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## Mediterranean Sea GIG: Coastal Waters - Seagrasses

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### 1. Introduction

- Five Member States (France, Italy, Spain, Cyprus, and Croatia) compared and harmonised their national assessment systems. Greece submitted a method (CymoSkew) but it was not included in the 2nd IC exercise. In Annex A Greece provide its arguments in relation to CymoSkew Intercalibration. Greek experts consider that the intercalibration of CymoSkew index with *Posidonia* metrics should be realized using as a common metric pressures and not the common metric used within the group.
- 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, and Croatia 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
Spain (Catalonia, Balearic Islands, Murcia, Andalusia)	POMI: <i>Posidonia oceanica</i> Multivariate Index	Yes
Spain (Valencia)	Valencian-CS: Valencian Classification System	Yes
France	PREI: <i>Posidonia oceanica</i> Rapid Easy Index	Yes
Italy	PREI: <i>Posidonia oceanica</i> Rapid Easy Index	Yes

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Cyprus	PREI: <i>Posidonia oceanica</i> Rapid Easy Index	Yes
Croatia	POMI: <i>Posidonia oceanica</i> Multivariate Index	Yes
Greece	CymoSkew	No
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 <sup>a</sup>	Disturbance sensitive taxa	(Diversity) *	Combination rule of metrics
Spain, Croatia POMI	Yes	Shoot density, meadow cover, shoot leaf surface	1 selected sensitive species, <i>Posidonia oceanica</i> + percent foliar necrosis, sucrose content in rhizomes, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ isotopic ratio in rhizomes, N content in epiphytes, Pb content in rhizomes	No, only 1 species	Yes, See description of the national method below
Spain Valencian-CS	Yes	Shoot density, meadow cover, dead matter cover, shoot leaf surface area	1 selected sensitive species, <i>Posidonia oceanica</i> + percent of plagiotropic rhizomes, rhizome baring/burial, percent of foliar necrosis, herbivore pressure, leaf epiphyte biomass	No, only 1 species	Yes, See description of the national method below
France, Italy, Cyprus PREI	Yes	Shoot density, shoot leaf surface area, maximum depth of the meadow (lower limit), type of lower limit	1 selected sensitive species, <i>Posidonia oceanica</i> , + ratio of epiphytic biomass and leaf biomass (E/L ratio)	No, only 1 species	Yes, See description of the national method below
Greece	Yes	Leaf length distribution asymmetry (shoot density is given as supplementary material)	1 selected sensitive species <i>Cymodocea nodosa</i>	No, only 1 species	See description of the national method below
Malta	No	-	-	-	-

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

Most of the seagrass (angiosperm) meadows in the Mediterranean are monospecific, thus classification systems are based on variables related to that species. Moreover, Mediterranean seagrass experts agreed to develop classification systems on the basis of one selected species, *Posidonia oceanica*, due to its wide distribution, its sensitivity, the existing knowledge and data on its responses to disturbance.

In addition, given the depth-dependence of most *Posidonia oceanica* descriptors, MS experts have agreed to collect data at  $15 \pm 1$  m depth.

**POMI and Valencian Classification System:** The metrics are combined using Principal Component Analysis (PCA). The EQR is calculated on the basis of the first component value with the following equations:

$$EQR'_x = (CI_x - CI_{\text{worst}}) / (CI_{\text{optimal}} - CI_{\text{worst}})$$

Where  $EQR'_x$  is the ecological quality of the site  $x$ ,  $CI_x$  is the score of the site  $x$  on the first component,  $CI_{\text{optimal}}$  is the score of the 'optimal' site (reference site) on the first component,  $CI_{\text{worst}}$  is the score of the 'worst' site on the first component.

The initial version of POMI was based on 14 metrics: shoot density, meadow cover, percent plagiotropic rhizomes, shoot leaf surface, percent foliar necrosis, P, N and sucrose content in rhizomes,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  isotopic ratio in rhizomes, N content in epiphytes, Cu, Pb, and Zn content in rhizomes (see Romero et al. 2007). After a few years of familiarization with the index, the method has now been simplified to include only 9 of the initial 14 variables (POMI 9). POMI 9 excludes phosphorous and nitrogen content, copper and zinc concentrations and plagiotrophic rhizomes. POMI 9 has been developed to minimize the redundancy of metrics, while still retaining the high sensitivity to change that POMI 14 had (Bennett *et al.*, 2011, Table 2). All those metrics have been observed to respond to a series of anthropogenic impacts including nutrient increase, organic matter increase, mechanical impact (anchoring, fish trolling), industrial pollution or fish farming among others (Martinez-Crego *et al.*, 2008).

The Valencian classification system is based on nine metrics: shoot density, meadow cover, dead matte cover, percent of plagiotropic rhizomes, rhizome baring/burial, shoot leaf surface area, percent of foliar necrosis, herbivore pressure, leaf epiphyte biomass (Fernandez-Torquemada et al. 2008, Table 2). All these metrics have been observed to respond to a series of anthropogenic impacts including nutrient increase, organic matter increase, mechanical impact (anchoring, fish trolling), industrial pollution, fishery or fish farming among others (Martinez-Crego et al. 2008).

**PREI:** The EQR is calculated according to the following equations:

$$EQR' = (N_{\text{density}} + N_{\text{shoot leaf surface area}} + N_{E/L} + N_{\text{lower limit}})/3.5$$

Where  $N_{\text{density}}$  = value measured/reference value;  $N_{\text{shoot leaf surface area}}$  = value measured/reference value;  $N_{E/L} = [1-(E/L)]*0.5$ ;  $N_{\text{Lower limit}} = (N'-17)/(\text{reference value}-17)$ .

PREI is based on five metrics: shoot density, shoot leaf surface area, the ratio of epiphytic biomass and leaf biomass (E/L ratio), maximum depth of the meadow (lower limit), and type of lower limit (Gobert *et al.*, 2009, Table 2). All these metrics have been observed to respond to a series of anthropogenic impacts including nutrient increase, organic matter increase, mechanical impact (anchoring, fish trolling), industrial pollution or fish farming among others (Martinez-Crego *et al.*, 2008).

**CymoSkew index** was estimated following the formula:

$$\text{Skewness index} = n \cdot M3 / [(n-1) \cdot (n-2) \cdot S^3]$$

where  $M3 = \sum (x_i - \text{Mean})^3$

$x$  = ln-transformed relative frequencies of adult and intermediate photosynthetic leaf lengths distinct values produced in frequency tables

$S$  = standard deviation

$n$  = ln-transformed relative frequencies of 60 adult and intermediate photosynthetic leaf lengths distinct values

At meadow level ranges from 1 (reference conditions) to 5 (degraded conditions). To ensure comparability in accordance with the WFD the CymoSkew can be transformed into ecological quality ratios (EQR, i.e. the ratio between the value of the observed biological parameter for a given surface water body and the expected value under the reference conditions), as follows:

$$\text{CymoSkew}_{\text{EQR}} = 1.25 - (0.25 \cdot \text{CymoSkew})$$

where  $\text{CymoSkew}_{\text{EQR}}$  values for coastal waters in Greece higher than 0.5 indicate sustainable ecosystems of good or high ecological status, whereas CymoSkew values lower than 0.5 indicate that the ecosystems should be restored to a higher ecological status class.

## 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	All : SCUBA Diving transect, quadrates
How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area?	Italy: annual sampling (August to September) France: annual sampling (April) Spain Catalan, Balearic Islands, Murcia, Andalucia + Valencia: annual sampling (September-October)
Sampling/survey months	Italy: August to September France: April

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<b>Information provided in the online WISER project assessment method questionnaires</b>	
	Spain Catalan, Balearic Islands, Murcia, Andalucia + Valencia: September -October
Which method is used to select the sampling /survey site or area?	Italy: expert judgement France: expert knowledge Spain Catalan, Balearic Islands, Murcia, Andalucia: expert judgement based on the presence of a meadow Spain Valencia: Expert knowledge
How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area?	Italy: e.g. station at 15 m: 18 replicates (shoots), 9 replicates (density), 3 replicates (visual census) – station lower limit: 6 replicates (shoots), 6 replicates (density), 1 replicates (visual census). France: e.g. station at 15 m: 20 replicates (shoots), 20 replicates (density), 1 replicate (visual census) for station lower limit Spain-POMI: sampling at 15 meters in 5 zones (nested design): at each zone 1 to 5 replicates are taken depending on the metric (minimum samples 5, maximum 27) Spain-CS Valencia: 3 sites with 3-5 replicates per site at each locality
Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based	Italy: station 15 m: estimate about 1600 square meters – station lower limit: along transept of 50 – 60 m Spain-POMI & Spain-CS Valencia: along a transect of 50 m x 10 meters (500 m <sup>2</sup> ) France: station 15 m: estimate about 1000 square meters – station lower limit: along transect of 50 – 60 m
Short description of field sampling/ survey procedure and processing (sub-sampling)	Italy-PREI: station at 15 m (nested design): sampling stations randomly positioned in 3 areas of 400 square meters distant one from each other about 10 meters France: station at 15 m: sampling stations randomly positioned in an area of 1000 square meters distant one from each other about 5 meters Spai -POMI: sampling at 15 meters in 5 zones (nested design): at each zone 1 to 5 replicates are taken depending on the metric (minimum samples 5, maximum 27). See Romero <i>et al.</i> 2007 for details on the methods for each variable. At each site, a transect is prepared following the 15 m isobath. Three sites, one at the origin (0 m), other intermediate (25 m) and the third terminal (50 m) are chosen and marked with pegs and buoys (for future samplings). Close to each bar, over an area of 25 m <sup>2</sup> , samples or measures are performed randomly (n=2-8, depending on the metric) resulting in a final sampling size per site of 3xn. Spain- Valencian CS: Sampling was done by scuba divers at 17 locations in the depth range 14-17 m. At each locality, three sampling sites separated by hundreds of metres were randomly selected to prevent spatial pseudo-replication. At

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### Information provided in the online WISER project assessment method questionnaires

each site, three 40 x 40 cm quadrates were randomly selected to measure shoot density, percentage of plagiotropic rhizomes, and rhizome baring. Living and dead *Posidonia* cover was estimated as the proportion of living and dead patches on three replicate 20 m transects. In addition, ten shoots were harvested at random and transported to the laboratory for further analysis.

