Contents

Annex

Mediterranean Sea GIG: Coastal waters - Benthic invertebrate fauna

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1. Introduction

- Six Member States (France, Italy, Spain, Cyprus, Greece and Slovenia) compared and harmonised their national assessment systems. All compliance criteria are met by Italy and Slovenia. In the case of Spain (all regions), Greece Cyprus, and France, their national methods do not include the diversity parameter. They justified the no use of diversity in basis on the problems of discontinuity associated with diversity measures. However, Italy and Slovenia include the diversity parameter in their national methods. Therefore, there was not a MEDGIG consensus about this subject, and two Parallel Intercalibration (IC) were carried out: Parallel IC 1 (Spain, France, Cyprus and Greece) and parallel IC 2 (Italy and Slovenia).
- In the Parallel IC 1 the Intercalibran “Option 3” was used- direct comparison of assessment methods using a common dataset via application of all assessment methods to all data available. In the Parallel IC 2, a Revised_Option3_Two Member States was used.
- In the Parallel IC 1, the comparability analysis showed that Spanish (Valencia, Andalusia and Murcia regions), and French national methods needed boundary adjustments; In the parallel IC 2 no boundaries adjustment was needed.
- The final results include EQRs of France, Italy, Spain, Cyprus, Greece and Slovenia 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	BENTIX	Yes, paralell IC 1
France	AMBI	Yes, paralell IC 1
Greece	BENTIX	Yes, paralell IC 1
Italy	M-AMBI	Yes, paralell IC 2
Slovenia	M-AMBI	Yes, paralell IC 2
Spain (Catalonia and Balearic Islands)	MEDOCC	Yes, paralell IC 1
Spain (Andalusia, Murcia, Valencia)	BOPA	Yes, paralell IC 1
Malta	No method	No
Croatia	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	Taxonomic composition	Abundance [a]	Disturbance sensitive taxa	Diversity	Biomass	Taxa indicative of pollution	Combination rule of metrics
Cyprus BENTIX	Yes (justification below)	Not in strict sense (only composition of 2 preclassified sensitivity classes)	Not in strict sense (only relative abundance of 2 preclassified sensitivity classes)	2 sensitivity classes	No (due to unimodal or no relationship)	No	Specific opportunistic species	No combination
France AMBI	Yes (justification below)	Not in strict sense (only composition of 5 preclassified sensitivity classes)	Not in strict sense (only relative abundance of 5 preclassified sensitivity classes)	5 sensitivity classes	No (due to unimodal or no relationship)	No	Specific opportunistic species	No combination
Greece BENTIX	Yes (justification below)	Not in strict sense (only composition of 2 preclassified sensitivity classes)	Not in strict sense (only relative abundance of 2 preclassified sensitivity classes)	2 sensitivity classes	No (due to unimodal or no relationship)	No	Specific opportunistic species	No combination
Italy M-AMBI	Yes	Not in strict sense (only composition of 5 preclassified sensitivity classes)	Not in strict sense (only relative abundance of 5 preclassified sensitivity classes)	5 sensitivity classes	Shannon –Wiener's index, species richness, linear model	No	Specific opportunistic species	Multivariate analysis performed on AMBI Index, Shannon Diversity H'and Species Richness S together
Slovenia M-AMBI	Yes	Not in strict sense (only composition of 5 preclassified	Not in strict sense (only relative abundance of 5	5 sensitivity classes	Shannon –Wiener's index, species richness,	No	Specific opportunistic	Factorial analysis calculating

Member State	Full BQE method	Taxonomic composition	Abundance [a]	Disturbance sensitive taxa	Diversity	Biomass	Taxa indicative of pollution	Combination rule of metrics
		sensitivity classes)	preclassified sensitivity classes)		linear relationship		species	vectorial distances to reference conditions
Spain MEDOCC	Yes (justification below)	Not in strict sense (the composition of 4 preclassified classes including all the species)	Not in strict sense (relative abundance of 4 preclassified classes)	4 sensitivity classes	No (due to unimodal or no relationship)	No	Specific opportunistic species	No combination
Spain BOPA	Yes (justification below)	Not in strict sense (only composition of 2 preclassified sensitivity classes for polychaetes & amphipods)	Relative abundance of opportunistic polychaetes and amphipods only	2 sensitivity classes for polychaetes and amphipods only	No (due to unimodal or no relationship)	No	Specific opportunistic species	No combination
Malta	-	-	-	-	-	-	-	-
Croatia	-	-	-	-	-	-	-	-

France – AMBI method

AMBI method (Borja et al., 2000) is the index proposed by France during the second phase of intercalibration. In the first intercalibration phase, France proposed the MAMBI index (Borja et al., 2004), but this was proposed based on expert judgement. In the second phase, according to recommendations of the MED-GIG, the correlation between pressures and indices was explored considering quantitative values of pressures and AMBI and MAMBI. It was demonstrated that MAMBI index was not related with pressure (i.e. %Organic Matter) but AMBI index obtained good and significant relationships with the pressure parameter. On the basis of these results, France experts decided to use the AMBI index. The description of the method is the following:

For the development of the AMBI, the soft bottom macrofauna is divided into five groups according to their sensitivity to an increasing stress:

I. Species very sensitive to organic enrichment and present under unpolluted conditions.
II. Species indifferent to enrichment, always in low densities with non-significant variations with time.
III. Species tolerant to excess of organic matter enrichment. These species may occur under normal conditions, but their populations are stimulated by organic enrichment.
IV. Second-order opportunist species, mainly small sized polychaetes
V. First-order opportunist species, essentially deposit-feeders.

The formula is the following:

$$AMBI = \frac{\{(0 \times \%GI) + (1{,}5 \times \%GII) + (3 \times \%GIII) + (4{,}5 \times \%GIV) + (6 \times \%GV)\}}{100}$$

The AMBI method is calculated using the AMBI software (http://www.azti.es).

The reference value (AMBI ≤ 1.2) is derived as dominance of sensitive and indifferent taxa in the abundance. The bad status value (AMBI > 5.5) is achieved when second order and first order opportunists dominate. The boundaries between the ecological classes are those identified by Borja *et al.,* (2000, 2003) and Muxica *et al.*, (2005). EQR is determined by subtraction of the boundary value divided by the maximal value 7 from 1.

Classification	AMBI index	EQR value
High	1.2 < AMBI < 0	> 0.83 - 1
Good	3.2 < AMBI < 3.2	> 0.53 - 0.75
Moderate	5 < AMBI < 3.2	> 0.39 - 0.53
Poor	5 < AMBI < 6	> 0.21 - 0.39
Bad	> 6	< 0.21

Greece and Cyprus – Bentix method

As in the first intercalibration phase, Greece has proposed the Bentix index (Simboura & Zenetos, 2002) for establishing the ecological status, having into account the benthic macroinvertebrates element. This index is based on the relative percentage of 'sensitive' (GS) and 'tolerant' (GT) species in the fauna weighted analogously to derive a single formula:

$$Bentix = \frac{(6 \times \%GS + 2 \times \%GT)}{100}$$

GS includes the sensitive taxa, and GT the tolerant taxa.

