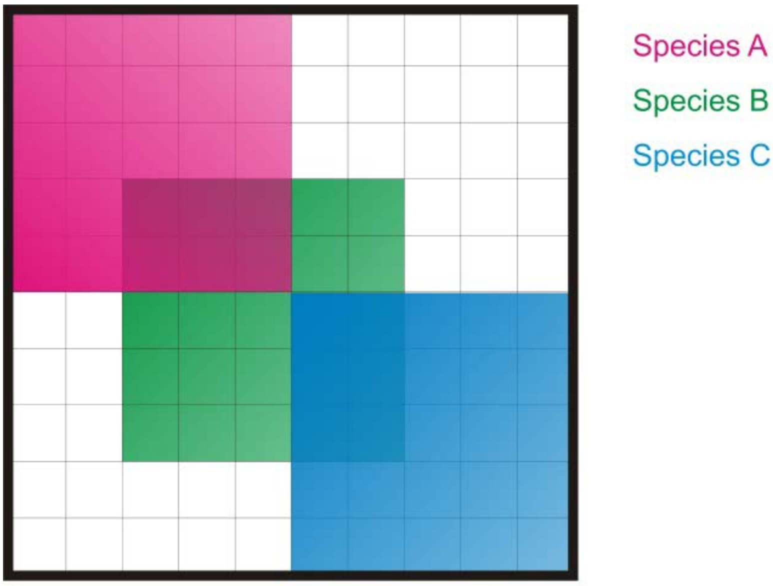


Figure A.1 Coverage (%) estimation of three different species by both abundance methods:

Method A: Species A=25%, Species B=13%, Species C=25%, Total coverage (%)=63%

Method B: Species A=25%, Species B=25%, Species C=25%, Total coverage (%)=75%

In the Common Metric dataset France has provided data using method A, Greece has provided data using method B, and Italy has provided data using both methods.

Although there is this discrepancy it has been decided to further proceed with IC option 3 between the three methods:

1. The coverage (%) estimation of benthic macrophytes has been provided at the site (an area of ca. 15 m x 15 m) scale (not at a sample scale). At this spatial scale the bias of coverage (%) estimation between the two methods used has a rather restricted impact on indices calculations.
2. The total coverage (%) of seagrass meadows can hardly exceed 100% with any method used. Yet, by using method A due to their canopy the seagrass abundance can

only be slightly underestimated in cases when a heavily load of epiphytes exist (Figure A.2).

3. The total coverage (%) of macroalgae in eutrophicated coastal lagoons can be underestimated by using method A but with low impact on indice calculation (see below, Figure A.3).
4. For MaQI application the total coverage boundary used is smaller/greater than 5% (all station in common dataset are characterized by total coverage greater than 5%). Moreover the relative abundance (Rhodophyta/Chlorophyta) assessment is not affected by differences in total coverage calculation. Therefore the different methods used to calculate the total coverage do not affect the correct application of MaQI in all stations of the IC common dataset (option 3 is applicable).
5. For EEI-c application values of Ecological Status Groups coverage higher than 60% have only a neglectful effect on index estimation.
6. For Exclame application higher than 100% total coverage values are adjusted to 100%.

Figure A.2 A moderately degraded meadow where the seagrass coexist with macroalgae

Figure A.3 A degraded lagoon site where the macroalgae dominate (bloom).