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Annex

A. Integration of data from Italy, Slovenia and Cyprus - Results of comparison analysis 46

Mediterranean Sea GIG: Coastal Waters - Macroalgae

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1. Introduction

- Seven Member States (France, Italy, Spain, Cyprus, Croatia, Slovenia and Greece) compared and harmonised their national assessment systems.
- Intercalibration "Option 2" was used - indirect comparison of assessment methods using a common metric.
- The comparability analysis show that national methods from all MS give a closely similar assessment (in agreement to comparability criteria defined in the IC Guidance), so no boundary adjustment was needed.
- The final results include EQRs of France, Italy, Spain, Cyprus, Croatia, Slovenia and Greece, assessment systems.

2. Description of national assessment methods

Table 2.1 Overview of the national assessment methods

Member State	Method	Included in this IC exercise
Cyprus	EEI-c = Ecological Evaluation Index (continuous)	Yes
Croatia	CARLIT = Cartography of Littoral and upper-sublittoral rocky-shore communities	Yes
France	CARLIT = Cartography of Littoral and upper-sublittoral rocky-shore communities	Yes
Greece	EEI-c = Ecological Evaluation Index (continuous)	Yes
Italy	CARLIT = Cartography of Littoral and upper-sublittoral rocky-shore communities	Yes
Slovenia	EEI-c = Ecological Evaluation Index (continuous)	Yes
Spain	CARLIT = Cartography of Littoral and upper-sublittoral rocky-shore communities	Yes

Malta	No method	No

2.1. Methods and required BQE parameters

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

Member State	Full BQE method	Abundance	Disturbance sensitive taxa	(Diversity)*	Combination rule of metrics
Croatia, France, Italy, Spain (CARLIT)	Yes	Cover (length of coast in meters) of 9 pre-classified sensitivity classes	Communities sorted into 9 sensitivity classes	No	See below the description of the method
Cyprus, Greece, Slovenia (EEI-c)	Yes	Percentage coverage (destructively collected sample - 400 cm^2) of 5 pre-classified sensitivity classes	Species sorted into 5 sensitivity classes	No	See below the description of the method
Malta	-	-	-	-	-

*The optional non-obligatory parameter diversity is put between brackets.

CARLIT method

EQR is calculated according to the formula:

$$EQR = \frac{\sum \frac{EQ_{ssi} * l_i}{EQ_{rsi}}}{\sum l_i}$$

where

i = situation (geomorphologically relevant situation)

EQ_{ssi} = EQ in the study site for situation i

EQ_{rsi} = EQ in the reference sites for the situation i

l_i = coastal length in the study coast for the situation i

EEI-c method

EQR is calculated according to the 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

$a, \ldots, f$ are the coefficients of the hyperbola:

$a = 0.4680 \quad b = 1.2088 \quad c = -0.3583$

$d = -1.1289 \quad e = 0.5129 \quad f = -0.1869$

2.2. Sampling and data processing

Table 2.3 Overview of the sampling and data processing of the national assessment methods included in the IC exercise.

Information provided in the online WISER project assessment method questionnaires	
Sampling/survey device	CARLIT: Visual sampling EEI: Greece & Cyprus: Metallic frame for coastal waters; Slovenia: scrape
How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area?	CARLIT: Once a year EEI: Two or three sampling occasions
Sampling/survey months	Sampling time for CARLIT is scheduled from April (Italy) or May (Spain) to June and for EEI-c all year during different periods of the year, preferably from April to November (Greece & Cyprus). Slovenia: May - June and August - September
Which method is used to select the sampling /survey site or area?	For CARLIT Spain all the length of rocky coast is surveyed. Alternatively you can make a stratified sampling. Italy: Only the water bodies which have the 80% of coast line as rocky shore EEI: Expert knowledge
How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area?	For CARLIT none if all the rocky coast is surveyed. Expert knowledge advice otherwise. EEI: 3-5 replicates per sampling occasion per site for two or three sampling occasions
Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of survey site or area is based	CARLIT: Around 400 km on study coast and 250 km on Reference Zone coasts EEI: Greece & Cyprus: Sum of at least 9-12 spatial replicates: ca. 0.289 m^2 for coastal lagoons, 0.625 m^2 for coastal waters. Slovenia: Sum of 3 spatial replicates= 3 x 400 cm^2 per station per season

Information provided in the online WISER project assessment method questionnaires

Short description of field sampling/survey procedure and processing (sub-sampling):

Both methods are developed on littoral rocky coasts and at a fixed depth (0-3 m). Visual mapping for CARLIT and destructive sampling with a metallic frame for EEI-c. The samples are sorted into 9 communities of different sensitivity for CARLIT, whereas into 5 functional groups of different sensitivity for EEI-c.

CARLIT:

A sampling unit is a sector of coast, at least 50 meters, with an homogeneous community category (corresponding to a single community or combination of communities). The sectors are translated on a graphical display. Rocky-shore coasts are sampled by visual census of the dominant macroalgal community carried out by small boats so as to sail along the coast as close as possible (3-4 m) to the rock face. The observed linear development of each macroalgal community is noted down on a cartographic support (aerial photography at 1:5000 scale) with the geomorphological features of the studied coast line; the minimum length of the sampling unit is 50m. For some species (*Cystoseira* spp.) the cover of macro-algal belt are recorded and some macroalgae talli samples are collected for the taxonomic identification.

EEI:

Greece & Cyprus: Sampling follows a nonaligned block design, in which a sample is located randomly within a representative permanent cell of dimensions 10 x 10 m. The sampling is destructive, using for coastal lagoons a metal hand-held box corer (17 x 17 x 15 cm; L x W x H), which is vertically pushed through the benthic vegetation and sediment. From each sample the existing vegetation (seaweeds, seagrasses leaves and roots, Cyanobacteria colonies) was carefully removed and placed individually in airtight plastic bags, where it was fixed in 4,5% formalin in sea water for a few seconds. The excess formalin solution was later removed from the plastic bag, which was then sealed, labelled, and stored in a plastic box. A similar procedure is followed in coastal waters using a metallic frame of 25 x 25cm.

Slovenia: At each sampling site, in a depth range of 2 to 4 m, three samples are randomly scraped from the bottom (20 x 20 cm). All samples are collected between 8 and 12 a.m.

2.3. National reference conditions

Table 2.4 Overview of the methodologies used to derive the reference conditions for the national assessment methods included in the IC exercise

Member State	Type and period of reference conditions	Number of reference sites	Location of reference sites	Reference criteria used for selection of reference sites
Spain	Expert knowledge, historical data in the Catalan coast before 1980's and in the adjacent Albères coast, least disturbed conditions from spring 2002	250 km of coast*	Only 3 reference zones in natural parks from Corsica and Balearic Islands considered representative for the entire Mediterranean Sea: Facade maritime du Parc Naturel Regional de Corse (France), Parc Natural de Ses Salines (Balearic Islands, Spain) and Reserva Marina del Nord de Menorca (Balearic Islands, Spain).	Undisturbed (or with only very minor disturbances) sites that cover a wide range of coastal geomorphologies, from different geological origins (volcanic, granite, calcareous, metamorphic) to different wave exposures and coastal morphologies. The references zones present no or very low pressures as appear in the benchmark definition.
France	Expert knowledge, historical data before 1980's	250 km of coast	Only 3 reference zones in natural parks from Corsica and Balearic Islands considered representative for the entire Mediterranean Sea: Facade maritime du Parc Naturel Regional de Corse (France), Parc Natural de Ses Salines (Balearic Islands, Spain) and Reserva Marina del Nord de Menorca (Balearic Islands, Spain).	Undisturbed (or with only very minor disturbances) sites that cover a wide range of coastal geomorphologies, from different geological origins (volcanic, granite, calcareous, metamorphic) to different wave exposures and coastal morphologies.
Italy	Existing near-natural reference sites (same as Spain)	250 km of coast	Same reference sites and values as Spain	Undisturbed (or with only very minor disturbances) sites that cover a wide range of coastal geomorphologies, from different geological origins (volcanic, granite, calcareous, metamorphic) to different wave exposures and coastal morphologies.

Member State	Type and period of reference conditions	Number of reference sites	Location of reference sites	Reference criteria used for selection of reference sites
Greece	Existing near-natural reference sites in Aegean Sea sampled once during the period 1999-2000	62 samples from 26 putatively pristine Aegean sites dominated by *Cystoseira* cf. *crinita* community as part of the Hellenic "NATURA 2000" data-base*	Natural parks/Natura 2000 sites in Aegean Sea, Greece.	Reference sites have been identified according to the low pressures and impacts they receive in accordance with Annex V of WFD. Criteria used: • population density: no settlement with more than 1000 in/km^2 in the next 15 km and/or more than 100 habitats/km^2 no in the next 3 km within that area (winter population); • no more than 10% of artificial coastline; • no harbour (more than 100 boats) in 3 km; • no beach regeneration within 1 km; • no industries within the 3 km; • no fish farms within the 1 km; • no desalination plants within 1 km; • no evidence of *Cystoseira* forest regression due to other unconsidered impacts; if there is evidence of *Cystoseira* regression (for example due to overgrazing), the quality element macroalgae index may not be applied, depending on the method used).
Cyprus	Existing near-natural reference sites	Cyprus sites/samples (Akamas and Cape Pyla	Natural parks/ proposed Natura 2000 site (Akamas) and Cape Pyla	Reference sites have been identified according to the low pressures and impacts they receive in accordance with Annex V of WFD. Selection criteria: (1) the presence of high representatively and conservation status of *Posidonia* meadows (priority habitat), and (2) the low human pressures (low inhabitant density, no industrial settlement in the water basin, marine protected area).
Slovenia	Existing near-natural reference sites spring-	One reference zone (MPA) is	MPA Strunjan Nature Reserve (Slovenia)	High ecological status of macroalgae; low pressures and impacts - natural coastal environment well preserved; no

Member State	Type and period of reference conditions	Number of reference sites	Location of reference sites	Reference criteria used for selection of reference sites
	summer 2006. One site in the MPA (RR1) is included in the monitoring program, sampled almost every year.	considered representative for Slovenian coastal waters		sources of anthropogenic pollution; no non-indigenous species that can affect autochthonous species and habitats.
Croatia	Existing near-natural reference sites in Croatian Adriatic Sea sampled in spring 2010	100 km of coast	Sites include remote islands, islands with very low population density and MPAs	Undisturbed (or with only very minor disturbances) sites that cover a wide range of coastal geomorphologies, from different geological origins to different wave exposures and coastal morphologies.
Malta	-	-	-	-

All the Member States agreed on the identification of reference sites according to the low pressures and impacts they receive in accordance with Annex V of WFD.

Criteria used:

- population density: no settlement with more than 1000 in/km^2 in the next 15 km and/or more than 100 habitats/km^2 in the next 3 km within that area (winter population)
- no more than 10% of artificial coastline
- no harbour (more than 100 boats) within 3 km
- no beach regeneration within 1 km
- no industries within 3 km
- no fish farms within 1 km
- no desalination plants within 1 km
- no evidence of *Cystoseira* forest regression due to other unconsidered impacts; if there is evidence of *Cystoseira* regression (for example due to overgrazing), the quality element macroalgae may not be applied, depending on the method used).