## 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 (Catalonia, Balearic Islands, Murcia, Andalusia) POMI	Modelling considering the 3 best metrics in the region (two regions considered: Islands and Continent)	No existing reference sites	No existing reference sites	Because no truly unimpacted reference conditions exist in the Mediterranean a composite "optimal" site was constructed based on the POMI metrics with the assumption that this hypothetical site would have ecologically ideal conditions in relation to each of the metrics (the same as the PREI method). In the case of POMI, the average metric values for the reference site were obtained with the three best values observed for each metric when all sites were included. In addition, a "worst" site was obtained with the average of the three worst values for each metric.
Croatia POMI	Modelling considering the 3 best metrics in the region	No existing reference sites	No existing reference sites	Idem as for POMI Spain
Spain (Valencia)- CS Valencia	Modelling considering the 3 best metrics in the region	No existing reference sites	No existing reference sites	Reference conditions were established in a manner identical to POMI. We did not have a real reference site so we composed a virtual site, with the best values observed for all individual metrics (highest values for "positive" metrics and lowest for the "negative" ones) to serve as a reference condition.
France PREI	Modelling considering the 3 best metrics in the region (two regions considered: Islands and Continent)	No existing reference sites	No existing reference sites	Because no truly unimpacted reference conditions exist in the Mediterranean, a composite "optimal" site was constructed based on the PREI metrics with the assumption that this hypothetical site would have ecologically ideal conditions in relation to each of the metrics. Contingent on the specific metric this normative ideal



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Member State	Type and period of reference conditions	Number of reference sites	Location of reference sites	Reference criteria used for selection of reference sites
				would correspond to the maximum or the minimum value obtained (e.g. maximum for shoot density, minimum for epiphytic biomass/leaf ratio). The three best values recorded for each metric were chosen when all sites were included, and the highest value excluded. The optimum value of each metric for the composite reference site was derived as the average of the remaining two values.
Cyprus PREI	Modelling considering the 3 best metrics in the region (the region considered is the same used by Italy)	No existing reference sites	No existing reference sites	Idem as for PREI France
Italy PREI	Modelling considering the 3 best metrics in the region	No existing reference sites	No existing reference sites	Idem as for PREI France
Greece	Existing near-natural reference site, extrapolating model results	1 meadow (2 sites, 4 areas) in the North Aegean Sea	Thasos (Kavala Gulf, North Aegean Sea)	Because no truly unimpacted reference conditions exist in the Mediterranean, we assumed that leaf length asymmetry values and other meadows relevant metrics (e.g. meadow density, epiphytes) varies similarly to one site identified as near existing natural reference site. Dense (mean shoot density-msd>950 shoots m <sup>-2</sup> *) monospecific meadow of <i>Cymodocea nodosa</i> without presence of opportunistic species (e.g. <i>Hincksia mitchelliae</i> ) epiphytes on adult leaves in autumn. In deeper waters coexistence with <i>Posidonia</i> .
Malta	-	-	-	-

All references are biogeographically corrected by the 3 intercalibrated methods (PREI, POMI and CS-Valencia) itself. These 3 methods consider the reference conditions as the best of the 3 parameters found in the region where it is applied. In two Member States (Spain and France) two additional

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references are used: one for the Islands and one for the Continent. In the case of Cyprus they have also included some references from Italy as very few data is available (for the size of the Island). Cyprus has only done few sites where the method has been applied but given the small size we believe that using some references from Italy is the best option (even in the future) as they use the same method and the geographical proximity should be completely within the range. Even if more data was included, is still better to use some reference sites from Italy as it encompasses a larger variation.

### 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-POMI	<p>Equidistant division of the EQR gradient.</p> <p>The class boundaries are established by assuming that the system responds to all pressures in a linear way. While it is possible that this assumption may not always hold, thus far, no clear thresholds or discontinuities have been identified between the element quality and the pressure gradient. Thus, following exactly the same boundary setting as PREI, the range from 0 to 0.099 was arbitrarily assigned to the bad ecological status corresponding to the absence (due to anthropic impacts) of the targeted seagrass (<i>P. oceanica</i>) and the other EQR boundaries were obtained dividing the remaining scale (from 0.1 to 1) into four categories of equal amplitude (0.225 each). Therefore, when <i>P. oceanica</i> exists, the EQR is computed as follows: <math>EQR = (EQR' + 0.11)/(1+0.10)</math></p>	Equidistant division	Equidistant division	Yes quantitative

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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-CS	The class boundaries are established by assuming that the system responds to all pressures in a linear way. While it is possible that this assumption may not always hold, thus far, no clear thresholds have been identified between element quality and the pressure gradient. Thus, following exactly the same boundary setting as PREI and POMI the range from 0 to 0.099 was arbitrary assigned to the bad ecological status and the other EQR boundaries were obtained dividing the remaining scale (from 0.1 to 1) into four categories of equal amplitude (0.225 each). Therefore, when <i>P. oceanica</i> exists, the EQR is computed as follows: $EQR = (EQR' + 0.11)/(1+0.10)$	Equidistant division	Equidistant division	Yes quantitative
France PREI	The class boundaries are established by assuming that the system responds to all pressures in a linear way. While it is possible that this assumption may not always hold, thus far, no clear thresholds have been identified between the element quality and the pressure gradient. <i>P. oceanica</i> is very sensitive to anthropogenic disturbances and meadows disappearance has been reported in environmental conditions where macrofauna can still survive, hence the bad class has been defined as the ecological status in which <i>P. oceanica</i> cannot survive. In other words, wherever and whenever a <i>P. oceanica</i> bed is able to survive, albeit in a heavily degraded state, the ecological status is above bad. However its absence is not necessarily related to degradation, therefore the bad class can only be attributed to areas which show evidence of a recent die-off of the meadow (< 5years). Consequently, the bad ecological status was arbitrarily assigned the range from 0 to 0.099. The other EQR boundaries were obtained dividing the remaining scale (from 0.1 to 1) into four categories of equal amplitude (0.225 each). Therefore, when <i>P. oceanica</i> exists, the EQR is computed as follows: $EQR = (EQR' + 0.11)/(1+0.10)$	Equidistant division	Equidistant division	Yes quantitative

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<b>Member State</b>	<b>Type of boundary setting: Expert judgment – statistical – ecological discontinuity – or mixed for different boundaries?</b>	<b>Specific approach for H/G boundary</b>	<b>Specific approach for G/M boundary</b>	<b>BSP: method tested against pressure</b>
Italy PREI	Idem as PREI France	Equidistant division	Equidistant division	Yes quantitative
Greece	Equidistant division of the EQR gradient by assuming that the index at meadow level ranges from 1 (reference conditions) to 5 (degraded conditions). The class boundaries are established by assuming that the system responds linearly to all pressures as follows: High ESC ( $1 \geq \text{CymoSkew} > 0.75$ ), Good ESC ( $0.75 \geq \text{CymoSkew} > 0.5$ ), Moderate ESC ( $0.5 \geq \text{CymoSkew} > 0.25$ ), Poor ESC ( $0.25 \geq \text{CymoSkew} >$ ), Bad ESC ( $=0$ ).	Equidistant division	Equidistant division	Yes quantitative
Cyprus PREI	Idem as PREI France	Equidistant division	Equidistant division	Yes quantitative
Croatia- POMI	Equidistant division of the EQR gradient. Idem as POMI Spain	Equidistant division	Equidistant division	Yes quantitative
Malta	-	-	-	-

## 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 <b>five classes</b> (high, good, moderate, poor and bad).	Italy, France, Spain-all regions, Croatia have 4 classes (high, good, moderate and poor). Bad is only considered when an existing meadow is lost because <i>P. oceanica</i> is very sensitive to anthropogenic disturbances
2. High, good and moderate ecological status are set in line with the WFD's <b>normative definitions (Boundary setting procedure)</b>	Italy, France, Spain-all regions, Croatia: yes
<ul style="list-style-type: none"> <li>• Scope of detected pressures</li> </ul>	Italy, France, Spain-all regions, Croatia: integrated pressures
<ul style="list-style-type: none"> <li>• Has the pressure-impact relationship of the assessment method been tested?</li> </ul>	Italy, France, Spain-all regions, Croatia: yes
<ul style="list-style-type: none"> <li>• Setting of ecological status boundaries: methodology and reasoning to derive and set boundaries</li> </ul>	Italy, France, Spain-all regions, Croatia: yes: equidistant division of the EQR gradient
<ul style="list-style-type: none"> <li>• Boundary setting procedure in relation to the pressure:</li> <li>• Which amount of data/pressure indicators have been related to the method and what was the outcome of the relation?</li> </ul>	The relationship of the integrated pressures with POMI (with Catalan coast), PREI (French Coast) and CS (with Valencian Coast) has been established with a regression and all of them were highly significant ( $p < 0.01$ )
<ul style="list-style-type: none"> <li>• 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</li> </ul>	Italy, France, Spain-all regions, Croatia: yes: the reference condition is a formula that includes the best different metrics
3. <b>All relevant parameters</b> indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A <b>combination rule</b> 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	Italy, France, Spain-all regions, Croatia: yes