BENTIX index can be applied using the Add-In (1.1. version) software package for MS Excel 2007 (http://bentix.ath.hcmr.gr/). Only a reference has been used to all data.

The resulting classification scheme and Ecological Quality Ratio (EQR) is the following:

Classification	Bentix index	EQR value
High	4.5 < Bentix < 6	> 0.75 - 1
Good	3.5 < Bentix < 4.5	> 0.58 - 0.75
Moderate	2.5 < Bentix < 3.5	> 0.42 - 0.58
Poor	2 < Bentix < 2.5	> 0.35 - 0.42
Bad	0	< 0.35

Italy – M-AMBI

The Multimetric AMBI method (M-AMBI) (Muxica et al., 2007) is the method selected by Italy. M-AMBI is a multimetric approach including the number of species (S), the Shannon diversity index (H’), and the AMBI index. Its procedure is based on a factor analysis including two virtual samples representing high and bad ecological quality status. M-AMBI method is calculated using the AMBI software (http://www.azti.es).

The M-AMBI is obtained by calculating the Euclidean distance between the projection of each station to the line connecting both high and bad reference stations (see Bald et al., 2005 for further details).

Reference conditions were defined for each metrics (AMBI, R, H’) that corresponds to the lowest values of the pressure indicators: Load_N (ton/Km2); Sum PAH (mg/kg); Pesticides (kg commercialized in the Province); TOC(%); TRIX; Stability (cycles/h); Corine land cover Urban land %; Corine land cover Agricultural land %.

On the basis of the reference values (S = 50; AMBI = 0,5; H’ = 4,8), the values of M-AMBI boundaries were calculated:

Classification	EQR value
High	> 0.96 - 1.17
Good	> 0.72 - 0.9
Moderate	> 0.49 - 0.72
Poor	> 0.24 - 0.49
Bad	< 0.2

Slovenia

As Italy, Slovenia has adopted the M-AMBI method for the establishment of the ecological status in its coastal water bodies. However, different reference conditions, and therefore different boundary values, were defined. In the Slovenian case, the reference conditions were determined according expert judgement since in the Slovenian sea there aren’t any proper reference sites. Four sampling sites from the area with minimal known human impact (SD4VT9, SD2VT4, SD_VT2_P1 and SD_VT2_P2) were taken into consideration when setting the reference conditions. Median value of the four above mentioned stations was calculated and adding 15 % on it has set the reference conditions (S = 91; AMBI = 1,34; H' = 5,87).

The H-G boundary value was calculated based on reference conditions, other boundaries were set equidistantly. The boundary values are the following:

Classification	EQR value
High	>0.83-1.00
Good	>0.62-0.83
Moderate	>0.41-0.62
Poor	>0.20-0.41
Bad	<0.20

Spain (Catalonia and Balearic islands) – MEDOCC method

The index MEDOCC has been developed and applied to the soft-bottom communities in the Catalan Coast and Balearic Islands in the Northwestern Mediterranean (Pinedo and Jordana, 2007). It classifies the species found in the community in four ecological groups: sensible, indifferent, tolerant, and opportunistic. The calculation of the index is based on the proportion of these four groups.

$$MEDOCC = \frac{\{(0 \times \%EGI) + (2 \times \%EGII) + (4 \times \%EGIII) + (6 \times \%EGIV)}{100}$$

where EGI, EGII, EGIII, and EGIV are sensitive, indifferent, tolerant, and opportunistic species, respectively. MEDOCC values can vary between 0 (only sensitive species are present) and 6 (opportunistic species are the 100% of the total abundance) (Pinedo and Jordana, 2007). Only one reference has been used to all data.

Classification	MEDOCC index	EQR value
High	0 < MEDOCC < 1.6	> 0.73 - 1
Good	1.6 < MEDOCC < 3.2	> 0.47 - 0.73
Moderate	3.2 < MEDOCC < 4.77	> 0.20 - 0.47
Poor	4.77 < MEDOCC < 5.5	> 0.080 - 0.20
Bad	5.5 < MEDOCC < 6	< 0.080

Spain (Murcia, Valencia and Andalusia regions)

BOPA method has been proposed by Spain (Valencia, Murcia and Andalusia regions) during the second phase of intercalibration. The description of the methods is the following:

BOPA index (Dauvin and Ruellet, 2007) was created after the study carried out by Gómez-Gesteira and Dauvin (2000) about the effectiveness of the opportunistic polychaete/amphipod ratio for identifying oil spill events. Dauvin and Ruellet, (2007) modified the ratio in the BOPA index to allow estuarine and coastal communities to be divided into the five classes suggested by the WFD. This index considers that opportunistic polychaetes are tolerant, indifferent or favoured by disturbances, and amphipods (except the genus Jassa) as a particular zoological group sensitive to significant increases in pressures.

The values of the BOPA are calculated from the benthic data series, using the following algorithm:

$$BOPA = log\left(\left(\frac{fp}{fa+1}\right)+1\right)$$

where *fp* is opportunistic polychaete frequency, and *fa* is amphipod (excluding G. *Jassa*) frequency. BOPA index varies between 0 (when *fp* = 0) and 0.30103 (when *fa* = 0).

Classification	BOPA index	EQR value
High	0 < BOPA < 0.045	> 0.85 - 1
Good	0.045 < BOPA < 0.139	> 0.54 - 0.85
Moderate	0.139 < BOPA < 0.193	> 0.36 - 0.54
Poor	0.193 < BOPA < 0.267	> 0.11 - 0.36
Bad	0.267 < BOPA < 0.301	< 0.11

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	Greece and Cyprus: Benthic grab (van Veen grab, Ponar grab or Box corer). Sampling surface does not affect reliability of the index to a high extent. Italy: van Veen Grab Slovenia: van Veen grab (0.1m2) Spain (Catalonia and Balearic Islands): van Veen Grab Spain (Valencia, Murcia and Andalucia regions: van Veen Grab France: Information not provided
How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area?	Greece and Cyprus: Once a year is sufficient - preferably warm season; two replicates per sampling. Italy: six-monthly (Spring/Fall) Slovenia: Two samplings per year (May/June and September); three replicates per site per sampling Spain (Catalonia and Balearic Islands): once a year is enough; two replicates per site per sampling Spain (Valencia, Murcia and Andalucia regions: once a year is enough France: Information not provided
Sampling/survey months	Greece and Cyprus: Spring-Summer Spain (Catalonia and Balearic Islands): Spring-Summer Italy: six-monthly (Spring/Fall) Slovenia: End of May/beginning of June and end of August/beginning of September Spain (Valencia, Murcia and Andalucia regions: Spring-Summer France: Information not provided
Which method is used to select the sampling /survey site or area?	Greece and Cyprus: Expert knowledge, sites most representative of water body) based on the knowledge of diffuse or point sources of pollution Italy: Expert judgment Slovenia: Expert knowledge Spain (Catalonia and Balearic Islands): expert judgment; sites representative