*Spain CARLIT: in 2001, with the first application of CARLIT, several water masses in NW Mediterranean Sea were selected (MPA of Menorca, Formentera, Corsica). More than 25 km of coastline were investigated following the cartographic approach of the method. The selected sites presented no or very few human pressures (low inhabitant density, no industrial settlement in the water basin, marine protected areas…).

*Greece EEI: from 2000 to 2002 a large scale ecological cartography has been carried out in the Greek Seas, for the implementation of the “habitat” Directive 92/43/EEC and the delimitation of the areas (sites) which will contribute to the European Natura 2000 network. 45 pristine sites of the Aegean Sea have been studied using as criteria (1) the presence of high representatively and conservation status of Posidonia meadows (priority habitat), and (2) the low human pressures (low inhabitant density, no industrial settlement in the water basin, marine protected area). From those sites having high representatively and ecological status of hard substrate (26 in total) 65 samples of benthic macrophytes were sampled and analysed.

The criteria for the reference site chosen in Slovenia are the following: low pressures and impacts (natural coastal environment well preserved; no sources of anthropogenic pollution; no non-indigenous species that can affect autochthonous species and habitats); high ecological status of macroalgae.

At present, Spain, France and Italy are using the reference values proposed by Spain (Ballesteros *et al.*, 2007). This situation may be ameliorated by adding new sites along the French and the Italian coasts, but an ad hoc study would be necessary for that.

2.4. National boundary setting

Table 2.5 Explanations for national boundary setting of the national methods included in the IC exercise

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	BSP: method tested against pressure
Spain	Boundaries are set according to biotic index and/or combined with the results of multivariate analysis. No statistical analysis exclusively to set boundaries. Calibrated against pre-classified sampling sites. Mixed. Continuum of possibilities with gradual disappearance/ appearance of different indicator species. Good-Moderate boundary indicated by the absence of Cystoseira	Equidistant division: expert judgment of 25% deviation from the reference = EQR 0.75	Equidistant division and modification: expert judgment of 40% deviation from the reference = EQR 0.6	Yes, quantitative
France	Not provided, BSP copied from Spain	BSP taken over from Spain	BSP taken over from Spain	Yes, quantitative
Italy	BSP taken over from Spain	BSP taken over from Spain	BSP taken over from Spain	Yes, quantitative
Greece	Boundaries are set according to biotic index (EEI-c) and to community structure (SIMPER analysis). The dominance of the late-successional species of the genera *Cystoseira* form communities indicative of pristine state, which is characterized by low nutrient and clear water conditions, whilst the dominance of opportunistic seaweeds as *Ulva* and *Gracilaria* and Cyanobacteria form communities indicative of degraded state, which is characterized by high nutrients, heavy metals and turbid conditions. The coexistence of the late-successional like *Cystoseira, Sargassum, Corallina* species with opportunistic like *Ulva, Cladophora, Gracilaria,* Cyanobacteria species form	Modelling and expert judgment on biological criteria (EQR=0.76±0.09SD): Equidistant division at 25%	Modelling and expert judgment on biological criteria (EQR=0.48±0.09SD): Equidistant division at 50%	Yes, quantitative

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	BSP: method tested against pressure
	communities that are indicative intermediate (moderate) conditions. Continuum of possibilities with gradual disappearance/ appearance of different indicator species. Equidistant division of the EQR gradient.			
Cyprus	BSP copied from Greece Equidistant division of the EQR gradient	BSP taken over from Greece	BSP taken over from Greece	Yes, quantitative
Slovenia	Boundaries taken over from the intercalibration exercise. Boundaries are set according to biotic index (EEI-c). No statistical analysis exclusively to set boundaries. No discontinuities. Continuum of possibilities with gradual disappearance/appearance of different indicator species.	BSP taken over from Greece	BSP taken over from Greece	Yes, quantitative
Croatia	Expert judgement (BSP taken over from Spain)	Equidistant division: expert judgment of 25% deviation from the reference = EQR 0.75	Equidistant division and modification: expert judgment of 40% deviation from the reference = EQR 0.6	Yes, quantitative
Malta	-	-	-	-

The boundaries were set up according to an equidistant division approach of a continuum as no evident discontinuities were detected.

2.5. Results of WFD compliance checking

Table 2.6 List of the WFD compliance criteria and the WFD compliance checking process and results of the national methods included in the IC exercise

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	EEI: yes CARLIT: yes
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	EEI: yes CARLIT: yes
• Scope of detected pressures	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	See section on national boundary 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.	EEI: yes CARLIT: yes
• Complete list of biological metric(s) used in assessment	See section on Required BQE parameters
• Data basis for metric calculation	
• Combination rule for multimetrics	See section on Required BQE parameters

Compliance criteria	Compliance checking conclusions
4. Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	EEI: yes CARLIT: yes
• 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?	No
5. The water body is assessed against **type-specific near-natural reference conditions**	EEI: yes CARLIT: yes
• Scope of reference conditions	CARLIT: Habitat-specific EEI: Habitat-specific
• Key source(s) to derive reference conditions	CARLIT: Expert knowledge, Historical data, Least Disturbed Conditions; EEI: Existing near-natural reference sites
• Number of sites, location and geographical coverage of sites used to derive reference conditions	CARLIT: 250 km of coast, only reference zones in natural parks from Corsica and Balearic Islands considered representative for the entire Mediterranean Sea (France & Spain) + Ligurian region (Italy) EEI: Greece & Cyprus: 62 Aegean sites in the Mediterranean Sea, Greek Aegean Islands; Slovenia: Strunjan Nature Reserve
• Time period (months+years) of data of sites used to derive reference conditions	CARLIT: Springtime 2002 (Spain), spring/summer 2000 (Italy) EEI: One year
• Reference site characterisation: criteria to select them	See section on National reference conditions
6. Is a true reference used for the definition of High status or an alternative benchmark estimation?	See section on National reference conditions
7. Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	EEI: yes CARLIT: yes
• Has the uncertainty of the method been quantified and is it regarded in the assessment ?	Yes, within the works carried out in the European WISER project
• Specify how the uncertainty has been quantified and regarded	To be done
8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	EEI: yes CARLIT: yes

Compliance criteria	Compliance checking conclusions
9. Selected taxonomic level achieves adequate **confidence and precision** in classification	EEI: yes CARLIT: yes
• Minimum size of organisms sampled and processed	CARLIT: Macroalgae (>1cm) EEI: Greece & Cyprus: All down to light microscope scale, Slovenia: 2 mm
• Record of biological data: level of taxonomical identification – what groups to which level: CARLIT: Genus, Species/species groups. Algal communities (or combination of communities), the main algal species and the mussel *Mytilus galloprovincialis* or other dominant macroinvertebrates. For Italy: The method is based on a simplification of more widespread macroalgal communities study in the upper infra-littoral fringe in the Mediterranean Sea. On the base of existing literature and expert judgement, 19 categories have been created (table updated in 2008 for the Italian application) with associated values ranging from 1 to 20. Such categories follow mostly a taxonomic scheme, grouping species, genus and orders. EEI: Greece & Cyprus: All plants are classified at a species and a functional group (sensu Orfanidis *et al.* 2001) level. Slovenia: Only species covering at least 1% of the sampling area are assessed.	

Conclusions of the compliance checking: Compliance criteria are met. CARLIT and EEI-c methods meet the requirements stated in the WFD IC Guidance (2.1. WFD compliance criteria).

Good ecological status boundaries of CARLIT and EEI-c methods comply with the WFD normative definitions.

3. Results IC feasibility checking

3.1. Typology

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

Method	Appropriate for IC types/subtypes
EEI-c	Rocky shore
CARLIT	Rocky shore

The Intercalibration is feasible in terms of typology. In fact, Typology is not relevant for BQE macroalgae in Mediterranean coastal waters. Common IC type: only one type: Entire Mediterranean Sea, no subdivision, rocky shore of all Member States

During the early stages of the CIS the Mediterranean working group agreed in using only 2 parameters to distinguish water types, namely substrate composition and depth. Most of other geomorphological parameters, described in Directive Annex II (1.2.4), were not relevant (i.e. tidal regime) to distinguish different water types in relation to their ecological "significance" for the Mediterranean Sea. Four main Types were then defined (Table 2.1.1). However, throughout the CIS, following data analyses for the different BQEs, these types did not actually proved to be relevant for the IC exercise, for some BQEs, as Mediterranean ecosystem is quite homogeneous in comparison to Northern Seas (some ecological differences do exist but within the Mediterranean scale).

For Macroalgae Intercalibration the methods used are applied to macroalgal communities (species composition and abundance) of the upper infralittoral zone (3.5 to 0.2 m depth) in rocky coasts, with no types distinction.

3.2. Pressures addressed

The relationship between pressures and EQR values has been calculated using the new MA-LUSI index. It has been developed as an improvement of LUSI index (Flo *et al*., 2011) specifically for shallow water macroalgae communities. The new MA-LUSI index includes not only the pressures from the original LUSI Index but some other pressures that affect macroalgae communities: Mariculture, Sewage outfall, Harbours or Irregular fresh water inputs. In some countries Background trophic status has been also included as a local pressure. MA-LUSI values were calculated using a 3 km buffer zone around the sampling site on a Corine land cover map, except in Croatia where a 1 km buffer zone was used, because of specific coastal morphology (presence of mountains close to the coast). For CARLIT method the site has been defined as 1 km of coastline.