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Compliance criteria	Compliance checking conclusions
<ul style="list-style-type: none"> <li>Complete list of biological metric(s) used in assessment</li> </ul>	<p>Italy-PREI: shoot density, Leaf surface area, Lower depth limit, Typology lower limit, Epiphyte biomass/leaf biomass.</p> <p>France-PREI: shoot density, Leaf surface area, Lower depth limit, Tipology lower limit, Epiphyte biomass/leaf biomass.</p> <p>Spain Catalan-POMI: shoot density, meadow cover, percent plagiotropic rhizomes, shoot leaf surface, percent foliar necrosis, P, N and sucrose content in rhizomes, <math>\delta^{15}\text{N}</math> and <math>\delta^{34}\text{S}</math> isotopic ratio in rhizomes, N content in epiphytes, Cu, Pb, and Zn content in rhizomes</p> <p>Spain Valencia-CS: shoot density, meadow cover, dead matter cover, percent of plagiotropic rhizomes, rhizome baring/burial, shoot leaf surface area, percent of foliar necrosis, herbivore pressure, leaf epiphyte biomass</p>
<ul style="list-style-type: none"> <li>Data basis for metric calculation</li> </ul>	<p>Italy-PREI: 42 sampling area/ 29 sampling area (Italian data set)/ Literature data</p> <p>France-PREI: for intercalibration 40 sites with common data</p> <p>Spain Catalan-POMI: 30 sampling areas (for intercalibration 14 sites of common data)</p> <p>Spain Valencia-CS: for intercalibration 10 sites common data</p>
<ul style="list-style-type: none"> <li>Combination rule for multimetrics</li> </ul>	<p>Italy-PREI: average of the metrics</p> <p>France-PREI: average of the metrics</p> <p>Spain Catalan-POMI: multivariate analysis (PCA)</p> <p>Spain Valencia-CS: multivariate analysis (PCA)</p>
<p>4. Assessment is adapted to <b>intercalibration common types</b> that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT</p>	<p>Italy, France, Spain-all regions, Croatia: yes</p>
<ul style="list-style-type: none"> <li>Is the assessment method applied to water bodies in the whole country?</li> </ul>	<p>Italy-PREI: yes</p> <p>France-PREI: yes</p> <p>Catalan-POMI: no- 4 regions: Catalan, Balearic Islands, Murcia, Andalusia (Spain)</p> <p>Valencia-CS: no- 1 region Valencia (Spain)</p>
<ul style="list-style-type: none"> <li>Specify common intercalibration types</li> </ul>	<p>Only one type by now for all the Mediterranean</p>
<ul style="list-style-type: none"> <li>Does the selection of metrics differ between types of water bodies?</li> </ul>	<p>Italy, France, Spain-all regions, Croatia: no</p>

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Compliance criteria	Compliance checking conclusions
5. The water body is assessed against <b>type-specific near-natural reference conditions</b>	Italy, France, Spain-all regions, Croatia: The water body is assessed in all methods against the best conditions of each individual parameter
<ul style="list-style-type: none"> <li>• Scope of reference conditions</li> </ul>	See section on National reference conditions: Site specific Least Disturbed Conditions, Modelling (extrapolating model results), Expert knowledge
<ul style="list-style-type: none"> <li>• Key source(s) to derive reference conditions</li> </ul>	Italy: average of the best values of descriptor/ Literature data France, Spain-all regions, Croatia: average of the best values of descriptor
<ul style="list-style-type: none"> <li>• Number of sites, location and geographical coverage of sites used to derive reference conditions</li> </ul>	Italy: 29 sampling sites and data collected for the entire Western Mediterranean Basin Spain:POMI: 30 sampling areas Spain: Valencian CS: 15 Localities in Valencian region
<ul style="list-style-type: none"> <li>• Time period (months+years) of data of sites used to derive reference conditions</li> </ul>	Italy: 2000 – 2008 Spain: POMI- we derive reference conditions every year (data from 2003-2009) France & Valencia: not reported
<ul style="list-style-type: none"> <li>• Reference site characterisation: criteria to select them</li> </ul>	Italy: reference site were determined on the basis of analysis significant pressures Catalan: reference site derived from the best values of a descriptor
<ul style="list-style-type: none"> <li>• Is a true reference used for the definition of High status or an alternative benchmark estimation?</li> </ul>	Italy: high status France, Spain Catalan + Valencia: alternative benchmark
6. Assessment results are expressed as <b>EQRs</b> : <ul style="list-style-type: none"> <li>• Are the assessment results expressed as Ecological Quality Ratios (EQR)?</li> </ul>	Italy, France, Spain-all regions, Croatia: yes
7. Sampling procedure allows for <b>representative</b> information about water body quality/ecological status <b>in space and time</b> See info from WISER Questionnaires:	Italy, France, Spain-all regions, Croatia: yes
<ul style="list-style-type: none"> <li>• Has the uncertainty of the method been quantified and is it regarded in the assessment ?</li> </ul>	France, Italy, Spain Valencia: no, to be done Spain Catalonia: yes- in progress of publication. <i>Specification of uncertainty consideration:</i> a) by choosing three sampling sites, distant 100 m one from each other, and performing the entire protocol at each site, in eight of the meadows of the monitoring network, in

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Compliance criteria	Compliance checking conclusions
	two different sampling periods and in two deeps b) by performing a sensitivity test of the POMI (adding random variation to the descriptors)
<ul style="list-style-type: none"> <li>Specify how the uncertainty has been quantified and regarded</li> </ul>	Spain:POMI: Uncertainty has been assessed with the program Starbugs (Clark 2004)-developed for rivers
8. All data relevant for assessing the biological <b>parameters</b> specified in the WFD's normative definitions are covered by the <b>sampling procedure</b>	Italy, France, Spain-all regions, Croatia: yes
9. Selected taxonomic level achieves adequate <b>confidence and precision</b> in classification	No taxonomy is needed for this methods
<ul style="list-style-type: none"> <li>Minimum size of organisms sampled and processed</li> </ul>	Spain: POMI:shoot Spain: Valencian CS: <i>P. oceanica</i> shoot (>10 cm)
<ul style="list-style-type: none"> <li>Record of biological data: level of taxonomical identification – what groups to which level</li> </ul>	Not necessary

**General conclusion of the compliance checking:** Compliance criteria are met

### 3. Results IC Feasibility checking

#### 3.1. Typology

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.

Method	Appropriate for IC types/subtypes	Remarks
POMI, Valencian CS and PREI	Used in entire Mediterranean Sea	The CymoSkew has not participated into the intercalibration



## Intercalibration of biological elements for transitional and coastal water bodies

### 3.2. Pressures addressed

Table 3.1 Pressures addressed by the national methods and overview of the relationship between national methods and the pressures.

Member State	Method/Metrics tested	Pressure	Pressure indicators	Amount of data	Strength of relationship
Spain	POMI	Eutrophication, organic matter and direct impact (direct habitat degradation)	Quantitative test: metrics for pressures were urban sewage discharge (Kg/day.Km coast), urban soil surface (ha/Km coast), tourism pressure (rooms/Km of coast), harbors pressure (number of boats/Km of coast). All variables were normalized and then added in one index.	14 sites	Linear regression $R^2 = 0.617$ $p < 0.05$
Spain	Valen-cian CS,	Eutrophication, organic matter and direct impact (direct habitat degradation)	Quantitative test: We correlated estimates of six anthropogenic pressures that were considered most relevant to Posidonia meadows: coastal construction (km artificial coastline per km of coastline by water body), beach regeneration (m <sup>3</sup> sand added km-1 coast), urban sewage (kg COD d-1 km-1 coast), industrial sewage (kg COD d-1 km-1 coast), pollution from rivers and channels (kg BOD5 d-1 km-1 coast), and pollution from agricultural soil use (ha mm precipitation year-1). All variables were normalized and then added in one index.	35 sites	Linear regression $R^2 = 0.651$ $p < 0.05$
France	PREI	Eutrophication, organic matter and direct impact (direct habitat degradation)	The anthropization index has been defined as a sum of 7 impact factors affecting the seawater quality and /or the biotope quality: fish farming (production in ton/year), industrial development (zoning area surface: km <sup>2</sup> ), agriculture (ha of land cover), tourism (number of camping/city; number of secondary house), fishing (number and type of boat/maritime area), commercial port (number of port and number of boat) and urbanization (habitants/km <sup>2</sup> ). Each impact factor was classified	36 sites	Linear regression $R^2 = 0.74$ $p < 0.05$