Information provided in the online WISER project assessment method questionnaires	
	of the water body Spain (Valencia, Murcia and Andalucia regions: expert knowledge; sites representative of the water body France: Information not provided
How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area?	Greece and Cyprus: two replicates per site are sufficient, number of sites depending on area. Italy: 3 replicates/sampling Slovenia: 3 replicates/sampling Spain (Catalonia and Balearic Islands): two per site are sufficient. Sites per area depends on the area Spain (Valencia, Murcia and Andalucia regions: two per site are sufficient. Sites per area depends on the area France: Information not provided
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	Greece and Cyprus: two spatial replicates (0.1 m^2) per station Italy: Average of three spatial replicates (0,1m^2). Slovenia: 0.3 m2/sampling (season) and 0.6m^2/year Spain (Catalonia and Balearic Islands): two spatial replicates (600 cm^2) per station Spain (Valencia, Murcia and Andalucia regions: Valencia: two especial replicates (625cm^2) per station); Murcia: 3 replicates (400 cm^2) per station; Andalusia: 3 replicates (500 cm^2 and 300 cm^2). France: Information not provided
Short description of field sampling/survey procedure and processing (sub-sampling)	Greece and Cyprus: Two replicate samples are collected on soft bottom in the infralittoral/sublittoral zone at each station using a Van Veen grab for the analysis of zoobenthos. Samples for fauna analysis are sieved on board through a 1 mm sieve and stored in 4 % formalin solution, stained with Rose Bengal. Samples are sorted in the lab and are grouped into the main benthic groups. Subsequently most of the specimens are identified to the species level and only when this was not possible (broken material) to a higher taxonomic level (genus or family). Organisms of the complete sample are identified. Italy: Two stations, along a transept off-coast. The first one on sandy sediment (% of sand equal or more than 75%). The second one on silty sediment (% of sand equal or less than 25%) Slovenia: Samples taken with van Veen grab on soft bottom in the depth of 7-9m. Replicates taken randomly and treated separately. Organisms in the complete sample are identified to the lowest possible level. Spain (Catalonia and Balearic Islands): Two replicate samples are collected at each station using a van Veen grab at 10-15 meters depth. Fine to muddy sediments are selected. Sediment and organisms kept in the 500 µm net are conserved in a bag with formaldehyde and Rosa Bengal (help to the separation of organisms in the lab.) Specimens are identified to the species level (only in few cases genus or family level is assigned). Subsamples are obtained for granulometric analysis, organic matter content, and heavy metals concentration Spain (Valencia, Murcia and Andalucia regions: replicate samples are collected at each station using a van Veen grab at 10-30 meters depth. Fine to muddy sediments are selected. Samples for fauna analysis are sieved a 0,5 mm sieve and stored in 4 % formalin solution, stained with Rose Bengal. France: Information not provided

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
Cyprus BENTIX	Existing near-natural reference sites	IC phase I: use of reference sites of Greece (BSP) plus 2 stations from Cyprus corresponding to ref. conditions (Posidonia oceanica meadows). In addition one extra national reference site has been set in a pristine area of Cyprus	Same as Greece, and South-Western Cyprus - Cape Greco (Natura 2000 site)	Absence of anthropogenic activities - Reference sites are set in pristine-undisturbed areas (Natura 2000) & the Greek criteria (as above)
France AMBI	Existing near-natural reference sites , Expert judgment	3 sites	Corsica, Provence-Alpes-Côte d'Azur (East of Rhône River. Languedoc Roussillon (West of Rhône River)	The lowest anthropogenic influences Biological criteria: Selecting the best situation (sample) where most species belong to EGI (sensitive species) and EGII (indifferent species). Expert judgment
Greece BENTIX	Existing near-natural reference sites Mixed soft sediments: 1985-1997 Muddy bottoms: 1992-1997	1st phase report: Mixed soft sediments: 20 stations in the Aegean & Ionian Seas Muddy bottoms: 5 stations in the Aegean sea Milestone 3: Around 10 sites corresponding to pristine conditions were used to validate the numerical value of the Bentix method under high status	Aegean and Ionian seas: Cyclades islands, Ionian coasts (western Greece)	Biological criteria: The sites are from undisturbed areas, the fauna is composed of mostly sensitive species (over 75%) = Bentix $\geq$ 5 for mixed soft sediments. The benthic fauna is usually very diverse and evenly distributed with no one species naturally dominating over 10%. The maximum value of the Bentix corresponds with the theoretical situation where the fauna is composed of only sensitive and indifferent species. Muddy bottoms: Bentix > 4, Sensitive species over 50%

Member State	Type and period of reference conditions	Number of reference sites	Location of reference sites	Reference criteria used for selection of reference sites
Italy M-AMBI	Identification of RC through data analysis and expert knowledge– 2008-2009 data on sandy soft-bottom sediments of Central Adriatic and Central Tyrrhenian (western Mediterranean)	No real reference sites.	No real reference sites.	The lowest anthropogenic influences. Definition of the multiple linear model of pressures/metrics relationship. Definition of reference values for each metrics (AMBI, R, H') that corresponds to the lowest values of the pressure indicators; Load_N (ton/Km2); Sum PAH (mg/kg); Pesticides (kg commercialized in the Province); TOC(%); TRIX; Stability (cycles/h); Corine land cover Urban land %; Corine land cover Agricultural land %. Reference condition values: AMBI= 0.5 R= 50 H'= 4.8
Slovenia M-AMBI	Expert knowledge and least disturbed conditions from 4 sites in year 2005	4 least impacted sites (similar level of pressure) used to derive theoretical reference	2 water bodies: Uvala svetega Jerneja, SI5-WB2, station 1, 2 and 4 Si5-WB4, station 9	The lowest anthropogenic influences Biological criteria: EG I and II represent over 60% of abundance, EG V almost absent: high taxa richness and diversity.
Spain MEDOCC	Expert knowledge, Least Disturbed Conditions (2002-03 in Catalonia and 2005 in Balearic Islands). Modelling (extrapolating model results)	All data set for each region (Catalonia and Balearic Islands) was used to find the best situation. This value was taken into account to create the virtual situation with only sensible, indifferent and tolerant species	Catalan and Balearic Islands coast	Biological criteria: Selecting the best situation (sample) where most species belong to EGI (sensitive species) and EGII (indifferent species). From these samples, a new theoretical situation was created where the fauna is composed of only sensitive (EGI: 90%) and indifferent species (EGII: 10%), in Catalonia and sensitive (80%),