Table 3.2 Pressures addressed by the national methods and overview of the relationship between national methods and the pressures. (Pressures: EU = eutrophication, GD = general degradation

Member State	Method/ Metrics tested	Pressure	Pressure indicators	Amount of data	Strength of relationship
Spain	CARLIT	EU, GD	MA-LUSI index Following pressures are included: a) Indirect pressures from Corine database (Urban, Commercial & Industrial, Agriculture) b) Direct pressures (Sewage outfall, Mariculture, Sediment nutrient release, Irregular freshwater inputs, Harbour)	40 sites	Linear regression ($p<0.05$)

Member State	Method/ Metrics tested	Pressure	Pressure indicators	Amount of data	Strength of relationship
			c) Confinement		
France	CARLIT	EU, GD	MA-LUSI index Following pressures are included: a) Indirect pressures from Corine database (Urban, Commercial & Industrial, Agriculture) b) Direct pressures (Sewage outfall, Mariculture, Sediment nutrient release, Irregular freshwater inputs, Harbour) c) Confinement	24 sites	Linear regression (p(<0,05)
Italy	CARLIT	EU, GD	MA-LUSI index Following pressures are included: a) Indirect pressures from Corine database (Urban, Commercial & Industrial, Agriculture) b) Direct pressures (Sewage outfall, Mariculture, Sediment nutrient release, Irregular freshwater inputs, Harbour) c) Confinement d) Background trophic status	75 sites	Linear regression ($p<0.05$)
Greece	EEI-c	EU, GD	MA-LUSI index Following pressures are included: a) Indirect pressures from Corine database (Urban, Commercial & Industrial, Agriculture) b) Direct pressures (Sewage outfall, Mariculture, Sediment nutrient release, Irregular freshwater inputs, Harbour) c) Confinement d) Background trophic status	12 sites	Significant linear correlations ($p<0.01$)
Cyprus	EEI-c	EU, GD	MA-LUSI index Following pressures are included:	3 sites	Significant linear correlations ($p<0.01$)

Member State	Method/ Metrics tested	Pressure	Pressure indicators	Amount of data	Strength of relationship
			a) Indirect pressures from Corine database (Urban, Commercial & Industrial, Agriculture) b) Direct pressures (Sewage outfall, Mariculture, Sediment nutrient release, Iregular freshwater inputs, Harbor) c) Confinement d) Background trophic status		
Slovenia	EEI-c	EU, GD	MA-LUSI index Following pressures are included: a) Indirect pressures from Corine database (Urban, Commercial & Industrial, Agriculture) b) Direct pressures (Sewage outfall, Mariculture, Sediment nutrient release, Iregular freshwater inputs, Harbor) c) Confinement d) Background trophic status	6 sites	significant linear correlation ($p<0.01$)
Croatia	CARLIT	EU, GD	MA-LUSI index Following pressures are included: a) Indirect pressures from Corine database (Urban, Commercial & Industrial, Agriculture) b) Direct pressures (Sewage outfall, Mariculture, Sediment nutrient release, Iregular freshwater inputs, Harbor) c) Confinement	27 sites	significant linear correlation ($p<0.01$)

The regression of each method in relation to pressures is presented below (Figure 3.1 to Figure 3.8):

France-CARLIT

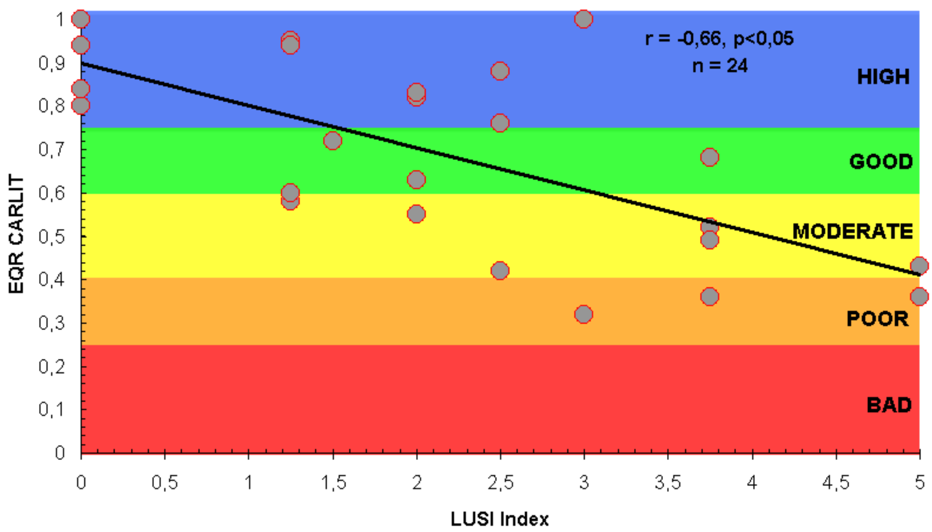

Figure 3.1 Relation between CARLIT and pressure data (expressed as MA-LUSI index) in French coastal waters

Italy-CARLIT

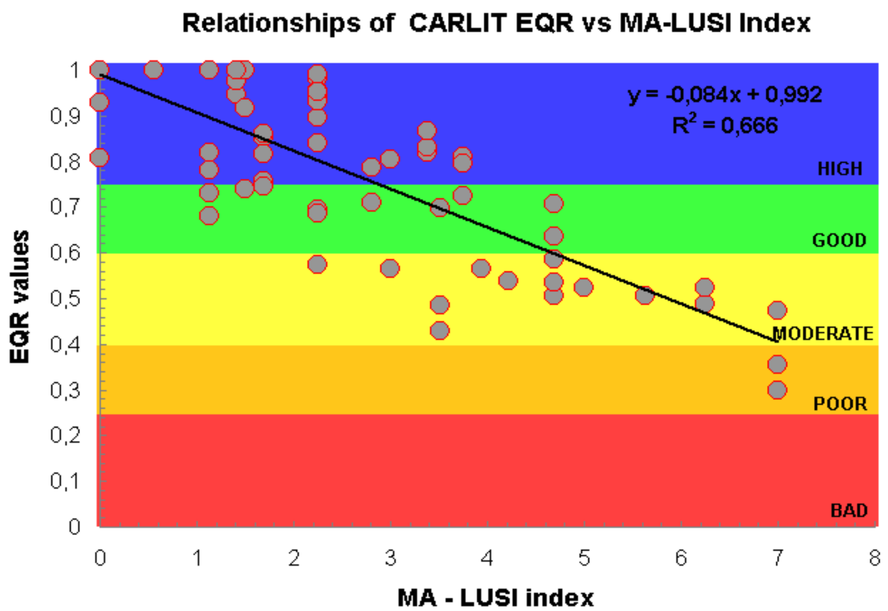

Figure 3.2 Relation between CARLIT and MA-LUSI in Italian coastal waters

Spain-CARLIT

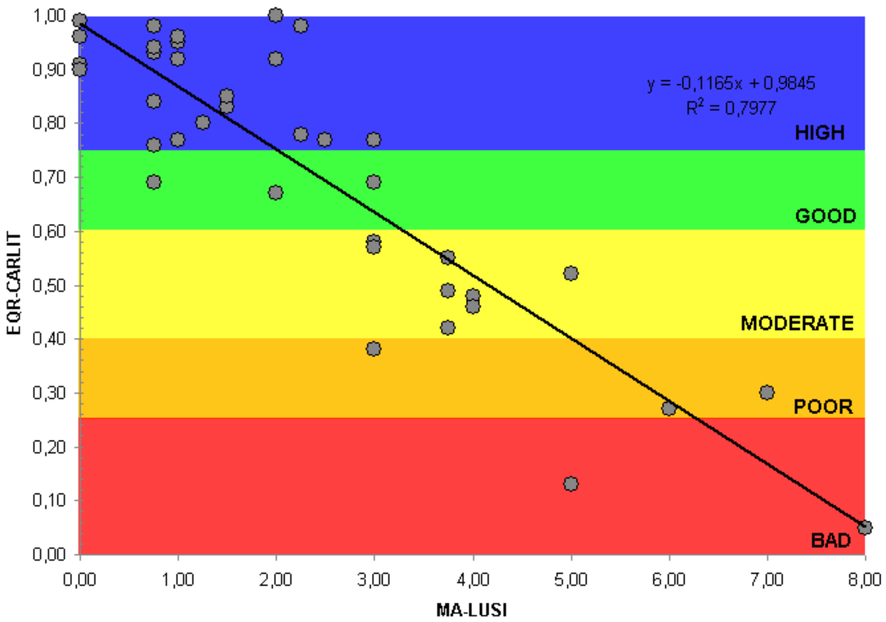

Figure 3.3 Relation between CARLIT and MA-LUSI in Spain coastal waters

Greece-EEI-C

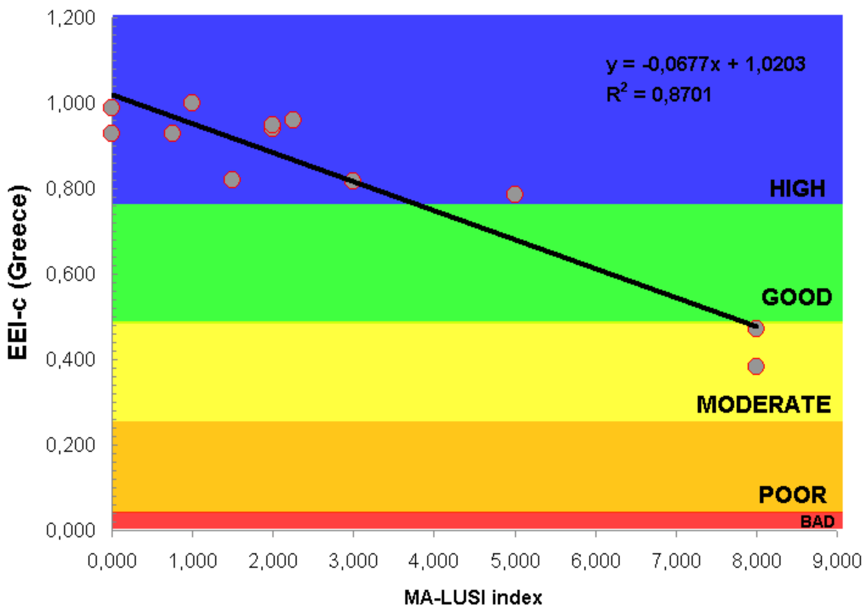

Figure 3.4 Relation between EEI-c and pressure data (expressed as MA-LUSI index) in Greek coastal waters

Cyprus-EEI-c

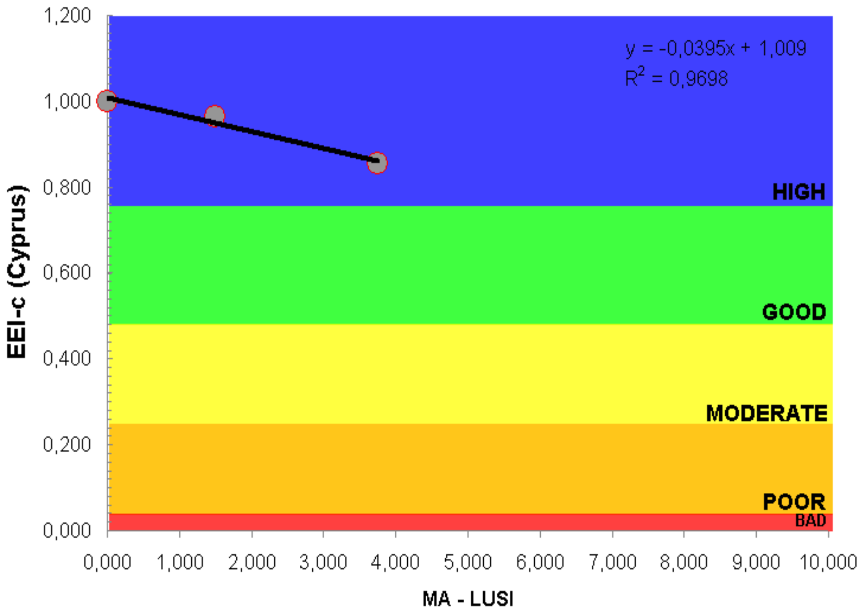

Figure 3.5 Relation between EEI-c and pressure data (expressed as MA-LUSI index) in Cyprus coastal waters

The following explanation for Cyprus is provided in addition:

The 3 points on the above graph (Figure 3.5) correspond to 3 sampling sites. However, they are not single-time values, but they are average values of 3 years of seasonal samplings, that is, 3 stations, 3 years, 3-4 samplings/year, 3-15 samples/sampling. That is, the above graph derived from a total of more than 60 samples.