**Intercalibration of biological elements for transitional and coastal water bodies**

Member State	Method/Metrics tested	Pressure	Pressure indicators	Amount of data	Strength of relationship
			from 0 (none impact) to 5 (dramatic effect on the meadow) (Goibert <i>et al.</i> , 2009)		
Italy	PREI	Eutrophication, organic matter and direct impact (direct habitat degradation)	A version of LUSI index (LUSIsg) that takes under consideration indirect (land based: urban, commercial and industrial, agriculture) and direct (sea based: mariculture, sewage outfall, harbour, sediment nutrient release) anthropogenic pressures along with confinement and background trophic status. All pressures have been quantified in a 3 km radius circle	22 sites	Linear regression $R^2=0.61$ , $p<0.05$
Greece	Cymo-skew	Eutrophication, organic matter and direct impact (direct habitat degradation)	A version of LUSI index (LUSIsg) that takes under consideration indirect (land based: urban, commercial and industrial, agriculture) and direct (sea based: mariculture, sewage outfall, harbour, sediment nutrient release) anthropogenic pressures along with confinement and background trophic status. All pressures have been quantified in a 3 km radius circle	11 sites (180 samples)	Linear regression $R^2=0.66$ , $p=0.002$
Cyprus	PREI	Eutrophication, organic matter and direct impact (direct habitat degradation)	A version of LUSI index (LUSIsg) that takes under consideration indirect (land based: urban, commercial and industrial, agriculture) and direct (sea based: mariculture, sewage outfall, harbour, sediment nutrient release) anthropogenic pressures along with confinement and background trophic status. All pressures have been quantified in a 3 km radius circle	7 sites	Linear regression $R^2=0.86$ , $p=0.02$
Croatia	POMI	Eutrophication, organic matter and direct impact (direct habitat degradation)	LUSI index that takes into consideration indirect (land based: urban, commercial and industrial, agriculture) and direct (sea based: mariculture, sewage outfall, harbour, sediment nutrient release) anthropogenic pressures along with confinement and background trophic status.	17 sites	Linear regression $R^2=0.72$ , $p<0.05$

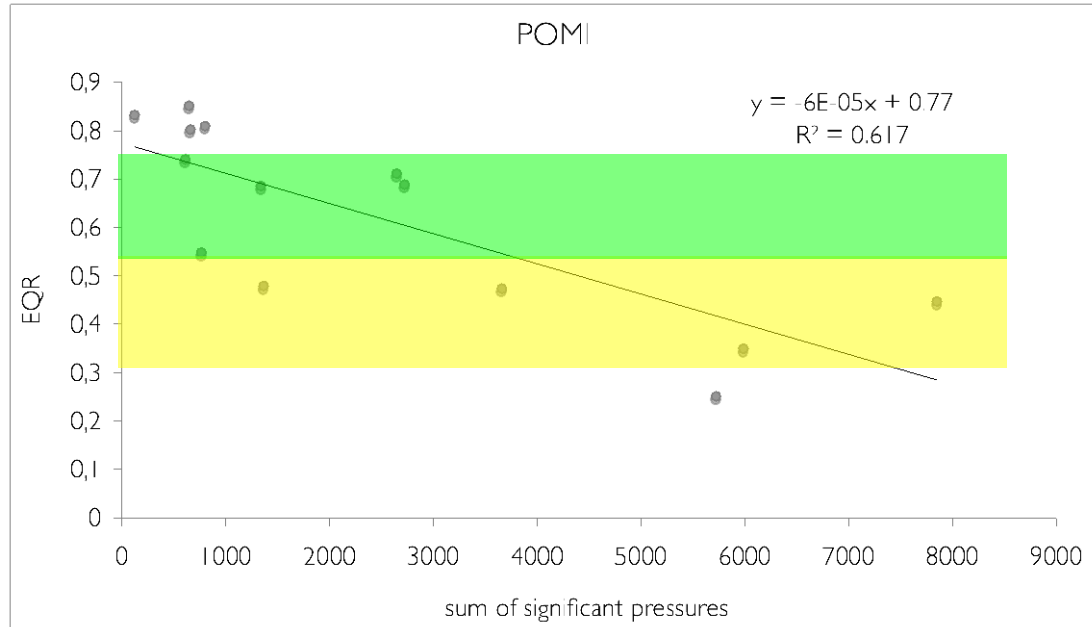
Intercalibration of biological elements for transitional and coastal water bodies

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Member State	Method/Metrics tested	Pressure	Pressure indicators	Amount of data	Strength of relationship
Malta	-	-	-	-	-

Relationships between the methods and the pressures (Figure 3.1 to Figure 3.6).

**Spain – Catalonia POMI:**



*Figure 3.1 Relationship between POMI in Spain and anthropogenic pressures, for which the combined pressure gradient is composed of the sum of significant pressures (i.e. sewage pressure, urban use pressures, tourism pressure and harbours pressure, see above for units).*

The good and moderate ecological class are indicated by the green, respectively yellow colour.

**Croatia-POMI:**

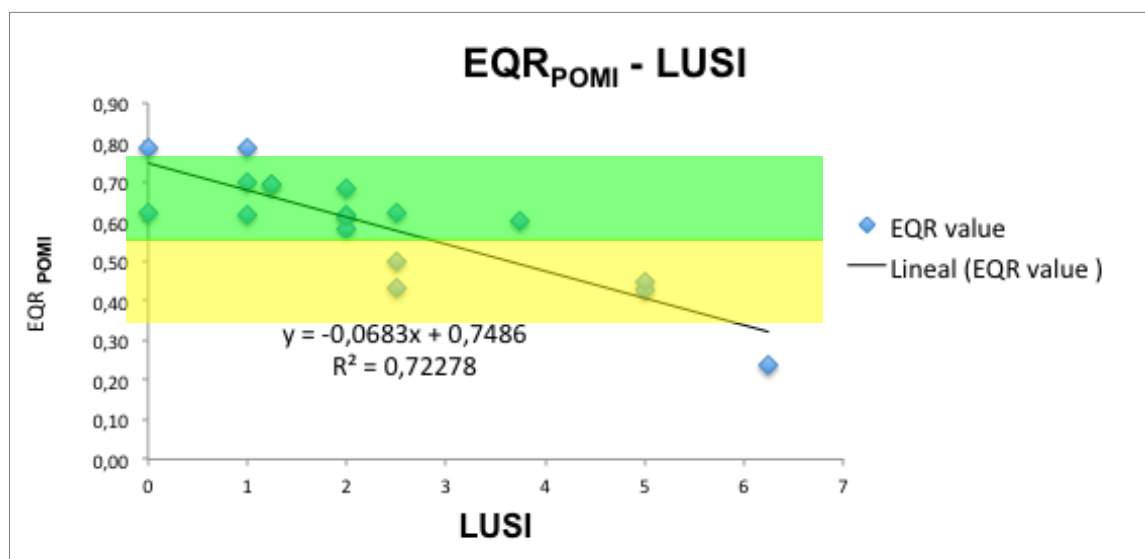
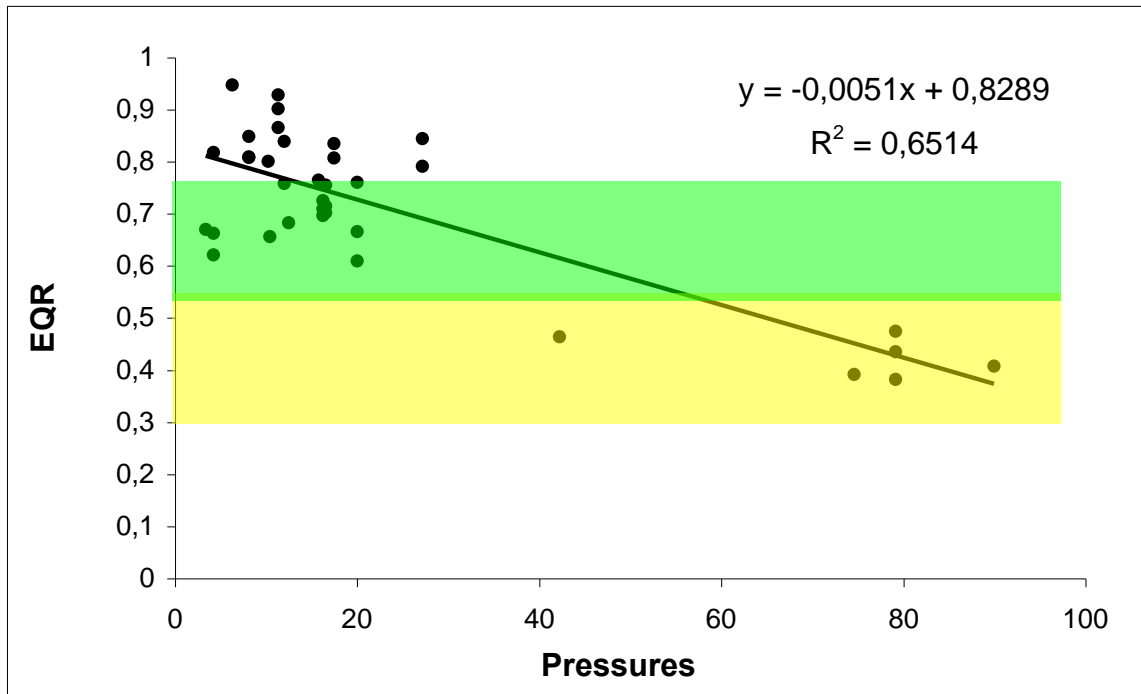


Figure 3.2 Relationship between POMI in Croatia and anthropogenic pressures, for which the combined pressure gradient is composed of the sum of significant pressures (LUSI Index).

The good and moderate ecological class are indicated by the green, respectively yellow colour.

**Spain Valencian CS:**



*Figure 3.3 Relationship between the Valencian CS and pressures. The pressures are calculated according to the procedure defined in the Catalan article 5 report, using the indicators coastal construction, beach regeneration, urban and industrial sewage, rivers and channels discharges, agricultural land use (Agencia Catalana del Aigua, 2006; Fernandez-Torquemada et al. 2008). The Valencian Classification system is presented as Component 1, before it is normalised on the EQR scale.*

The good and moderate ecological class are indicated by the green, respectively yellow colour.