Member State	Type and period of reference conditions	Number of reference sites	Location of reference sites	Reference criteria used for selection of reference sites
				indifferent (15%), and tolerant (5%) in Balearic Islands. Catalonia; MEDOCC=0.2 Balearic Islands; MEDOCC= 0.5
Spain BOPA	Expert knowledge, Least Disturbed Conditions (2005-2008 in Valencia; 2002-2003, 2006- 2007, 2009 in Murcia, and 2009 Andalusia)	12 sites (28 samples) corresponding to low anthropogenic conditions were used to validate the numerical value of BOPA index under high status	Murcia, Valencia and Andalucia regions	The lowest anthropogenic influences Biological criteria: The fauna is only composed by sensitive species (amphipod group excepting Jassa genus) and there are no opportunistic polychaetes
Malta	-	-	-	-
Croatia	-	-	-	-

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	Specific approach for H/G boundary	Specific approach for G/M boundary	BSP: method tested against pressure
Cyprus BENTIX	Boundaries were defined using paired metrics (the two general Ecological Groups percentages) that respond in different ways to the influence of the pressure.	Same as Greece (BSP)	Same as Greece (BSP)	Yes, quantitative tested against a gradient of increasing distance from pressure points
France AMBI	Boundaries were defined using the discontinuities of the five Ecological Groups metrics that respond in different ways to the influence of the pressures.	EQR-AMBI=0.83 . Taken from Borja et al. 2000	EQR-AMBI=0.53 from Borja et al. 2000	Yes, Quantitatively
Greece BENTIX	Boundaries were defined using paired metrics (the two general Ecological Groups percentages) that respond in different ways to the influence of the pressure.	Biological criterion: Mixed soft sediments: Bentix = 4.5 (in the Good to High boundary sensitive species become over 60% and the tolerant species less than 40%). The middle or class centre of good class is the cross-line point of the two lines (sensitive – tolerant species) corresponding to the value of BENTIX=4 where the two ecological groups of tolerant and sensitive share the fauna by 50 % each. This point corresponds with the ecotone point of the transitional zone, middle of good class. Muddy bottoms: Bentix = 4	Biological criterion: Mixed soft sediments: Bentix = 3.5. At the G/M boundary, the percentage of tolerant species becomes over 60% (roughly 2/3 of the fauna) and the sensitive taxa less than 40% (1/3 of the fauna) Class centre good bentix = 4 (sensitive and tolerant species = 50%) = ecotone point of the transitional zone from sensitive to tolerant species. Muddy bottoms: Bentix = 3	Yes quantitative
Italy M-AMBI	Boundaries were defined by the equidistant division of the EQR gradient, expert judgement and statistical approaches.	Equidistant division of the EQR gradient H/G=0,81	Equidistant division of the EQR gradient G/M=0,61	The quantitative relationship among M-AMBI index and the pressure/state indicators was performed using

Member State	Type of boundary setting	Specific approach for H/G boundary	Specific approach for G/M boundary	BSP: method tested against pressure
				multiple linear regression analysis
Slovenia M-AMBI	H/G boundary derived from metric variability at near-natural reference sites. Equidistant division of the EQR gradient for the other classes M/P boundary on 0,41 and P/B boundary on 0,20	Natural variability, presumed to be around 20%, defines width of High class, so upper and lower limit of High class differ for 20%. H/G boundary (lower limit) was calculated by taking median from EQR values of the 4 stations used in calculating reference conditions and subtracting additional 5% from this value. Subtracting 5% was needed because median of the actual data lays 15% from upper limit, so to get the lower limit, which differs from upper for 20% this subtraction must be done. H/G boundary EQR = 0,83	Other boundaries were set equidistantly from the H/G boundary (0,83): G/M boundary on 0,62	Yes qualitative
Spain MEDOCC	Boundaries were defined using the discontinuities of the four Ecological Groups metrics that respond in different ways to the influence of the pressure.	MEDOCC = 1.6: sensitive ecological group (EGI) accounting for more than 40% of total abundances	MEDOCC = 3.2: tolerant ecological group (EGIII) accounts for 50%, but sensitive taxa (EGI) are also present (10%)	Yes Quantitative
Spain BOPA	Boundaries were defined using the discontinuities of the frequency of two taxonomic Groups (amphipod excepting *Jassa* genus and polychaete opportunistic species) that respond in different way to the influence of the pressure	BOPA= 0.046: dominance of sensitive ecological group (amphipod group excepting Jassa genus) and low frequency of polychaete species	BOPA= 0.13; low frequency of amphipod species	Yes Quantitative
Malta	-	-	-	-
Croatia	-	-	-	-

* Expert judgment – statistical – ecological discontinuity – or mixed for different boundaries?

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).	**Spain, Slovenia, France, Italy, Greece, Cyprus:** Yes
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	**Spain, Slovenia, France, Italy, Greece, Cyprus:** 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 on Pressures addressed at national level
• 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	Yes, for all MS, as defined in the WFD. 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	**Spain, France, Greece and Cyprus:** No, diversity is not considered. These MS have demonstrated that their methods are sufficiently indicative of the status of the QE. **Italy, Slovenia:** Yes
• Complete list of biological metric(s) used in assessment	See section on required BQE parameters
• Data basis for metric calculation	**Greece and Cyprus:** Data from single sampling/survey occasion and aggregated data from multiple surveys in time (108 stations x 2 replicates). **Italy:** 19 (222 samples) and 24 western Mediterranean sites **Slovenia:** Aggregated data from multiple spatial replicates **Spain (Catalonia and Balearic Islands):** Data

Compliance criteria	Compliance checking conclusions
	from single sampling/survey occasion and aggregated from multiple spatial replicates (105 samples x 2 replicates) **Spain (Valencia, Murcia and Andalusia regions):** 284 samples Data from single sampling/survey occasion and aggregated data from multiple surveys in time and from multiple spatial replicates
• Combination rule for multimetrics	**Greece and Cyprus**: No **Italy:** Multivariate analysis **Slovenia:** Multivariate analysis **Spain (Catalonia and Balearic Islands):** No **Spain (Valencia, Murcia and Andalusia regions):** No **France:** No
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	Yes
• Is the assessment method applied to water bodies in the whole country?	MEDOCC: No, only in Catalonia and Balearic islands BOPA: No, only in Murcia, Valencia and Andalusia regions. AMBI-France: Yes MAMBI-Italy: Yes MAMBI-Slovenia: Yes BENTIX-Greece: Yes BENTIX-Cyprus: Yes
• Specify common intercalibration types	**Greece, Cyprus, France, Italy, Slovenia, and Spain (all regions):** Typologies are not relevant in the MedGIG ecosystem as it was concluded in MedGIG CW benthic invertebrate working group. Assessment was applied to common intercalibration types**:** CW-M2, CW-M3 as initially defined but is also applying to other types as EQR boundaries are not dependent on these types in the Mediterranean ecoregion 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**	**Spain, Slovenia, France, Italy, Greece, Cyprus:** Habitat-specific
• Scope of reference conditions	See section on national reference conditions
• Key source(s) to derive reference conditions	**Greece and Cyprus:** Existing near-natural reference sites **Italy:** Expert knowledge, Historical data. **Slovenia:** Existing near-natural reference sites and expert knowledge