More specifically, for the first site (Code=CY_5-C1-S1, Name=Akamas) the EEI-c value on the graph (1,00) is the average of 3 years (2007, 2008, 2009) of seasonal samplings, that is of a total of 12 samples. For the other 2 sites (Code=CY_22-C3-S1 and CY_23-C3-S1, Names=CP3 and CP4) the EEI-c values on the graph are the averages of 3 years (2008, 2009, 2010) of seasonal samplings, that is of a total of 30 samples for CP3 and 24 samples for CP4. That is, 66 samples in total. The standard deviations are in the same order: EEI-c Average= 1,00 (STD= 0), EEI Average= 0,95 (STD= 0,026) and EEI-c Average= 0,88 (STD= 0,04)

The reason why these average values were used:

As it is clear from the STDs, there was not any considerable between-years variance of the EEI-c values, so, since plotting each year's values against MA-LUSI had no added value compared to the averages, we chose to plot the averages instead. To make this more clear, below find: (i) Data table showing the number of samples, per site, per year of sampling, with the corresponding MA-LUSI values, the EEI-c values and the EEI-c EQRs, and (ii) The resulting graph (Figure 3.6), which represents the above-mentioned. (N= 9, where the 9 data points derive from the seasonal sample-averages per site, per year of sampling) and is identical with the initial graph with the averages.

No samples	Site	Year	MA-LUSI	EEI-c	EEI-c EQR
6	Akamas	A_07	0	10	1
3	Akamas	A_08	0	10	1
3	Akamas	A_09	0	10	1
15	CP3	CP3_08	1,5	9,73	0,97
9	CP3	CP3_09	1,5	9,33	0,92
6	CP3	CP3_10	1,5	9,7	0,96
9	CP4	CP4_08	3,75	8,98	0,87
9	CP4	CP4_09	3,75	9,39	0,92
6	CP4	CP4_10	3,75	8,81	0,85

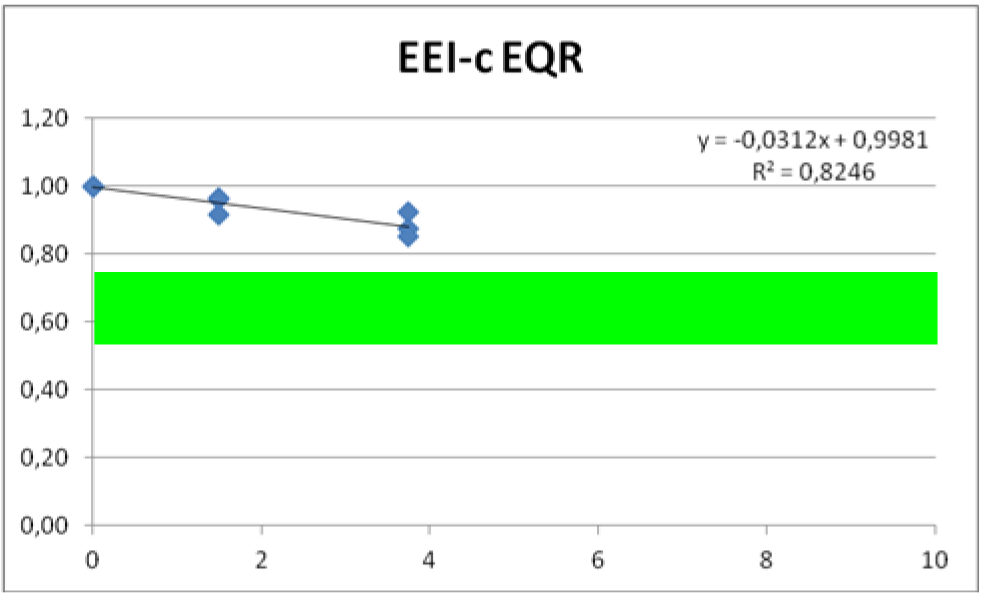

Figure 3.6

In basis on the explanation given above, it is clarified what the 3 points on the graph represent and thus the trend represented on the graph can be considered well-enough established and consistent through the 3-year sampling period.

Despite the sufficient (as clarified above) amount of data, the commented situation regarding the H/G boundary and the corresponding MA-LUSI does not change: it is a fact that Macroalgae are still in a High status at a relative high pressure level. Even so, the trend is overall as expected and similar to other MSs, that is negative correlation between pressures and impacts.

MA-LUSI= 4 (mainly due to mariculture: cages and hatchery and to irrigated agriculture in the specific site CP4), is indeed a relatively high LUSI, however one should interpret this taking into account many interacting parameters:

1. It is a well-established scientific finding that in the Eastern Mediterranean and specifically in the Levantine nutrient input from land-based and other sources does not have the expected impact on chlorophyll nor does it necessarily result in eutrophication phenomena. The ultra-oligotrophic character of the marine waters around Cyprus is a well-known fact in the scientific literature (e.g. Por, 1978; Krom *et al*., 1991; Krom *et al.,* 1992; Tselepides *et al.,* 2000; Karydis & Kitsiou, 2011). The scientific discussions about the causes of this 'paradox' are still ongoing and many interacting factors have been proposed as causative like narrow continental shelf, limited availability of nutrients in the marine waters, high salinities and temperatures which prohibit primary production, etc (Lakkis *et al*., 2000). In addition, the absence of big rivers of permanent flow in the area, in combination with the construction of the Aswan Dum contribute in this extreme oligotrophism of the Levant.
2. In Cyprus all coastal cities have sewage systems and sewage water is treated and reused (UNEP/MAP/WHO, 2004).
3. Absence of closed bays – mainly open sea with high-energy waters and hydrodynamic conditions which disperse rapidly any inputs.
4. Aquaculture cages sited in a minimum distance of 1km from shore and at depths of more than 30 m, interacting with point 3).
5. Many pressures are recently developed in Cyprus (e.g. mariculture activities), thus acting on the coastal waters only for a few years. We agree with Dr Orfanidis expressed view that the factor of duration of the pressures is important and should be taken into account.

Based on the above, we believe that:

1. The specific prevailing hydromorhological and physico-chemical conditions of CY Coastal waters eliminate a significant part of the effect of the pressures, resulting in a lower-than expected impact.
2. Many pressures are relatively recent and have been acting on CY coastal waters for a short time-period.
3. Facts i & ii are mainly responsible for the better-than expected ecological status of the macroalgae.

Despite this specific situation, the established methods for Macroalgae are sensitive enough to be considered useful in reflecting the pressures (although not in the extend which is expected like in other MSs), since:

1. They give an overall correct trend (negative correlation between pressures-impacts).

2. Although, all sites are at a High status, the 2 reference sites have given systematically higher EQRs than the other station with the higher MA-LUSI. Even a lower, but still High EQR value could be a useful sign of possible environment degradation.

Method	Pressure
CARLIT, EEI-c	Eutrophication, aquatic habitat destruction, general degradation, pollution by organic matter
Conclusion According to the available data and the results shown, the IC is feasible in terms of pressures. All the MS present a good correlation between MA-LUSI and EQR values.	

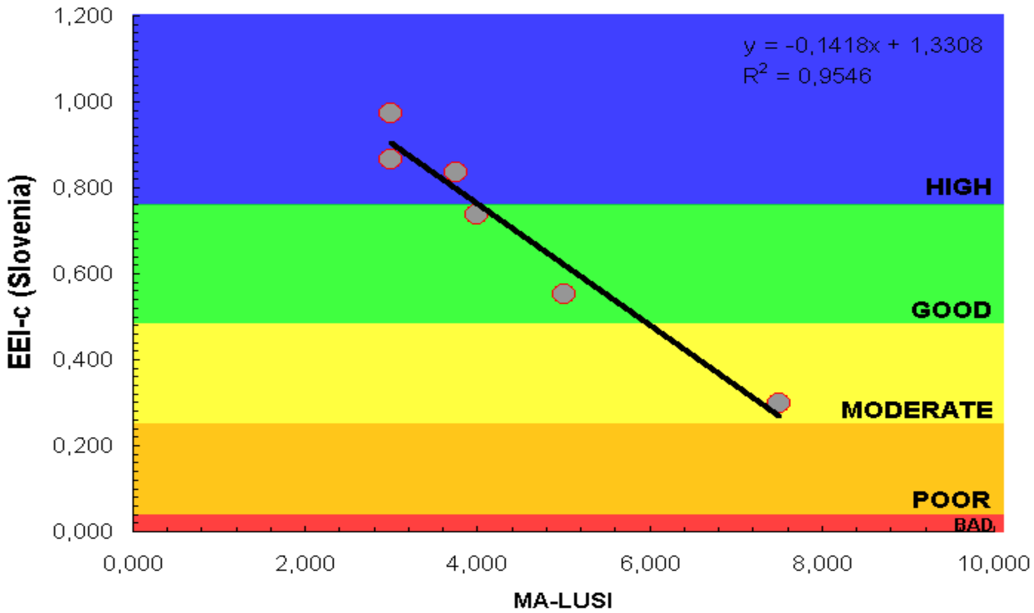

Figure 3.7 Relation between EEI-c and pressure data (expressed as MA-LUSI index) in Slovenian coastal waters

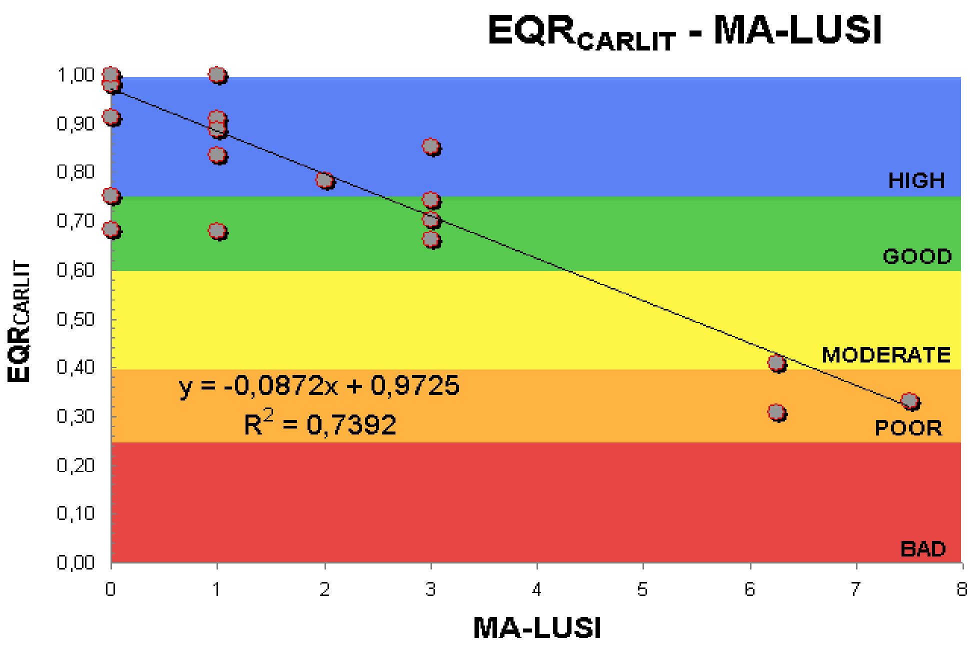

Figure 3.8 Relation between CARLIT and pressure data (expressed as MA-LUSI index) in Croatian coastal waters

3.3. Assessment concept

Method	Assessment concept	Remarks
CARLIT	Proportion of sensitive communities	The macroalgae assessment with both methods is restricted to hard substrate of 0 m to 3 m depth.
EEI-c	Late-successional vs. opportunistic species proportion	

The intercalibration is feasible in terms of assessment concept as both methods measure coverage of macroalgae and reveal the response of benthic macrophytes to anthropogenic stress. Both methods focus on the same zone (littoral and upper infralittoral of the rocky shores) and on the same life forms. EEI-c quantifies functional-morphological groups on a micro scale (coverage by vertical projection from destructively sampled quadrate of 400 cm^2) and CARLIT quantifies sensitive communities on a macroscale (cover by visual estimation on at least 50 m linear coastline).