### France PREI:

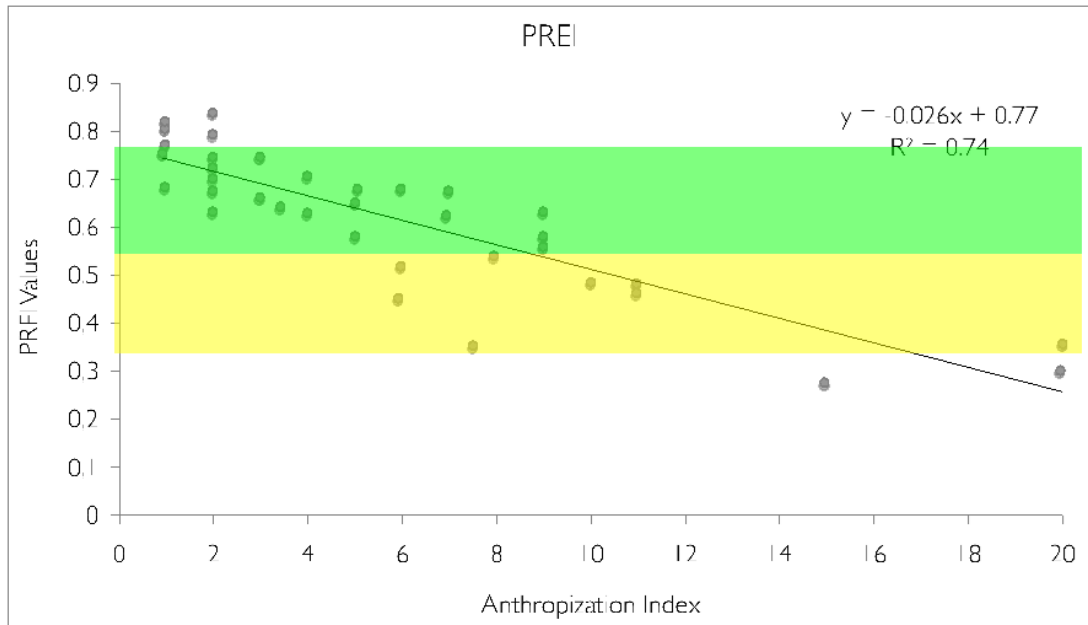


Figure 3.4 Relationship between the PREI classification system and pressures in France. The anthropization index has been defined as a sum of 7 impact factors affecting the seawater quality and /or the biotope quality: fish farming, industrial development, agriculture, tourism, fishing, commercial port and urbanization (see above for units). Each impact factor was classified from 0 (none impact) to 5 (dramatic effect on the meadow) (Gobert et al., 2009)

The good and moderate ecological class are indicated by the green, respectively yellow colour.

Italy PREI:

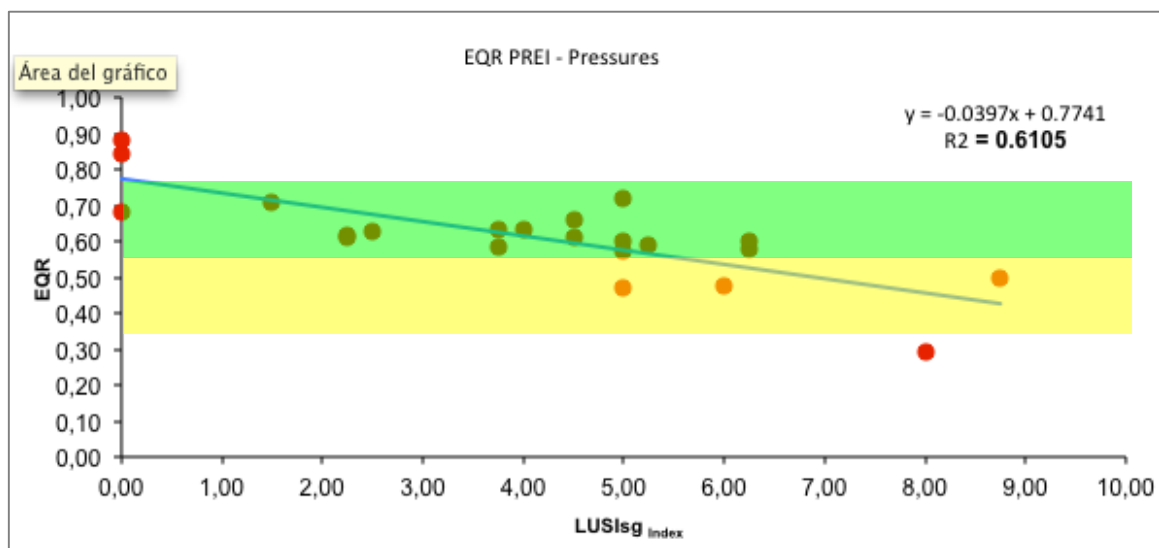


Figure 3.5 Relationship between the PREI classification system and pressures in Italy, for which the combined pressure gradient is composed of the sum of significant pressures (LUSI Index).

The good and moderate ecological class are indicated by the green, respectively yellow colour.



### Cyprus PREI:

In the case of Cyprus, only few locations have been measured (n=7), but we consider that given the size of the island this is a good representation of all the pressures occurring in the island.

While investigating the relation between the PREI EQR values with the Pressures, Cyprus evaluated both LUSI and MA-LUSI indices, since there were some discussions within the Angiosperm Group about the possible suitability of the latter. Early on the evaluation of MA-LUSI Cyprus encountered some methodological issues and decided to abandon it and proceed with LUSI index, similarly with other MSs in the group. The G/M boundary corresponds to a LUSI value of about 4.5.

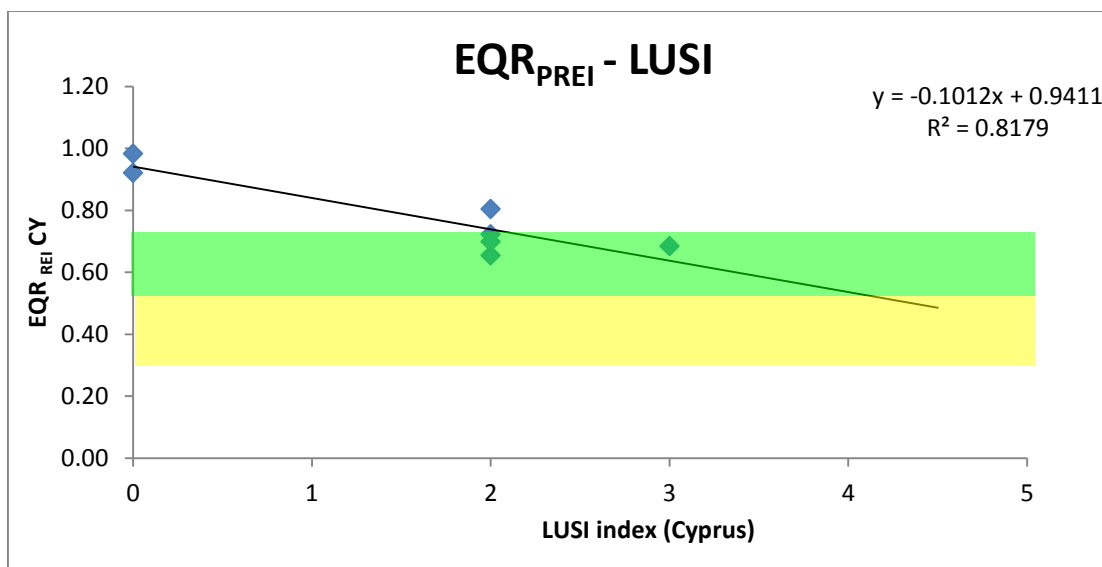


Figure 3.6 Relationship between the PREI classification system and pressures in Cyprus, for which the combined pressure gradient is composed of the sum of significant pressures (**LUSI Index**). It has to be noted that only 7 sites have been included in this relationship but considering the size of the island the number of locations is considered to be enough.

The good and moderate ecological class are indicated by the green, respectively yellow colour.

### Greece CYMOSKEW:

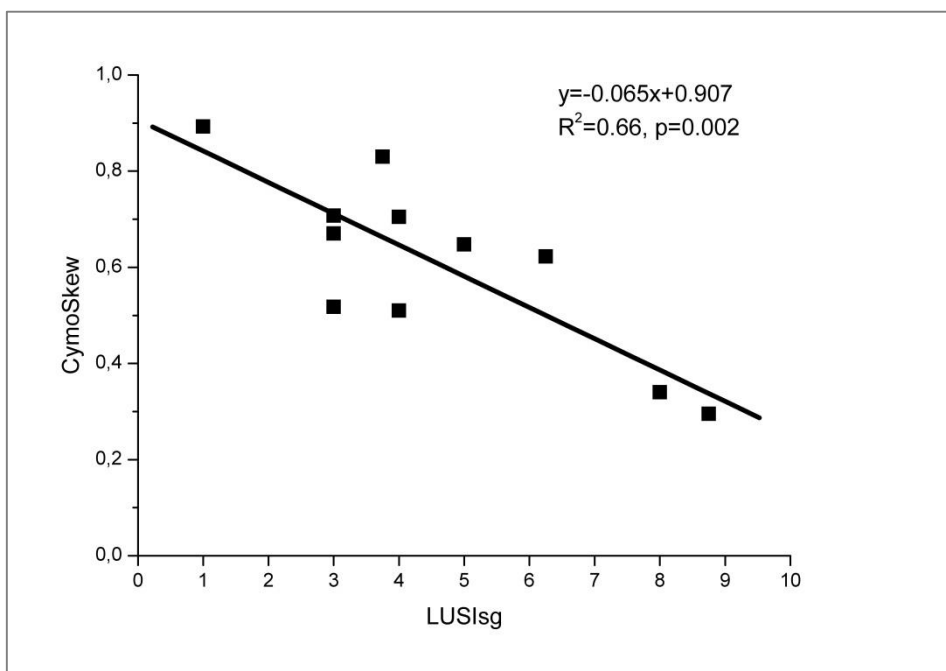


Figure 3.7 Relationship between the CymoSkew classification system and pressures index LUSIsg. The LUSIsg index takes under consideration indirect (land based: urban, commercial and industrial, agriculture) and direct (sea based: mariculture, sewage outfall, harbour, sediment nutrient release) anthropogenic pressures along with confinement and background trophic status.