Compliance criteria	Compliance checking conclusions
	Spain (Catalonia and Balearic Islands): Expert knowledge, and Least Disturbed Conditions **Spain (Valencia, Murcia and Andalucia regions:** Existing near-natural reference sites and expert knowledge **France:** Existing near-natural reference sites and expert knowledge
• Number of sites, location and geographical coverage of sites used to derive reference conditions	See section on national reference conditions
• Time period (months+years) of data of sites used to derive reference conditions	See section on national reference conditions
• Reference site characterisation: criteria to select them	See section on national reference conditions
• Is a true reference used for the definition of High status or an alternative benchmark estimation?	**Greece and Cyprus:** High status or reference conditions are derived from true reference undisturbed areas **Italy:** Virtual reference condition **Slovenia: A**n alternative benchmark estimation **Spain (Catalonia and Balearic Islands):** Virtual reference condition, using a community composed of mostly sensitive species (90%) **Spain (Valencia, Murcia and Andalucia regions):** Virtual reference condition **France:** Virtual reference condition
6. Assessment results are expressed as **EQRs:** • Are the assessment results expressed as Ecological Quality Ratios (EQR)?	**Spain, Slovenia, France, Italy, Greece, Cyprus:** Yes
7. Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	**Spain, Slovenia, France, Italy, Greece, Cyprus:** Yes
• Has the uncertainty of the method been quantified and is it regarded in the assessment?	**Greece, Cyprus, Slovenia, Italy, France, Spain (all regions):** Not statistically but on the basis of the methods' limitations.
• Specify how the uncertainty has been quantified and regarded	**Greece, Cyprus, Slovenia, Italy, France, Spain (all regions):** The software (in the case of BENTIX, MAMBI and AMBI) and/or the explanations (in published works) for the calculation of the methods set the limits of parameters under which the results are not within the confidence limits. These parameters are based on the lowest number of scores species and the lowest number of species in a matrix that is needed to calculate the methods.
8. All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure	**Spain, Slovenia, France, Italy, Greece, Cyprus:** Yes

Compliance criteria	Compliance checking conclusions
9. Selected taxonomic level achieves adequate confidence and precision in classification	**Spain, Slovenia, France, Italy, Greece, Cyprus:** Yes
• Minimum size of organisms sampled and processed	**Spain (Catalonia and Balearic islands):** Organisms retained in a 0.5 mm sized mesh. **Spain (Murcia, Andalusia regions):** Organisms retained in a 0.5 mm sized mesh. **Spain (Valencia):** Organisms retained in a 0.63 mm sized mesh. **Slovenia:** Organisms retained in a 1 mm sized mesh. **Italy:** Organisms retained in a 1 mm sized mesh. **Greece:** Organisms retained in a 1 mm sized mesh. **Cyprus:** Organisms retained in a 0.5 mm sized mesh. **France:** information not provided
• Record of biological data: level of taxonomical identification – what groups to which level	**Spain, Slovenia, France, Italy, Greece, Cyprus:** All groups identified to species level (or lowest reliable taxonomic level)

General conclusion of the compliance checking:

All compliance criteria are met by Italy and Slovenia. In the case of Spain (all regions), Greece Cyprus, and France, their national methods do not include the diversity parameter.

Spain, Greece, Cyprus and France justified the no use of diversity, in basis on that most of the biotic indices designed for the marine and estuarine invertebrate benthic communities are based on the Pearson-Rosenberg model of succession in relation to organic enrichment and pollution (Quintino *et al.*, 2006). According to this model, diversity does not show a monotonic trend along both spatial and temporal gradients of pollution. When moving away from the source of pollution, the peak of opportunists is often followed by a maximum value in diversity, which then stabilizes at a slightly lower level. This means that, in a gradient of pollution, the highest values for the diversity index may be recorded when the number of species is still low and the community is still in an early stage of recovery (Pearson & Rosenberg, 1978). These observations were extended to gradients of chemical contaminants (Thompson & Lowe, 2004).

Given the problems of discontinuity associated with diversity measures above mentioned, Spain, Greece, France and Cyprus consider that the diversity is not a good parameter to establish ecological status as stated in the WFD. However, Italy and Slovenia have included the diversity parameter in their national methods. Therefore, there is not a MEDGIG consensus about this subject.

Justification argued by Greece, Cyrus, Spain and France on the no use of diversity in the establishment of ecological status in coastal waters:

The following results show the weak response of the diversity descriptor in gradients of increasing anthropogenic disturbance in the coastal waters from Greece, Cyprus, Spain and France. The anthropogenic disturbance was measured through the organic matter content in the sediment and an integrative index (LUSI index) of the existing pressures in a water body. The LUSI index was constructed by Flo *et al.* (2011) and modified by Romero (2011), and it was successfully applied in the MED GIG phytoplankton group in order to ascertain the relationships between anthropogenic pressures and biological indicators.

Figure 2.1 shows the application of the pressure Index LUSI (Flo *et al.*, 2011) in the Greek water bodies.

Results show the variation of the indices along a gradient of water bodies ordered by increasing LUSI index. As it was expected, the trend line shows a decline of the EQR BENTIX values with increased values of LUSI index. However, the diversity measured by Shannon Wiener index is not able to distinguish the gradient of pressures affecting the water bodies. Linear regression plots (Figure 2.2 and Figure 2.3) show the good and significant relationships between BENTIX and LUSI index, and the absence of a significant relationship between the diversity measure and the pressure index.