4. Collection of IC dataset and benchmarking

4.1. Dataset description

Table 4.1 Description of data collection within the GIG

Size of common dataset: total number of sites	62 sites, 40 sites for Spain, 22 for Greece
Number of Member States	2
Repackage/disaggregation of samples/ WB results?	No
Gradient of ecological quality	Fully covered (only for Spain 5 classes)
Coverage per ecological quality class	For Spain the 5 ecological quality classes are covered, for Italy, France and Croatia 4 classes (no bad status), for Greece and Slovenia 3 classes (high, good, moderate) and for Cyprus only high status.

Table 4.2 Overview of the number of sites/samples/data values

Member State	Number of sites or samples or data values		
	Biological data	Physico- chemical data	Pressure data
Cyprus	EEI (3 sites, 71 sample)		X (according to MA-LUSI)
Greece	EEI (16 sites, 168 samples)	Yes, from the monitoring sites	X (according to MA-LUSI)
Italy	CARLIT (75 sites*)	Yes, from the monitoring sites	X (according to MA-LUSI)
Slovenia	EEI (51 sites for the first assessment; 7 monitoring sites twice per year)	Yes, from the same WBs, but not collected in the monitoring sites	X (according to MA-LUSI)
Spain	CARLIT: Cartography of Catalonia, Balearic Island and Valencia coast. More than 100 sample sites* in the entire Spanish Mediterranean coast	In Catalonia yes, from the same WBs, but not collected in the monitoring sites	X(according to MA-LUSI)
Croatia	CARLIT (250 km of coastline, more than 100 sites*)	Yes, from the same WBs, but not collected exactly in the monitoring sites	X (according to MA-LUSI)
France	CARLIT (> 4500 km of coastline, more than 100 sites*)	Yes, from the same WBs, but not collected in the monitoring sites	X (according to MA-LUSI)
Malta	-	-	-

* for CARLIT method a site is defined as 1km of coastline.

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 (obligatory and optional)	Both methods are developed on littoral rocky coasts and at a fixed depth (0-3 m). Sampling time for CARLIT is scheduled from May to June and for EEI-c during different periods of the year.
The sampling and analytical methodology	Visual mapping for CARLIT and destructive sampling with a metallic frame for EEI-c.
Level of taxonomic precision required and taxalists with codes	The samples are sorted into 9 communities of different sensitivity for CARLIT, whereas into 5 functional groups of different sensitivity for EEI-c.
The minimum number of sites / samples per intercalibration type	-

Sufficient covering of all relevant quality classes per type	All quality classes are covered sufficiently.

4.3. Common benchmark

The group has defined common reference conditions, true reference sites. The sites present no or very low pressures following the next criteria:

- population density: no settlement with more than 1000 inhabitants/km^2 in the next 15 km and/or more than 100 inhabitants/km^2 in the next 3 km within that area (number of inhabitants is restricted to winter population).
- no more than 10% of artificial coastline
- no harbour (more than 100 boats) within 3 km*
- no beach regeneration within 1 km *
- no industries within 3 km*
- no fish farms within 1 km*
- no desalination plants within 1 km*
- no evidence of *Cystoseira* forest regression due to other unconsidered impacts; if there is evidence of *Cystoseira* regression (for example due to overgrazing), the quality element macroalgae may not be applied, depending on the method used).

* all distances are linear

A list of reference sites data for each MS is provided below:

Table 4.4 List of reference sites

MS	Number of sites	EQR ± SD	Name of sites	Coordinates (WGS84)	Date of sampling
Italy	9 sites for benchmarking (but use of the same reference sites as Spain for EQR calculation)	0.97±0.06000 0.913±0.186 1.00± 0.000	Montecristo Favignana Carbonara	42°19′26′′LAT - 10°17′23′′ LONG 37°56′53′′LAT - 12°18′48′′ LONG 39°08′00.38′′LAT -09°36′17.68′′ LONG	June 2009 June 2009 June 2009
France	10 sites for benchmarking (but use of the same reference sites as Spain for EQR calculation)	1.00±0.00	Corse Corse Corse Corse Corse Corse Corse Port-cros (Var) Port-cros (Var) Port-cros (Var)	8°36'16,478"E 42°23'47,276"N 8°33'18,143"E 42°21'5,607"N 8°36'29,578"E 41°53'45,729"N 8°40'58,692"E 41°44'25,18"N 8°50'45,372"E 41°31'4,554"N 9°5'48,526"E 41°23'32,093"N 9°18'57,75"E 42°50'35,196"N 6°23'51,162"E 43°0'53,935"N 6°24'34,837"E 43°0'0,454"N 6°23'52,743"E 42°59'39,022"N	June 2010 June 2009 June 2009 June 2009 June 2009 June 2009 June 2009 June 2007 June 2007 June 2007
Greece	Site 1 Site 2 Site 3 Site 4 Site 5 Site 6 Site 7 Site 8	0.94±0.05	Pyrgos, Kavala Limenaria, Thassos Sxinias, Attica Sounio, Attica Lemnos Island Agios Eustratios Island Sifnos Island Despotiko Island	404422.6N, 240840.1E 403809.2N, 243127.4E 380747.6N, 240311.1E 373917.6N, 240125.5E 395439.5N, 252238.6E 393330.7N, 245953.6E 365722.0N, 240442.9E 365757.2N, 245850.0E	July 2010 June 2010 March 2002 September 2009 August 1999 August 1999 October 2000 October 2000

MS	Number of sites	EQR ± SD	Name of sites	Coordinates (WGS84)	Date of sampling
Croatia	20 sites	0.96±0.08	20 randomly selected sites (1 km long sectors) inside MPA	sites selected in a stretch of coast between start point (42°44′02.39′′, 17°31′31.90′′) and stop point (42°47′59.95′′, 17°23′44.68′′)	CARLIT: Spring 2010

MS	Number of sites	EQR ± SD	Name of sites	Coordinates (WGS84)	Date of sampling
Spain	6 sites	0.94±0.08	Catalan coast: 5, 17, 21,47, 54, 72	X Y 525625 4684715 523308 4678208 519449 4645757 513752 4694591 514911 4635747 495111 4618869 (Datum: ED50 31N)	Spring 2009
Cyprus	Site 1 Site 2	0.98±0.04	Akamas (CY_5-C1_S1/B2) Cape Pyla–Station3 (CY_22-C3_S1/B2)	35°01' 122N, 32°17' 697E 34°57.210 N, 33°53.392E	Various periods from summer 2007 – summer 2010
Slovenia	2 sites	0.92±0.08	RR1 Pa2	RR1 (45°32’403 N, 13°37’085 E) Pa2 (45°31'607 N, 13°35'365 E)	Various periods from summer 2006 – summer 2010

Screening of the biological data for impacts caused by pressures not regarded in the reference criteria to make sure that true reference sites are selected: Of course some large-scale pressure cannot be avoided (for example climate change). We did not screen the biological assemblages in order to select reference sites, because we think that the choice of reference sites has to be independent on the biological assemblages.

Detailed description of setting reference conditions (summary statistics used): The reference conditions for the infralittoral fringe macroalgal assemblages, in the Mediterranean Sea, are generally considered mostly the *Cystoseira* spp. assemblages (but not exclusively, for example also *Lithophyllum byssoides*). The group did not apply any statistic, because this is largely described in the scientific literature (both old and recent).

4.4. Benchmark standardisation

The benchmark standardization has been performed automatically with the data-excel sheet provided by the intercalibration group: IC_Opt2_sub v1.24.

The subtraction option was used for the EQR normalization as the pressures behave in a parallel way.

From the table it is clear that the Spanish reference sites have a higher EQR than the Greek reference sites, measured with the same biological common metric.

The pristine conditions of the Greek sites were confirmed by a general categorical pressure indicator to compare the pressure environment with the Spanish reference sites. In the same way, The average of the common metric COMA at Spanish and Greek reference sites indicates a biological difference between the Spanish and Greek benchmark sites.

An analysis of the species similarities and dissimilarities by SIMPER in the Greek and Spanish data indicated that the differences were mainly related to a higher presence of *Cystoseira* spp. and *Jania* spp., and to lower presence of *C. compressa* in the Catalan reference sites. To assess the variability of these species in pristine conditions, the species distribution at the reference sites of Slovenia, Croatia and Italy were also investigated. It was concluded that these species also occur at pristine sites of other Member States. Therefore the difference between Spanish and Greek reference sites were considered to be related to geographical differences and not to a difference in the pressure conditions between the chosen Spanish and Greek reference sites. Therefore an automatic benchmark standardization between Spanish and Greek sites on the common metric scale was legitimate.

Table 4.5 EQR and ICM values in Spain and Greece reference sites

National Method	EQR	ICM	National Method	EQR	ICM
Spain: CARLIT	0.940	0.94255424	Greece: EEI-c	0.934	0.69908542
Spain: CARLIT	0.986	0.96985254	Greece: EEI-c	0.967	0.82175535
Spain: CARLIT	0.913	0.816958	Greece: EEI-c	0.940	0.69599775
Spain: CARLIT	0.927	0.9549124	Greece: EEI-c	0.940	0.66299229

Spain: CARLIT	0.950	0.80170709	Greece: EEI-c	1.000	0.88556511
Spain: CARLIT	0.939	0.83854881	Greece: EEI-c	0.940	0.81347832
			Greece: EEI-c	0.980	0.85993015
			Greece: EEI-c	0.820	0.80259296
Average ICM		0.89			0.78

5. Comparison of methods and boundaries

5.1. IC option and common metrics

The IC Option used was the Option 2 using a common metric as there are differences in data acquisition and different numerical evaluation.