Method	Pressure
PREI (France, Italy, Cyprus)	Combination of pressures that include: fish farming, industrial development, agriculture, tourism, fishing, commercial port and urbanization
POMI (Spain-Catalonia;Balearic Islands;Murcia;Andalucia and Croatia)	Combination of pressures that include: sewage pressure, urban use pressures, tourism pressure and harbours pressure
Valencian CS (Spain-Valencia)	Coastal construction, beach regeneration, urban sewage, industrial sewage, pollution from rivers and channels, and pollution from agricultural soil use
CymoSkew (Greece)	Combination of pressures that include: indirect (land based: urban, commercial and industrial, agriculture) and direct (sea based: mariculture, sewage outfall, harbour, sediment nutrient release) anthropogenic pressures along with confinement and background trophic status.
<b>Conclusion</b>	
The Intercalibration is feasible in terms of <b>pressures</b> addressed. The combination of the pressures is almost the same for all the methods All methods have a very similar combination	

of the pressures that include in large terms eutrophication, organic matter and direct impacts to the meadows (i.e. direct extraction)

### 3.3. Assessment concept

Method	Assessment concept
PREI & VALENCIAN-CS	Based on a set of metrics related to structural and functional attributes of the system
POMI	Based on a set of metrics related to structural, functional and physiological attributes of the system
Cymoskew	Based on morphological and dynamic attributes of the system

The Intercalibration is feasible in terms of assessment concept. The three methods (Cymoskew has not been included in the intercalibration phase) follow a very similar philosophy in which a set of metrics is combined and that includes structural and functional attributes of the ecosystem. Additionally, POMI includes physiological attributes that have been observed to be highly correlated to the functional and structural attributes but with a faster capacity of response.

## 4. Collection of IC dataset and benchmarking

### 4.1. Dataset description

*Table 4.1 Description of the data collection within the GIG.*

Size of common dataset: total number of sites	85 sites 63 sites used in the comparison calculation FR: 38 sites (high, good, moderate status) ES-Cat: 16 sites (high, good, moderate status) ES-Val: 7 sites (high, good, 1 moderate site)
Number of Member States	2
Repackage/disaggregation of samples/WB results?	No, EQRs were used at the usual integrated level of the site
Gradient of ecological quality	The upper 3 (in the analysis) or 4 classes are covered
Coverage per ecological quality class	High, good and moderate status in the analysis. Italy, France and Spain Catalonia also have some poor sites.

*Table 4.2 Overview of the data set*

Member State	Number of sites or samples or data values		
	Biological data	Physico- chemical data	Pressure data
France - PREI method	36 sites	available	36 sites

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Spain - POMI method	14 sites	available	14 sites
Spain - CS- method	35 sites	available	35 sites

Other countries as Italy, Cyprus or Croatia also have data for the IC in Phase 2. This data are not being processed in the intercalibration because no common metrics are available for those countries. These countries are applying the same methods already included in the IC Phase 1 and no intercalibration is needed. However, the integration of these new datasets is shown in Annex B where all the different benchmarks for each method between countries have been compared.

The data set used in the intercalibration includes variables that are 'deep-independent'. *Posidonia* is very susceptible to depth and any data that includes shoot density or deep limit will be very difficult to be intercalibrated unless reference conditions are locally corrected (see previous explanation about reference conditions). To avoid this problem the variables chosen for the intercalibration where 'deep-independent' variables: form of the deep limit and shoot biomass. Only three regions (that included the 3 methods) had these two common metrics collected (form of the deep limit and shoot biomass). These regions are Catalonia-Spain, France and Valencia-Spain, and that is the reason why those are the ones used in the Option 2 (common metric) intercalibration procedure. However, we truly believe that when intercalibrating the methods itself, POMI Spain, PREI France and CS are very good representative dataset as are regions large enough to encompass all the variability of the methods itself. Additionally we have added the ANOVA results summarizing the differences between regions within each method of the countries/regions not included in the Option 2 intercalibration procedure.

### 4.2. Data acceptance criteria

*Table 4.3 List of data acceptance criteria used for the data quality control and describe the data acceptance checking process and results*

Data acceptance criteria	Data acceptance checking
Data requirements (obligatory and optional)	All methods are developed in <i>Posidonia oceanica</i> habitats (no other seagrasses have been included in this intercalibration exercise), in the same season (generally after summer) and at a fixed depth that in general is around 15 meters.
The sampling and analytical methodology	All methodologies include diving in the <i>Posidonia oceanica</i> meadow where several structural parameters are measured (shoot density, cover, deep limit, etc.); additionally some shoot samples are also required for all methodologies. All sampling methods include at least 3 samples (up to 20 depending on the parameter) that are randomly collected in the meadow.

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Level of taxonomic precision required and taxalists with codes	Taxonomy is not required in any method.
The minimum number of sites / samples per intercalibration type	The minimum number of sites is 14 for POMI.
Sufficient covering of all relevant quality classes per type	All quality classes are covered except for the bad class (red) that is only included if a previous existing meadow of <i>P. oceanica</i> disappears. This is based on the fact that <i>P. oceanica</i> is very sensitive to anthropogenic pressures and can not live under high level of disturbances.

### 4.3. Common benchmark

The group has defined alternative benchmark conditions of high status.

The alternative benchmark (high status) is defined as a location on the basis of a low impacted area (see below). The three methods are using an alternative benchmark presenting very low pressures that responds to following the selected criteria:

- low population density: no settlement in the next 3 km (or less than 10 habitats/ha within that area)
- mooring density lower than 2 mooring ha<sup>-1</sup>
- no harbour or mooring facility in 3 km
- no beach regeneration within 10 km
- no trawling in the area
- no industries within the 3 km
- no fish farms
- no desalination plants
- no evidence of meadow degradation due to other unconsidered impacts.

Identification of the **alternative benchmark sites** for each Member State in each common IC type:

POMI-Spain (Catalonia):

Jugadora (42°19.003'N; 3°18.875'E)

St. Feliu (41°46.343'N; 3° 01.629'E)

POMI-Spain (Balearic Islands):

Cala Deià (39,76277222 N; 2,642469444E)

Cala Marmacén (39,53516667N; 2,376E)

Cala Matzoc (39,75833333N; 3,405E)

Cala Millor (39,60063333N; 3,390166667E)

Cala Murtra-Formentor (39,93916667N; 3,184E)

Es Castell (39,15266667N; 2,93E)

Santa Maria-Cabrera (39,15116667N; 2,948666667E)

Ses Salinas (39,277N; 3,040816667E)

POMI-Spain (Murcia):

Punta de la Azahía (37°33'09.13"N; 1°10'39.91"O)

Punta Parda (37°25'20.28"N; 1°29'56.08"O)

POMI-Spain (Andalucía):

Carboneras-Isla San Andrés (36,99388889, -1,8825)

San José-Cala Higuera (36,76416666666667; -2,091944444444444)

POMI-Croatia:

Palmizana (Lat 43°09'56.80"; Long 16°23'49.40")

Rukavac (Lat 43°01'18.80"; Long 16°12'57.73")

PREI-France:

Bravone: (42,20239 N/ 9,56936 E)

Erbalunga: (42,76906N/ 9,47487E)

Levant: (43,00347N/ 06,26486E)

Giens: (43,01158N/06,09020E)

PREI-Italy

Asinara (Lat 41° 00' 52"; Long 08° 15' 53")

Capo Carbonara (Lat 39° 06' 58"; Long 09° 31' 57")

Carbonifera (Lat 42° 49' 44"; Long 10° 45' 29")

Maraone-Isole Egadi (Lat 37° 59' 31"; Long 12° 24' 38")

Punta Mesco-Parco Cinque Terre (Lat 44° 08' 19"; Long 09° 38' 34")

Punta Tresino (Lat 40° 20' 29"; Long 14° 57' 08")

PREI-Cyprus:

Cayo Greco (34° 57.401'N; 34° 05.208'E)

CS-Spain:

Cap Santa Pola (38° 12' 44.7" N; 000° 28' 52.8" W)

Tabarca (38° 09' 36.1" N; 000° 28' 38.7" W)

**Validation of the selection of the alternative benchmark with biological data:** In each benchmark it was established the EQR

**Description of boundary setting procedure set for the common IC type:** For each method and Member State, the boundaries were set up according to an equidistant division of a continuum as no evident discontinuities were detected. As a consequence, the division of the ecological gradient in ecological quality classes with the class boundaries is very similar for the different methods/Member States. Since every Member State has selected its own reference sites to derive the reference conditions for the different parameters included in the method, the comparability of the high status class in relation to the pressures is checked through benchmarking.

#### 4.4. Benchmark standardization

The benchmark standardization has been performed automatically with the data-excel sheet provided to the intercalibration group: IC\_Opt2\_sub v1.24. The EQRs of the unimpacted benchmark sites were very similar, so no further adjustment of the common metric EQRs was needed as no geographical differences could be detected in the biological EQR values of the common metric in the same pressure environment. For the normalization was used the subtraction option as the pressures behave in a parallel way.