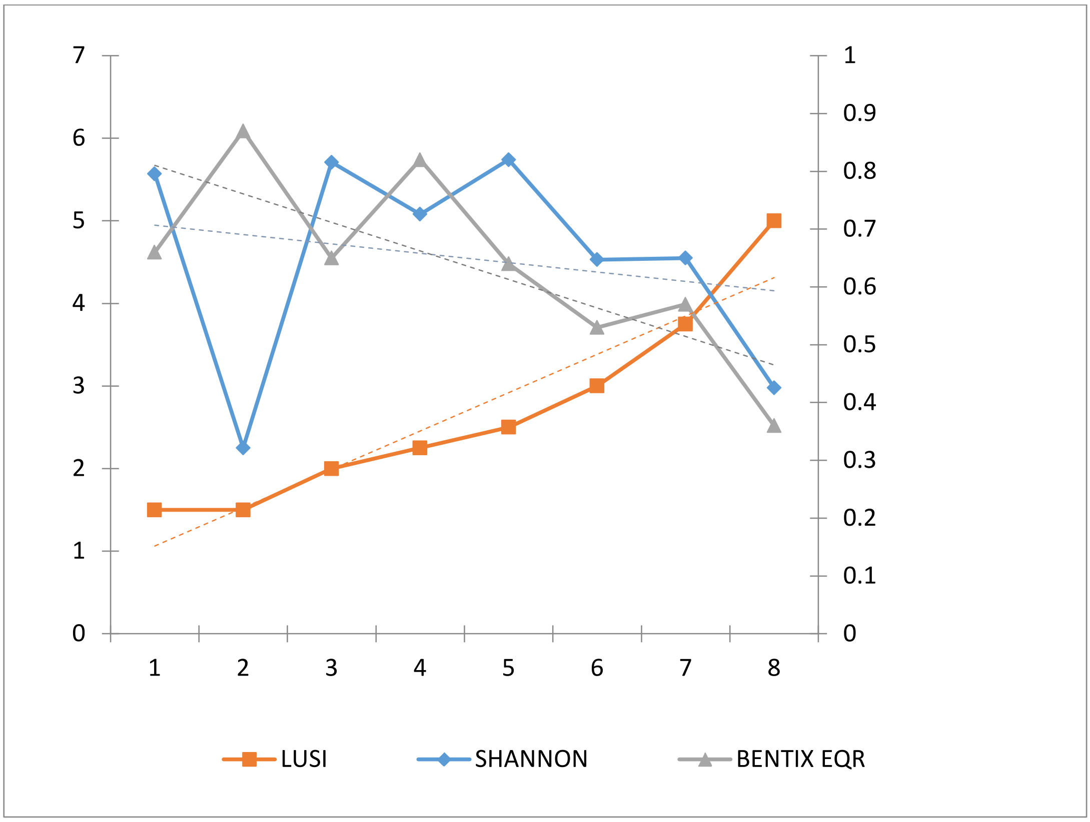

Figure 2.1 Variation of Shannon-Wiener and BENTIX indices along a pressures gradient

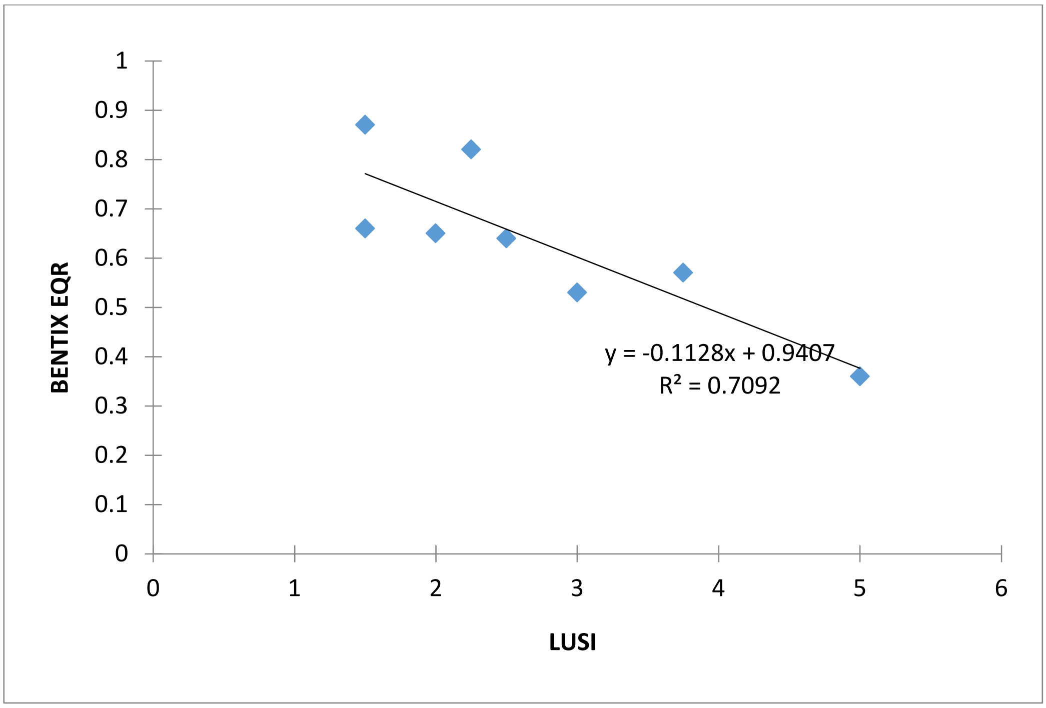

Figure 2.2 Relationship between BENTIX and pressure index

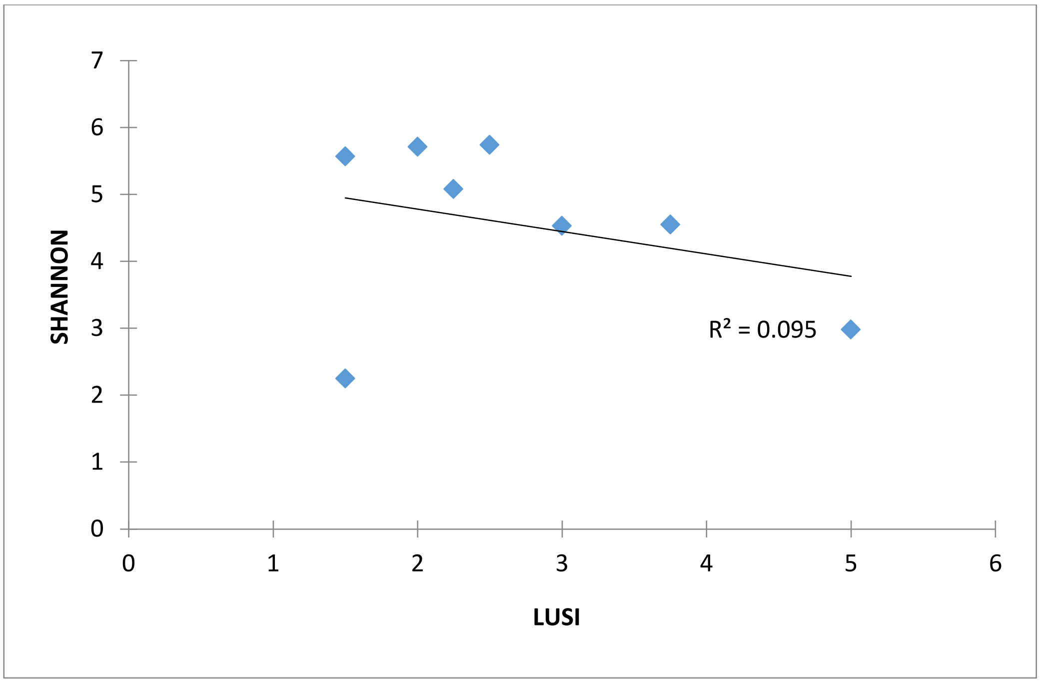

Figure 2.3 Relationship between Shannon-Wiener index and pressure index

Cyprus

The pressure Index LUSI was calculated for the IC coastal water bodies-stations of Cyprus according to the methodology described by Flo *et al.* (2011). Calculation was based on the "CLC2006 classes for Cyprus". River pressures are not applicable in the case of Cyprus due to the absence of any significant rivers with permanent flow in the island. Figure 2.4 shows the high and significant relationship between the BENTIX index and the pressures index. The absence of a relationship between the Shannon-wiener and the LUSI indices is highlighted in Figure 2.5. The Figure 2.6 shows the good response of the BENTIX EQR to the LUSI index, and the bad performance of the Shannon index in the establishment of different pressure levels.