The differences in data acquisition:

CARLIT: is based on the quantification of dominant communities (sensitive-opportunistic-tionitrophylous) at the landscape scale (more than 20 possible categories, measured in a continuous way, with a minimum variability of 50 m). Calculation of an EQR is based on reference values measured in existing reference sites.

EEI-c: is based on community (composition and abundance) quantification of species classified according to their morphology/physiology/distribution in two main ecological groups: ESG I comprises thick perennial (IA), thick plastic (IB) and shade-adapted plastic (IC) coastal water species, and ESG II comprises fleshy opportunistic (IIB) and filamentous sheet-like opportunistic (IIA) species. The mean group coverage (%) for an assemblage is estimated as follows: ESG I (% coverage) = [(IA*1)+(IB*0.8)+(IC*0.6)], ESG II (% coverage) = [(IIA*0.8)+(IIB*1)]. The EEI-c values range from 2 to 10 can be transformed into Ecological Quality Ratios from 0 to 1 as follows: EEI-c_{EQR}=1.25*(EEI-cvalue/RCvalue)-0.25, where RC=10, an ideal value very close to real reference values.

Explanation for the choice of the common metric and description:

One of the two methods and the common metric used in the first IC phase were not valid (because not giving continuous EQR values). Since then, the group has tried to improve both the method (new version of EEI sent to the group on the 10th of February 2011) and the common metric (two new common metric methods proposed during the meeting in Rome on the 21st and the 22nd of February 2011). Unluckily, both new common metrics proposed were not considered valid. Suggested way forward (by the JRC steering group members Nigel Willby and Wendy Bonne) was the creation of a common metric as the 1st axis of a multivariate analysis using a joint analysis of Spanish and Greek data with a reduced species list and a simplified abundance estimation for both data sets (the BENTHOS dataset of Spanish sites and the EEI-c dataset of Greek sites in one analysis).

This resulted in development of the COMMA (Common Metric Macroalgae) common metric.

The IC Common Metric (COMMA) used was the 1st axis of a multivariate analysis (PCO) (Figure 5.1) of a common dataset, which was built as follows:

1. The countries Greece and Spain submitted to the group two independent datasets from studies carried out along water quality gradients in Greek and Catalan coastlines, respectively.
2. In Greece, the macroalgal community (species diversity and abundance) of twenty two (22) sampling sites was studied at the upper infralittoral zone by means of a metallic frame 20cmx20cm or 25cmx25cm in size. One hundred eighty six (186) 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 (6-12 samples per site) at the site scale. In Spain, the macroalgal community (species diversity and abundance) of sixty seven (67) sampling sites was studied at the upper infralittoral zone by means of a metallic frame 20cmx20cm in size. All destructive samples (67) were sorted carefully in the laboratory and the surface covered by each species in horizontal projection was quantified as % of coverage. The total coverage of both counties usually exceeded 100% due to the presence of different layers in the vegetation (canopy, bushy layer, crusts and epiphytes).
3. In order to combine (merge) the two datasets, where the species due to biogeographical reasons and the species abundance due to different estimation approaches of the % coverage were different, the following steps were undertaken: (a) the taxonomically different or difficult taxa were consistently summarized to genus level as 'spp'. For the genus *Cystoseira* only the stenoecious species were included in one group (*Cystoseira* spp.) (b) Species abundance data of each dataset were separately transformed in % of maximum value.
4. From the dataset the most representative sites of rocky coasts (22 from Greece, 40 from Catalonia coasts) were selected to establish the Common Database of one hundred thirty three (133) taxa. Eight sites from Greece and six sites from Spain were characterized as benchmarks of pristine conditions.
5. In order to delimit the multivariate analysis two theoretical samples were incorporated representing the "ideal" degraded and pristine conditions in the Mediterranean Sea rocky coasts. This degraded and pristine station was chosen as such that it was located at both ends of the gradient on the PCO analysis, with all the other real samples spread in between.
6. Un-transformed data of the Common Database were analysed with Multivariate analysis (PCO-Principal Coordinates Analysis) using PRIMER 6 software. Samples were distributed along the plane defined by the two first axes account for 55.3% of the total variance of the data set (40.1% and 15.2%, for axes I and II, respectively). The values of each site on the 1st axis of a multivariate analysis (PCO) were accepted as values of the IC Common Metric (COMMA) through a transformation of the axis values to a scale of 0 to 1.

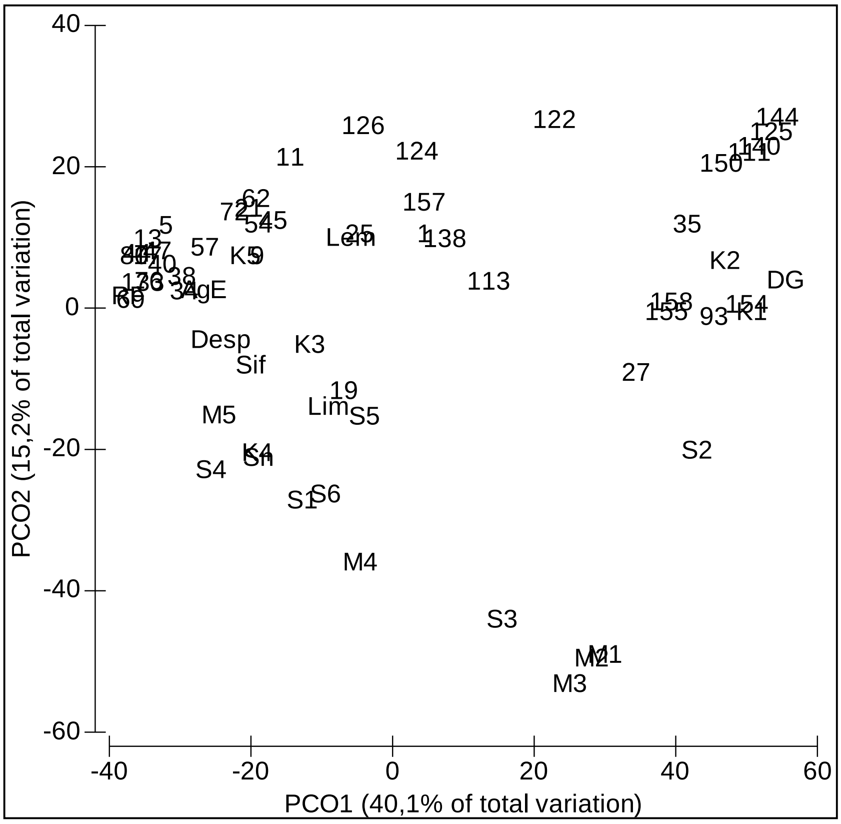

Figure 5.1 PCO ordination of the common dataset used for the calculation of the common metric COMMA

5.2. Results of the regression comparison

Overview of the results of regression comparison are shown in Table 5.1 and Figure 5.2 and Figure 5.3.

Both methods present a good correlation with the IC common metrics, therefore all of them are included in the IC exercise.

The Pearson correlation coefficients fulfil the requirement that r ≥ 0.5.

The slope of the regression fulfil the requirement that the slope should lie between 0.5 and 1.5.

Checking of methods comparability: No parameter fee statistical test have been performed in addition to the regression analysis,

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

Member State/Method	R^2	p
Spain:CARLIT	R^2=0.8, n=40	p<0.0001
Greece-Slovenia (pooled data)/EEI	R^2=0.76, n=22	p<0.0001

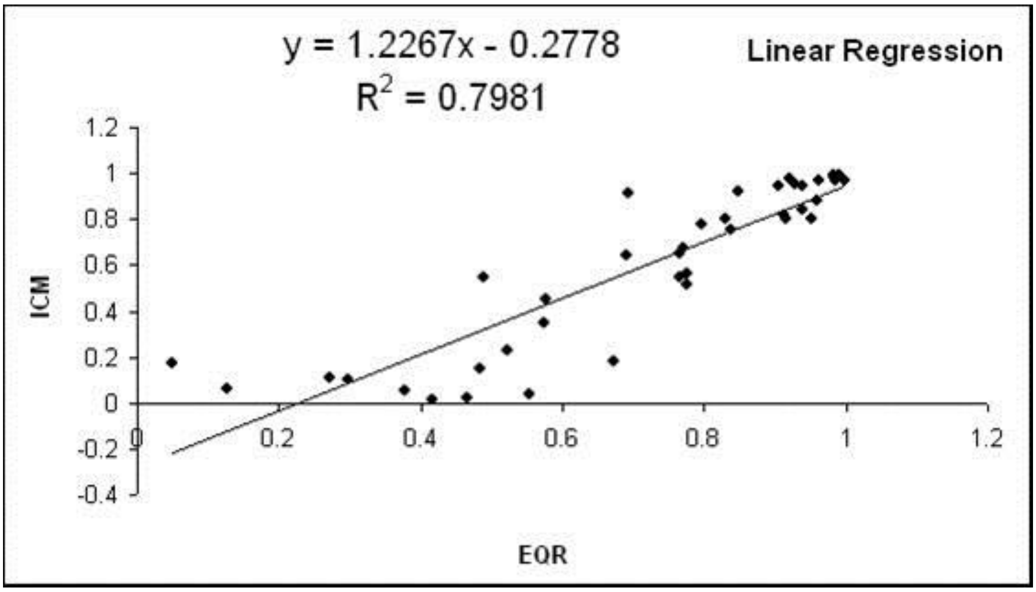

Figure 5.2 Spanish CARLIT EQR on X-axis versus the ICM EQR on Y-axis

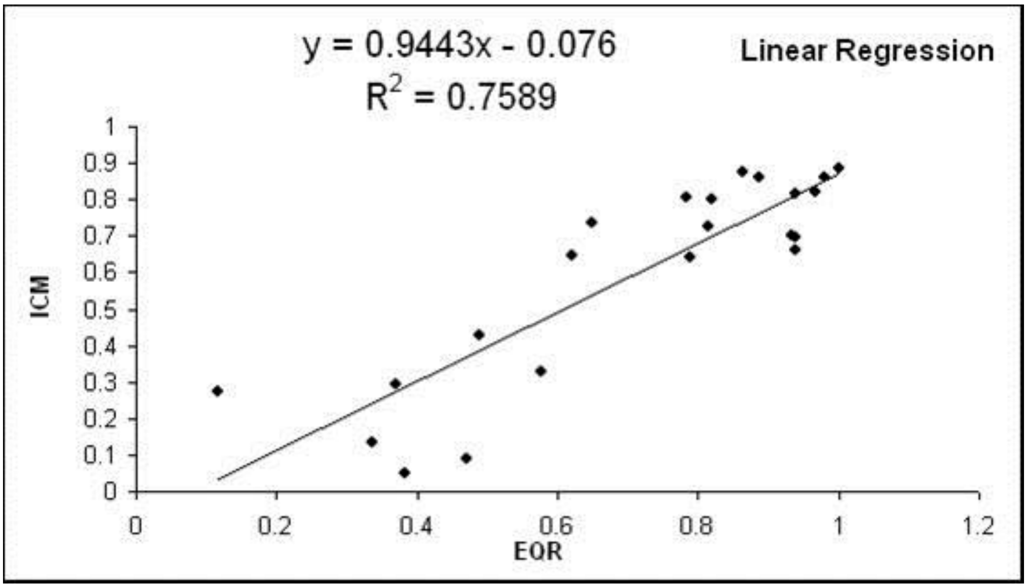

Figure 5.3 Greek EEI-c EQR on X-axis versus ICM EQR on Y-axis

5.3. Comparability criteria

Assessing level of boundary bias

The comparison has been done with the excel sheet IC_Opt2_sub v1.24. No adjustment is needed (Figure 5.4 and Figure 5.5).