### 5. Comparison of methods and boundaries

#### 5.1. IC option and common metrics

The IC option use was the Option 2, as different metrics and different integration methods are used, and there are differences in the data acquisition.

The IC common metric used was a biological common metric: the type of lower limit of the meadows and leaf surface area per shoot.

#### 5.2. Results of the regression comparison

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

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

The Pearson correlation coefficients fulfill the requirement that  $r \geq 0.5$ .

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

Checking of methods comparability: No parameter free 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 <sup>2</sup>	r	p	slope
PREI	0.55	0.745	<0.05	1.167
POMI	0.82	0.905	<0.05	0.836
Valencian-CS	0.80	0.892	<0.05	1.141



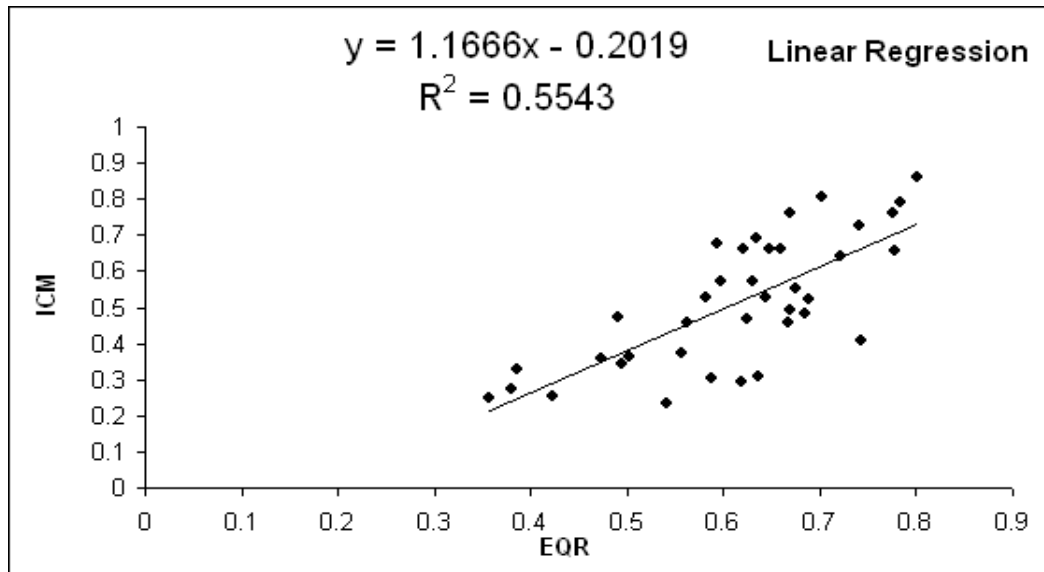


Figure 5.1 France: French PREI EQR on X-axis versus ICM EQR on Y-axis

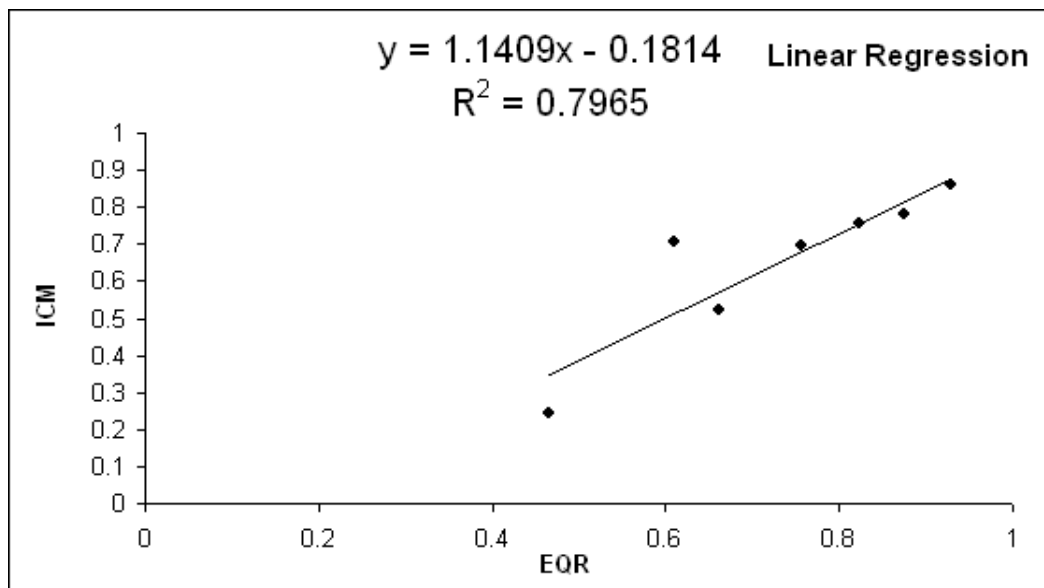


Figure 5.2 Spain: POMI on X-axis versus ICM EQR on Y-axis

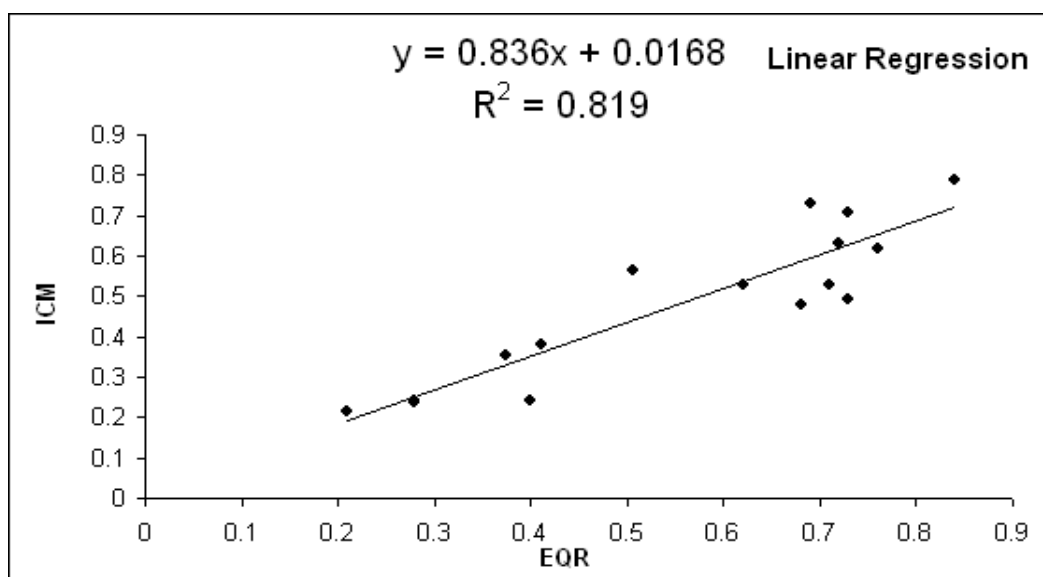


Figure 5.3 Spain: CS-Valencian on X-axis versus ICM EQR on Y-axis

### 5.3. Comparability criteria

#### Assessing level of boundary bias

The comparison has been done with the excel sheet IC\_Opt2\_sub v1.24 (Figure 5.4 and Figure 5.5).

No adjustment is needed.

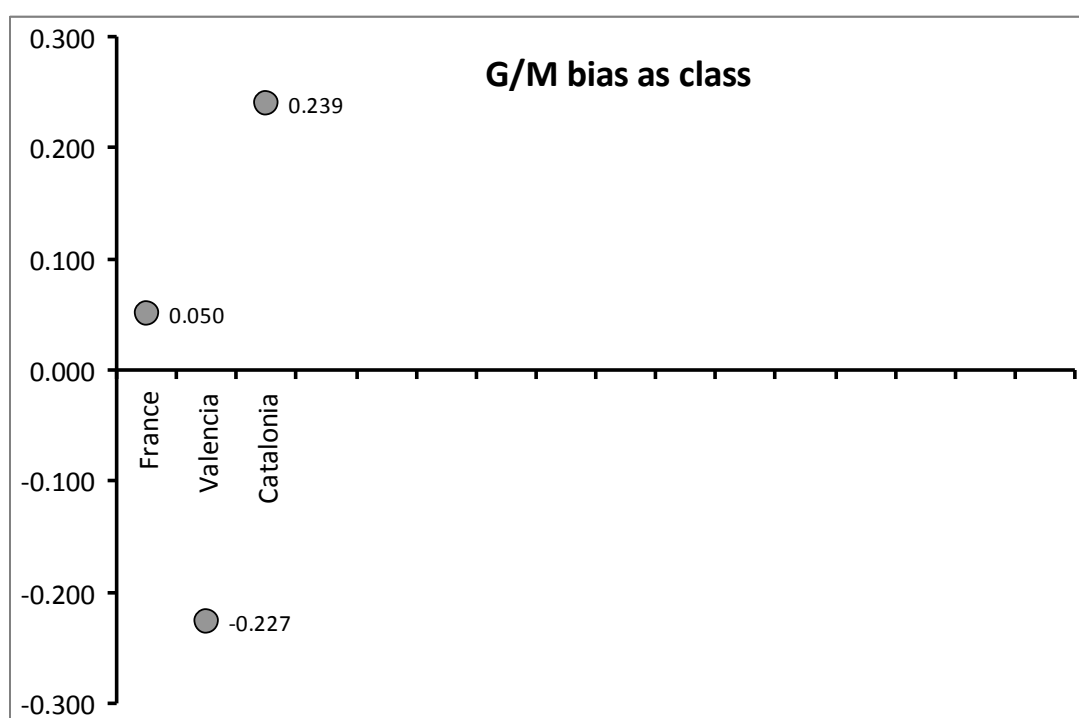


Figure 5.4 Comparison of the methods: GM boundary biases (GM- Good-Moderate class boundary).