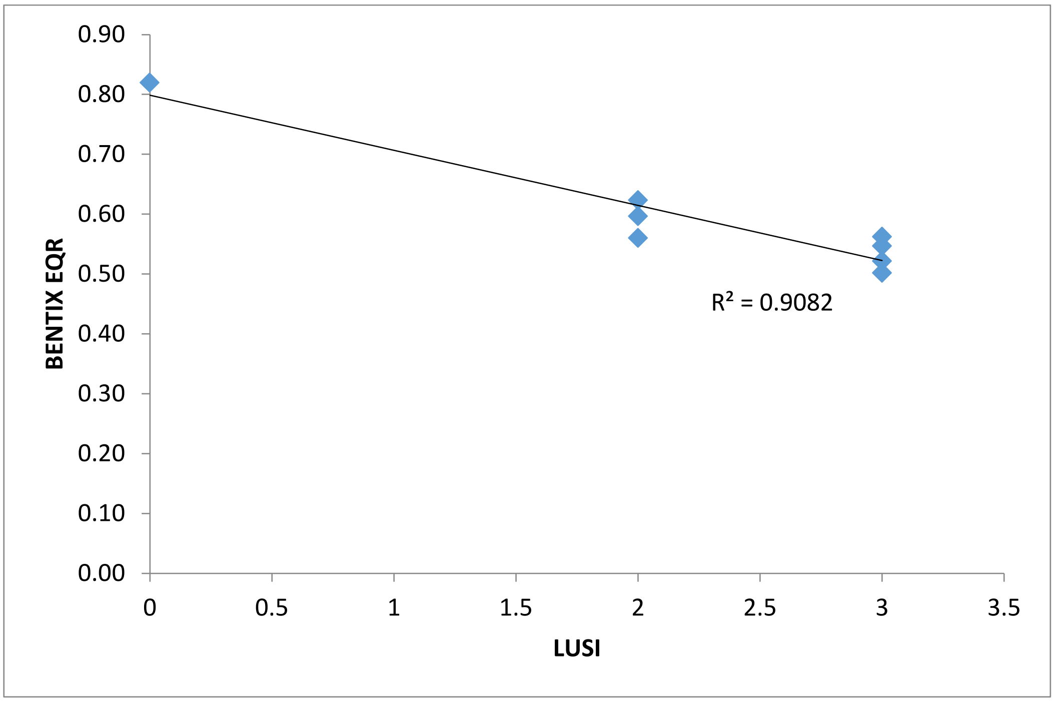

Figure 2.4 Relationship between BENTIX and pressure index

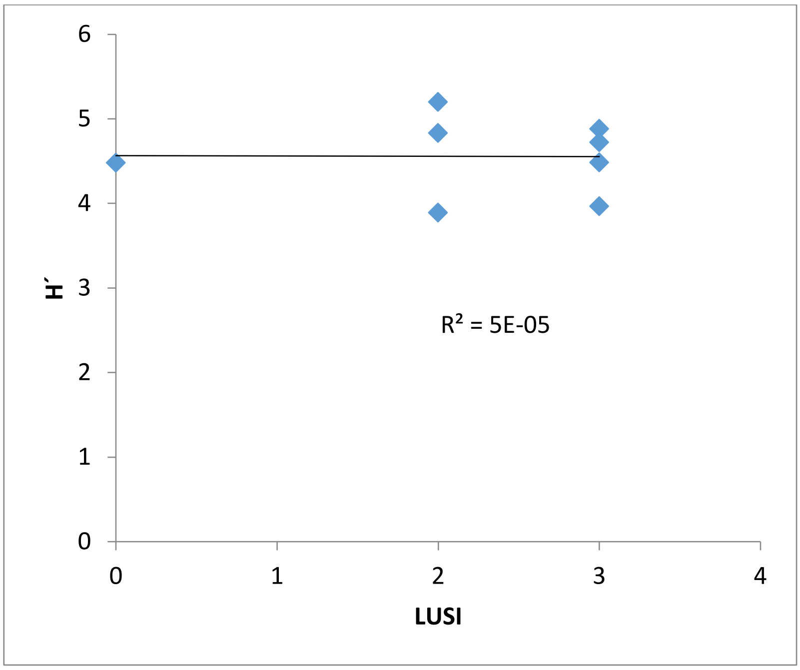

Figure 2.5 Relationship between Shannon-Wiener index and pressure index

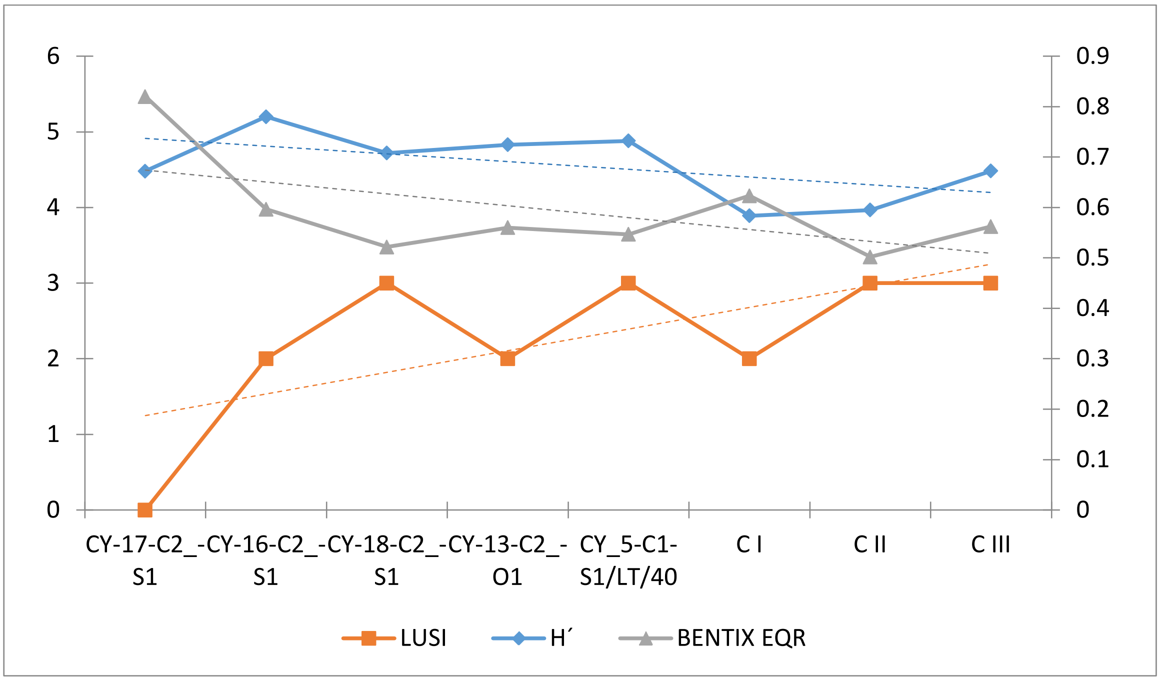

Figure 2.6 Variation of Shannon-Wiener and BENTIX indices along a pressures gradient

Spain (Valencia, Murcia and Andalusia regions)

Figure 2.7 and Figure 2.8 show the good response of the BOPA index to an increasing gradient of the pressures. In the Figure 2.7, the water bodies from Valencia, Murcia and Andalusia are ordered by an increasing gradient of the pressures affecting