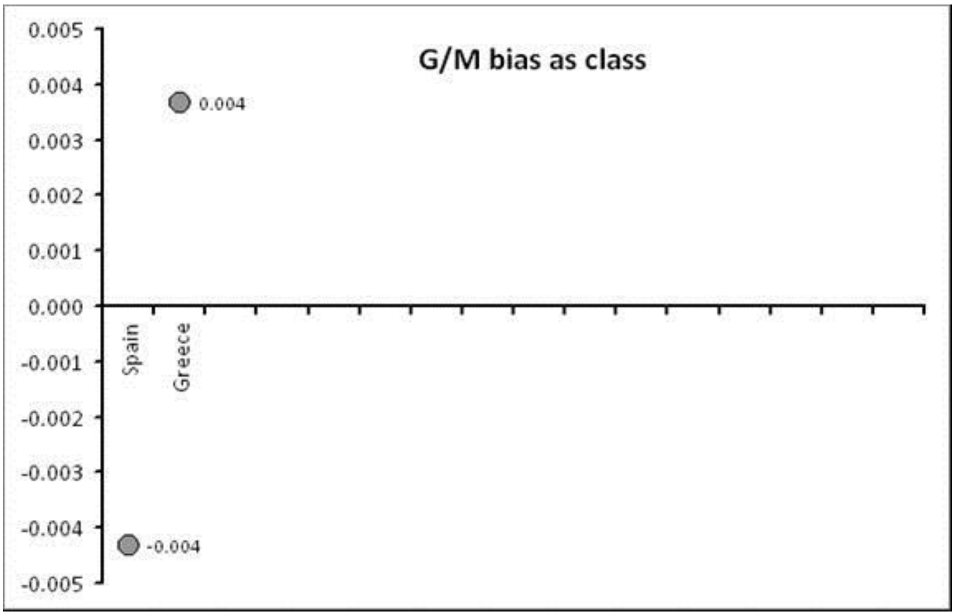

Figure 5.4 Comparison of the methods: GM boundary biases (GM- Good-Moderate class boundary)

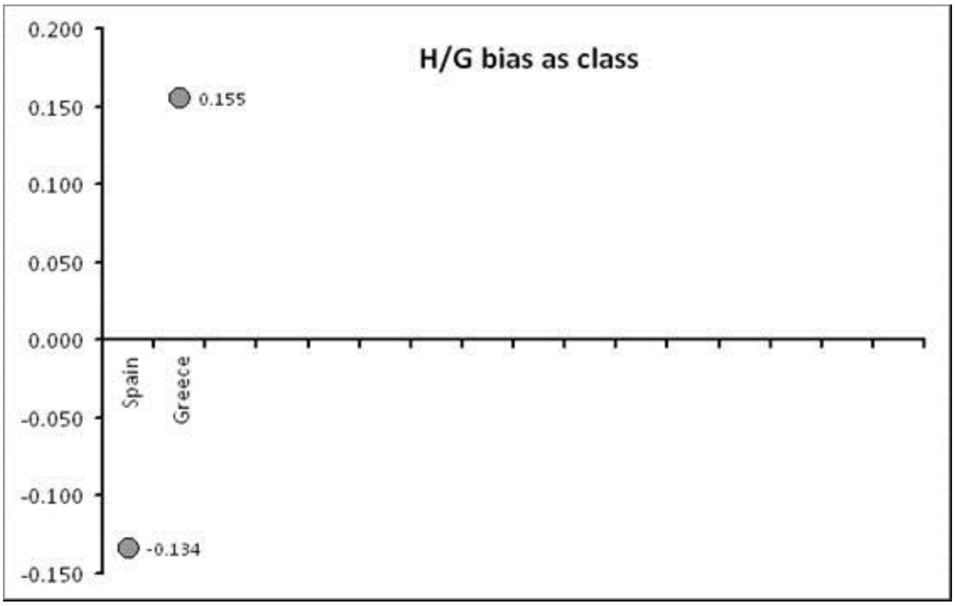

Figure 5.5 Comparison of the methods: HG boundary biases (HG- High-Good class boundary)

Assessing class agreement: No class agreement could be checked in the IC Option 2.

6. Final results to be included in the EC

6.1. Table with EQRs

Table 6.1 Overview of the IC results for the national methods

Biological Quality Element		**Macroalgae**	
Results coastal waters: Ecological quality ratios of national classification systems The following results apply to the upper infralittoral zone (3.5 – 0.2 m depth) in rocky coasts:			
Country	**National classification systems intercalibrated**	**Ecological Quality Ratios**	
		High-Good boundary	**Good-Moderate boundary**
Cyprus	EEI-c - Ecological Evaluation Index	0.76	0.48
France	CARLIT - Cartography of Littoral and upper-sublittoral rocky-shore communities	0.75	0.60
Greece	EEI-c - Ecological Evaluation Index	0.76	0.48
Italy	CARLIT - Cartography of Littoral and upper-sublittoral rocky-shore communities	0.75	0.60
Slovenia	EEI-c - Ecological Evaluation Index	0.76	0.48
Spain	CARLIT - Cartography of Littoral and upper-sublittoral rocky-shore communities	0.75	0.60

6.2. Correspondence common types versus national types

It is no necessary the transformation of common intercalibration types and common boundaries into the national typologies/assessment systems. The results are directly applicable to the national types that belong to the common type.

6.3. Gaps of the current intercalibration

- Additional analyses have been done in the present IC exercise to integrate data from other countries such as Italy, Slovenia and Cyprus. An analysis of variance between the benchmarks at bilateral (Spain, Greece) and international (all countries) level for each methodology is presented in Annex A.
- It is worth stressing that, in the studied zones, in the infralittoral fringe, there are no major problems due to biological indirect effects (for example invasive species and overfishing). These indirect effects can bias the estimation of the ecological status independently of the presence of human pressures in the neighbourhood, also in reference sites. This problem will probably appear in the future, with the

addition of new sampling sites. The group is already discussing several possible solutions to this problem.
- Malta still need to do all the intercalibration process.

7. Ecological characteristics

7.1. Description of reference or alternative benchmark communities

The common views for reference conditions can be summarized as follows:

1. Macroalgal communities of high diversity (more than 20 species (Eastern) and 40 species (Western) both in reference and in benchmark sites) should be dominated by brown algae mainly of the order Fucales in high irradiance sites and red algae of the order Corallinales (or other sciaphilic species) in vertical cliffs.
2. Dense well-developed macroalgal communities thriving in the upper infralittoral zone with most characteristic species belonging to the genera *Cystoseira, Sargassum, Lithophyllum, Peyssonnelia, Corallina* and *Padina*. Other common species belong to the genera *Halopteris, Stypocaulon, Dictyota, Dictyopteris, Laurencia, Cladophora* and *Jania*.
3. In the shadow zones (exposed steep vertical cliffs) *Lithophyllum byssoides* develops, forming important organogenic structures (trottoir). In marine caves with scarce light conditions a sciaphilic vegetation of red and green algae is dominant.
4. Spatio-temporal variability of the community's composition and abundance affected by hard substrata availability, intense and frequency of natural disturbances, e.g. hydrodynamism, grazing, by seasonal cycle of light period and intense, and by limiting factors like nutrients.

<u>CARLIT</u>:

Rocky shores places exposed to high irradiance levels and characterized by dense communities of several *Cystoseira* species: *C. mediterranea/amentacea var. stricta*, C. crinita, C. *brachyparpa var. balearica*, *C. foeniculacea/barbata/spinosa* var. *tenui*or/*compressa var. pustulata*. Alternatively, in shadow zones (steep vertical cliffs, high hydrodynamic conditions) *Lithophyllum byssoides* develops, forming important organogenic structures (trottoir).

<u>EEI</u>:

For the description of macroalgal community of the rocky upper infralittoral zone reference conditions in Greek coastal waters 62 samples from 26 putatively pristine Aegean sites dominated by *Cystoseira cf. crinita* community as part of the Hellenic "NATURA 2000" database (see Panayotidis *et al.*, 2001) in combination with the biotic index Ecological Evaluation-EEI Index (Orfanidis *et al.*, 2001; 2003) were used. The aim was (1) to develop an objective and statistically valid virtual" list of the most common algal species in the Aegean under undisturbed conditions, and (2) to test the conceptual model and the EEI recently developed by Orfanidis *et al.* (2001, 2003) for the implementation of Water Framework Directive (2000/60/EC) in Greek coasts. In total 113 taxa (73 Rhodophyceae, 25 Phaeophyceae, 15 Chlorophyceae) were identified in *Cystoseira cf. crinita* community of the Aegean Sea (Panayotidis *et al*., 2007). Nine (9) major taxa (except *C. cf. crinita*) contributed cumulatively by 90% in the community: *Haliptilon virgatum*, *Cystoseira compressa*, *Jania rubens*, *Padina pavonica*, *Herposiphonia secunda*, *Corallina elongata*, *Cladophora* spp., *Sphacelaria cirrosa* and *Titanoderma cystoseirae*. Moreover, 34 taxa contributed cumulatively by 99%. Under-storey layer considerably dominated to the community with most common representatives the red coralligenous algae *Haliptilon virgatum*, *Corallina elongata* and *Jania rubens*, and the brown alga *Padina pavonica*. It was followed by *C. crinita* epiphytes distinguished in: 1) filamentous green (*Cladophora* spp.), brown (*Sphacelaria cirrosa*) and red (*Herposiphonia secunda*) algae, and 2) in encrusting red algae (*Titanoderma cystoseirae* and *Hydrolithon* spp.). *Cystoseira compressa* contributed significantly (23.08%) to *C. crinita* community indicating that these species share common habitat resources in the Aegean Sea."