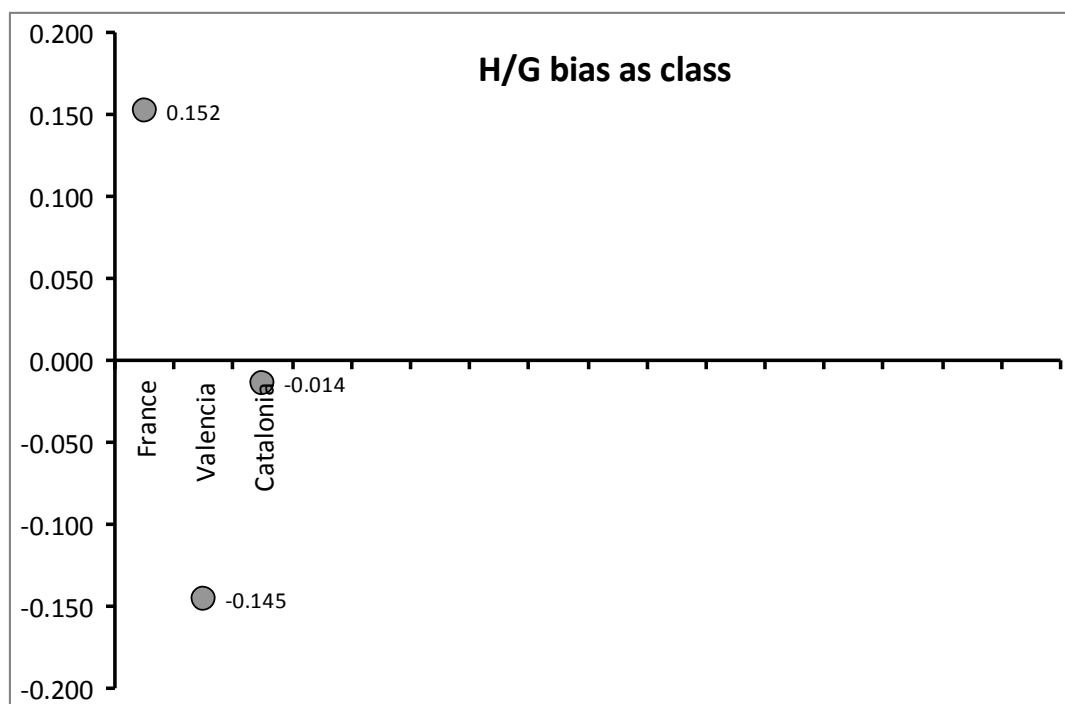


Figure 5.5 Comparison of the methods: HG boundary biases (HG- High-Good class boundary).

**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		Seagrasses	
Results coastal waters: Ecological quality ratios of national classification systems			
Country	National classification systems intercalibrated	Ecological Quality Ratios	
		High-Good boundary	Good-Moderate boundary
Cyprus	PREI - <i>Posidonia oceanica</i> Rapid Easy Index	0.775	0.55
France	PREI - <i>Posidonia oceanica</i> Rapid Easy Index	0.775	0.55
Italy	PREI - <i>Posidonia oceanica</i> Rapid Easy Index	0.775	0.55

Spain (Catalonia, Balearic Islands, Murcia, Andalusia)	POMI - <i>Posidonia oceanica</i> Multivariate Index	0.775	0.55
Spain (Valencia)	Valencian-CS	0.775	0.55

## 6.2. Correspondence common types versus national types

It is not 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

- This last aspect includes the integration of the data of other countries: Italy, Croatia, Cyprus and data from all regions in Spain (Balearic Islands, Andalusia and Murcia), where the intercalibration is not needed) in the intercalibration exercise. The integration has been done in the present IC exercise and the analyses results are shown in the Annex B.
- Malta and Greece still need to do the intercalibration process

# 7. Ecological characteristics

## 7.1. Description of reference or alternative benchmark communities

The benchmark sites considered do not have biogeographical differences.

The reference conditions at each region are very different and as a result, even the values of the borderline conditions between G/M of specific parameters differ in each region where the method is applied. So, just to put an example, if this data has to be provided will include for each parameter analysed (shoot density, cover, deep limit, shoot length, N content, etc..) the different values in each region (G/M) where different reference conditions have been considered (POMI Spain Continental, POMI Spain Islands, CS-Spain, PREI France Continental, PREI France Islands, PREI Italy, POMI Croatia, PREI Cyprus). Most of the parameters have only been measured for one method, but not for the others. Also, in each method the weight of each parameter is different. We think that this information will add more confusion than help to find trends, which are already included in the method itself.

## 7.2. Description of reference or alternative benchmark 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).

At the borderline conditions between good and moderate the communities are characterised by presenting in general an state of conditions of some of the considered metrics (cover, density, shoot length, etc..) that is at list 30% worse than the high status for that area (the references of each method are biographically corrected).

## 8. References

Bennett, S., G. Roca, J. Romero, T. Alcoverro, 2011. Ecological status of seagrass ecosystems: An uncertainty analysis of meadow classification based on the *Posidonia oceanica* multivariate index (POMI). *Marine Pollution Bulletin*: 62: 1616-1621.

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Romero, J., B. Martinez-Crego, T. Alcoverro & M. Perez, 2007. A multivariate index based on the seagrass *Posidonia oceanica* (POMI) to assess ecological status of coastal waters under the Water Framework Directive (WFD). *Marine Pollution Bulletin* 55: 196-204.

## Annexes

### A. Explanation provided by Greek experts arguing reasons why CymoSkew index should be inter-calibrated with the Posidonia indices using pressures as a Common Metric

*Posidonia oceanica* and *Cymodocea nodosa* are forming extensive of high productivity patchy meadows in Greek coasts peaked in summer regulated mainly by seasonal changes in light and temperature (Orfanidis *et al.*, 2005<sup>1</sup>, Orfanidis *et al.*, 2010<sup>2</sup>). The meadows of *Posidonia* extend to 20 (25) meters depth in the North Aegean, but they are able to reach 30 meters and 45 meters depth in the Central and South Aegean, respectively. Southern of Crete the maximum depth was recorded at 55 m. Since water transparency increases in the Aegean southwards (PAR attenuation coefficient (k) ranges from 0.12-0.13 at Macedonian coasts to 0.06-0.09 at Chios and Fourni islands, central Aegean), the deeper growth limit of *Posidonia* seems to be related with light penetration. In the Ionian Sea the lower depth limit approaches 50 meters depth. The upper limit of the *Cymodocea nodosa* meadows ranges from 0.2 m depth in sheltered to 3 m depth in semi-exposed coasts. The lower limit ranges from 5 to 10 (15) m depths. Mixed meadows of *Cymodocea* with *Posidonia* or *Zostera noltii* may also exist occasionally under relative pristine or relative degraded shallow waters, respectively.

Taking under consideration the above presented information we argue that the intercalibration of CymoSkew index with *Posidonia* metrics should be realized using as a common metric pressures and not the common metric already used within the group. This latter common metric includes parameters such as the type of lower limit of the meadow and the leaf surface per shoot that can vary naturally along the Greek coasts.

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<sup>1</sup> Orfanidis S., Panayotidis P., Siakavara A. 2005. Benthic macrophytes: main trends in diversity and distribution. In *State of the Hellenic Environment*, Chapter VI. *Biota of the sea bed*, 226-235 pp. (Eds, Papathanassiou E., Zenetos A.), HCMR Publication.

<sup>2</sup> Orfanidis S., Papathanasiou V., Gounaris S., Theodosiou Th. 2009. Size distribution approaches for monitoring and conservation of coastal *Cymodocea* habitats. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 177–188.

## B. Integration of data from Italy, Croatia, Cyprus and Spain (Balearic Islands, Andalusia and Murcia regions), where the intercalibration is not needed, in the IC exercise - Results of comparison analysis

Analysis of the variance between the benchmarks of the different new datasets within each method were performed.

Table show the comparison of POMI-Spain (already successfully intercalibrated) with POMI applied to dataset from another MS (Croatia) and from all other regions in Spain. Additionally, it is also includes the comparison between PREI-France (already successfully intercalibrated) and PREI applied to dataset from Italy.

The analysis of variance (Table A.1 and Table A.2) shows that there are no significant differences between the benchmarks in the different MS using POMI, nor in the different MS using PREI. So, we consider that Spain, France, Italy and Croatia have been successfully intercalibrated. Cyprus presents only one benchmark and has not been included in this ANOVA, but because they are using the Italian benchmarks their intercalibration can also be considered to be successful.

*Table A.1 Analysis of the variance between the different benchmarks for each region within each method. For POMI we have included Catalonia-Spain, Balearic Islands-Spain, Andalusía-Spain, Murcia-Spain and Croatia. For PREI we have included France and Italy.*

ANOVA	Factors	df	F	P
POMI	Regions	4	1.815	0.196
	Error	11		
PREI	Regions	1	0.008	0.457
	Error	8		

*Table A.2 Average and standard deviations values for the different benchmarks at each region.*

	POMI Balearic	POMI Catalonia	POMI Murcia	POMI Andalusia	POMI Croatia
Average	0.85	0.79	0.65	0.76	0.81
SD	0.07	0.08	0.19	0.23	0.12

	PREI France	PREI Italy
Average	0.77	0.72
SD	0.02	0.11