7.2. Description of good status communities

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

Species of the genus *Cystoseira* (Fucales, Cystoseiraceae) dominate Mediterranean upper-infralittoral communities (Feldmann, 1937; Boudouresque, 1971) and are particularly sensitive to any natural (Gros, 1978; Verlaque, 1987) or anthropogenic stress (Bellan-Santini 1966; Ballesteros *et al*., 1984; Hoffmann *et al*., 1988; Soltan *et al*., 2001) and, therefore, have experienced profound changes and decline over extensive areas (Thibaut *et al*., 2005). The highly structured and productive *Cystoseira mediterranea/stricta/crinita* communites are observed in hydrodynamic environments and non-polluted waters along the Northwestern Mediterranean coasts (Boudouresque, 1969; Ballesteros, 1988). Increasing concentrations of organic matter and nutrients drives *Cystoseira*-dominated communities to be replaced by the red alga *Corallina elongata* (Bellan-Santini, 1965, 1968; Ballesteros *et al*., 1984; Giaccone, 1993) and the mussel *Mytilus galloprovincialis* (Bellan-Santini, 1965, 1968; Bellan and Bellan-Santini, 1972). Green ephemeral algae begin to dominate in highly disturbed environments and near freshwater discharges: Ulva (Golubic, 1970; Bellan and Bellan-Santini, 1972; Rodriguez-Prieto and Polo, 1996), *Cladophora* (Belsher, 1977) or *Enteromorpha* (Ballesteros *et al*., 1984; Kadari- Meziane, 1994). Finally, the dominance of blue-green algae (*Oscillatoria, Lyngbya, Phormidium*) indicates very degraded environments (Golubic, 1970; Littler and Murray, 1975; Murray and Littler, 1978).

Based on these ecological changes, along pollution gradients, the boundary between High and Good conditions is defined when *Cystoseira* communities occur in patches and do not make extensive-continuous assemblages, and the *Lithophyllum byssoides* belt displays symptoms of degradation. Samples of *Cystoseira* assemblages indicate lower biomass of *Cystoseira* spp. and the substitution of the sciaphilic species inhabiting the underlayer dense *Cystoseira* assemblages by *Corallina elongata* or *Mytilus galloprovincialis*. The disappearance of these sensitive species and its replacement by stress tolerant species such as *C. elongata* and *M. galloprovincialis* defines the boundary between Good and Moderate situations

Nevertheless, the presence and abundance of littoral and sublittoral communities respond not only to water quality but also to other anthropogenic disturbances and natural geomorphological and physical factors variability of the coast. In situations defined as high water quality conditions, the sensitive species can be replaced by the stress tolerant ones, where other stress factors (e.g. low irradiance or sand abrasion) are affecting them.

Despite the communities representing the borderlines are the same all around the Mediterranean coastlines, exist some important differences that has to be considered. *Cystoseira compressa* is a plastic *Cystoseira* species that can leave either in pristine or in moderately degraded coastal waters. This phenomenon is a bit more pronounced in Aegean Sea and in the Gulf of Trieste (North Adriatic), which are in comparison to other Mediterranean parts, very shallow. In a recent paper (Orfanidis *et al.* 2011) it has been quantified using SIMPER analysis the contribution of *C. compressa* to the *C. crinita* community in the Aegean pristine sites to 7.17 (%) (Mean coverage 15.5). Therefore the abundance of *Cystoseira compressa* in the Eastern High and Good sites is considerably higher than in the Western sites.

Ecological Evaluation Index (EEI)

There are slight changes in the composition and abundance of macroalgal taxa compared to the type-specific communities. Such changes do not indicate any accelerated growth of phytobenthos or higher forms of plant life resulting in undesirable disturbance to the balance of organisms present in the water body or to the physicochemical quality of the water. This condition corresponds with slightly polluted sites (unbalanced). At the good status as is indicated by the EEI, the ESG I group may range from 30 to 60% while the ESG II from 0 to 30% of the macroalgae coverage, or the combination may thus that ESG I accounts for over 60% and ESG II between 30 and 60% of the total macroalgae coverage.

Upper infra-littoral macroalgal communities of Kavala and Saronikos Gulfs, Greece, were determined under moderate and good ESC by multivariate (Figure 7.1) and SIMPER (Table 7.1) analyses based on mean coverage (%) data. To moderate ESC group belong sites which were dominated by *Corallina elongata*, *Ulva rigida*, *Dictyopteris membranacea* and *Jania rubens* as well as by other species of the genera *Dictyota*, *Sargassum* etc. To good ESC group belong sites dominated by *Cystoseira* cf. *crinita*, *Sargassum vulgare* and *C. compressa* forming a consistent canopy layer, as well as by other species of the genera *Jania, Lithophyllum, Corallina, Ulva* etc.

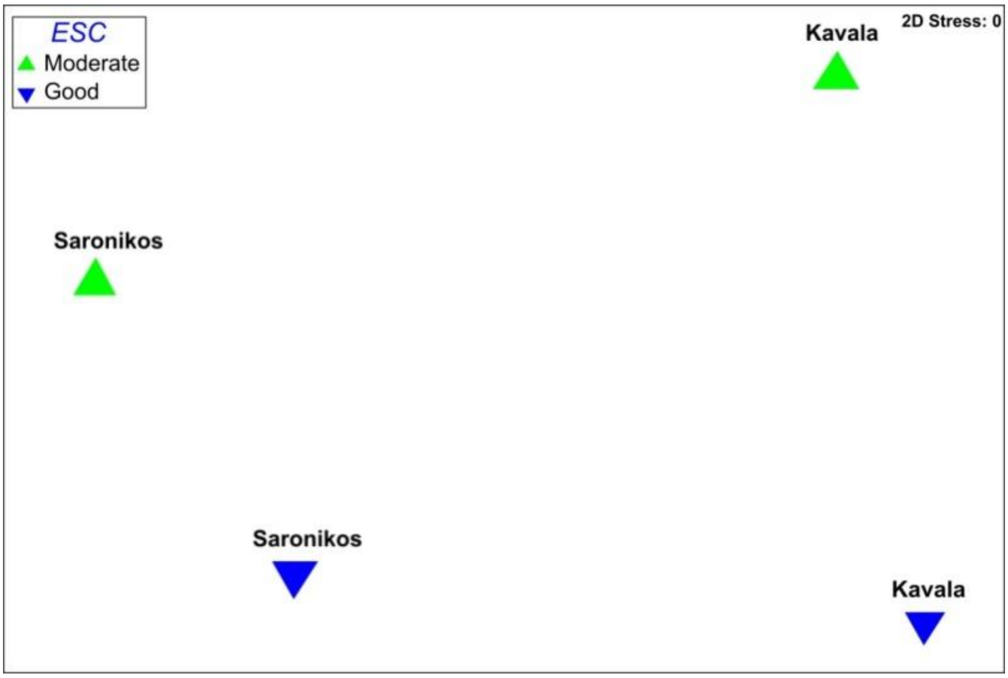

Figure 7.1 Two-dimensional MDS plot of the ESC in Kavala and Saronikos Gulfs using 4th-root transformated coverage (%) data

Table 7.1 Simper analysis of Good-Moderate ESC of seascapes Kavala-Saronikos Gulfs, Greece using non transformated coverage (%) data (Average dissimilarity 75.88)

Species	Group Moderate Mean Abundance	Group Good Mean Abundance	Mean Dissimilarity	Diss/SD	Contribution (%)	Cummulative (%)
Cystoseira cf. crinita	3,58	67,65	19,6	4,01	25,84	25,84
Corallina elongata	35,2	3,86	10,14	1,36	13,36	39,2
Jania rubens	9,85	11,92	3,46	0,99	4,56	56,73
Sargassum vulgare	4,78	9,44	2,63	1,25	3,47	60,2
Cystoseira compressa	1,89	6,76	1,71	1,37	2,26	67,84
Lithophyllum spp.	3,36	5,29	1,64	1,3	2,16	70
Ulva rigida	27,91	8,33	5,31	1,21	7	46,2
Dictyopteris membranacea	16,63	4,79	4,53	1,12	5,97	52,17
Dictyota dichotoma	8,62	1,12	2,05	1,22	2,71	62,91
Stypocaulon scoparium	4,96	8,14	2,02	1,35	2,67	65,58
Acanthophora delilei	5,5	0,13	1,45	0,88	1,91	71,91
Ulva spp.	0,82	4,8	1,27	1,02	1,68	73,59
Schizymenia dubyi	3,49	0	1,2	0,86	1,59	75,17
Sphacelaria cirrosa	0,04	3,61	1,16	1,14	1,53	76,7
Petalonia fascia	4,46	3,63	1,11	1,15	1,47	78,17
Cladophora sericea	0	3,1	1,04	0,85	1,37	79,54
Herposiphonia secunda	2,65	0,09	0,91	0,87	1,2	80,74

Our main hypothesis to explain the macroalgal community pattern across a good-moderate degradation gradient is: Under nutrient excess and turbid conditions, species composition shift from perennial slow-growing macroalgae to dominance of opportunistic and often bloom forming macroalgae due to the efficient nutrient assimilation of opportunistic macroalgae and their non-linear and self-accelerating response after crossing certain nutrient boundaries (Orfanidis *et al.* 2011). Furthermore, opportunistic macroalgae demand lower light quantities for growth than perennial canopy forming macroalgae, which under oligotrophic and highly transparent conditions they can luxury store large quantities of nutrients to support growth during periods of nutrient shortage. Slow growing calcareous species known as shade and grazing tolerant can develop dense coralline turf when a wave removes fleshy and canopy macroalgae or water turbidity increases. Within this group *Corallina* spp. deserve greater attention due to its notorious presence and wide distribution across degradation gradients. It can inhabit pristine conditions when canopy forming species due to disturbance or artificial substrate are absent to moderate degraded conditions utilizing plenty of nutrients under optimum light conditions. This maintenance of high degree of fitness over broad ranges of environmental conditions may facilitate through compensatory plastic response

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Annex

A. Integration of data from Italy, Slovenia and Cyprus - Results of comparison analysis

The analysis of variance shows that there are significant differences between the Common Metric benchmarks values of the different methods (CARLIT, EEI-c) at bilateral Spain-Greek scale (see Table A.1. below). The Common Metric benchmark EQR values were assessed by CARLIT (0.89) was higher than those of EEI-c (0.78) across Mediterranean Sea. This difference may due to: 1) the different sampling strategies implemented: while the sampling of CARLIT method realized during the high growth period of *Cystoseira* species may estimate maximum EQR values of the benchmark sites, the sampling of EEI-c method realized during the whole year may estimate average EQR values of the benchmark sites and 2) *Cystoseira compressa* is more abundant in the Eastern Mediterranean benchmark sites (more than 8% in average) than in the Western ones (less than 1% in average).

No differences were found within the benchmark EQR values of each method across the Mediterranean Sea (Table A.2).

Table A.1 Analysis of variance of Common Metric benchmark EQR values between Greece and Spain based on EEI-c and CARLIT, respectively

ANOVA	Factors	df	F	P
Spain-Greece	Methods	1	6.123	0.029
data	Error	12		

Table A.2 Analysis of variance between the benchmark EQR values within countries for EEI-c (a) and CARLIT (b) methodologies

(a) EEI-c

ANOVA	Factors	df	F	P
Greece, Slovenia, Cyprus, Croatia	Methods	3	0.403	0.75
data	Error	10		

(b) CARLIT

ANOVA	Factors	df	F	P
Spain, Italy, Croatia, France	Methods	3	1.282	0.294
data	Error	40		