them. The Figure 2.9 shows the absence of response of the diversity to different gradients of anthropogenic pressures. Figure 2.10 shows the good response of the BOPA index to the organic matter % along a gradient of stations ordered by increasing organic matter values. However, diversity measure is not able to detect differences among different levels of organic matter (Figure 2.11).

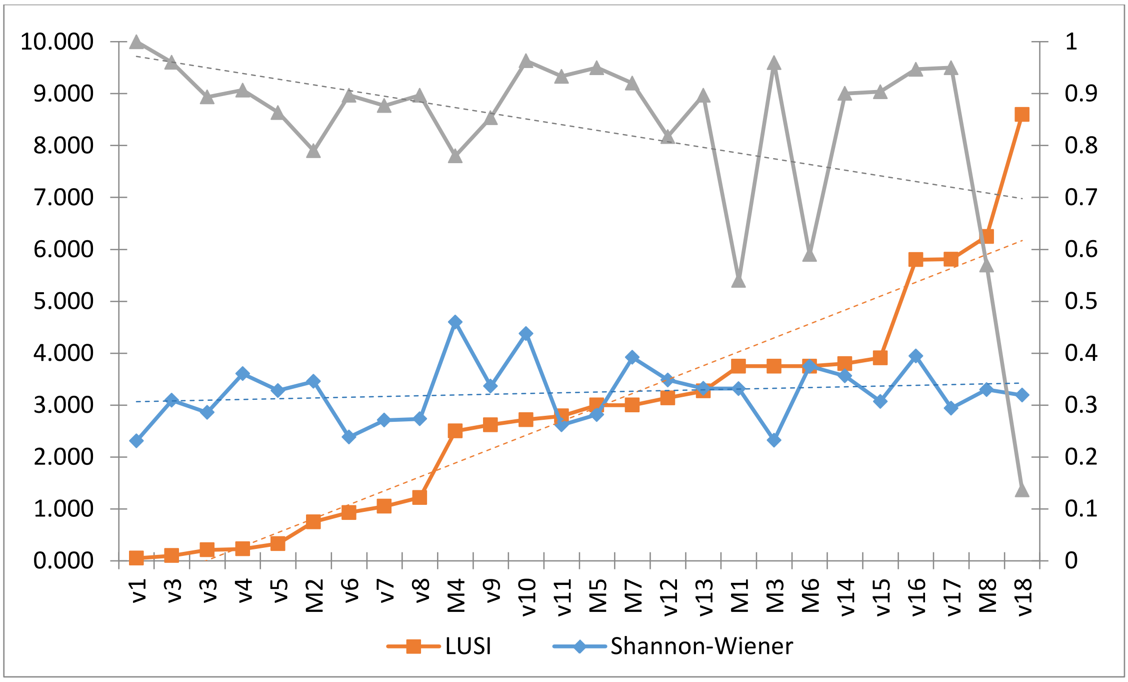

Figure 2.7 Variation of Shannon-Wiener and BOPA EQR values es along a pressures gradient

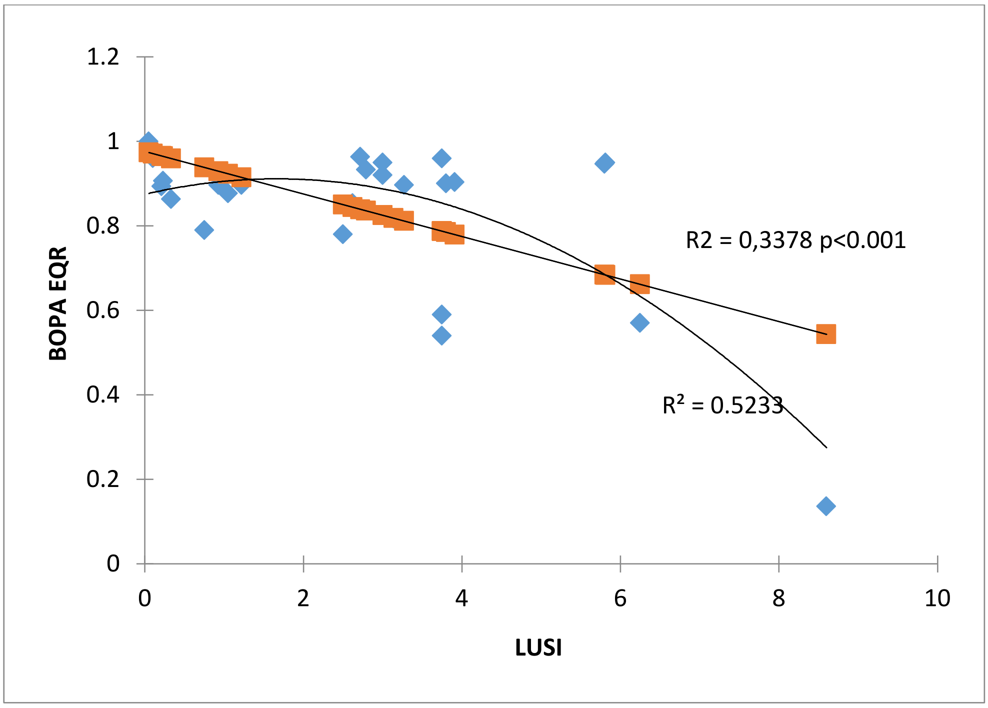

Figure 2.8 Relationship between BOPA EQR values and pressure index

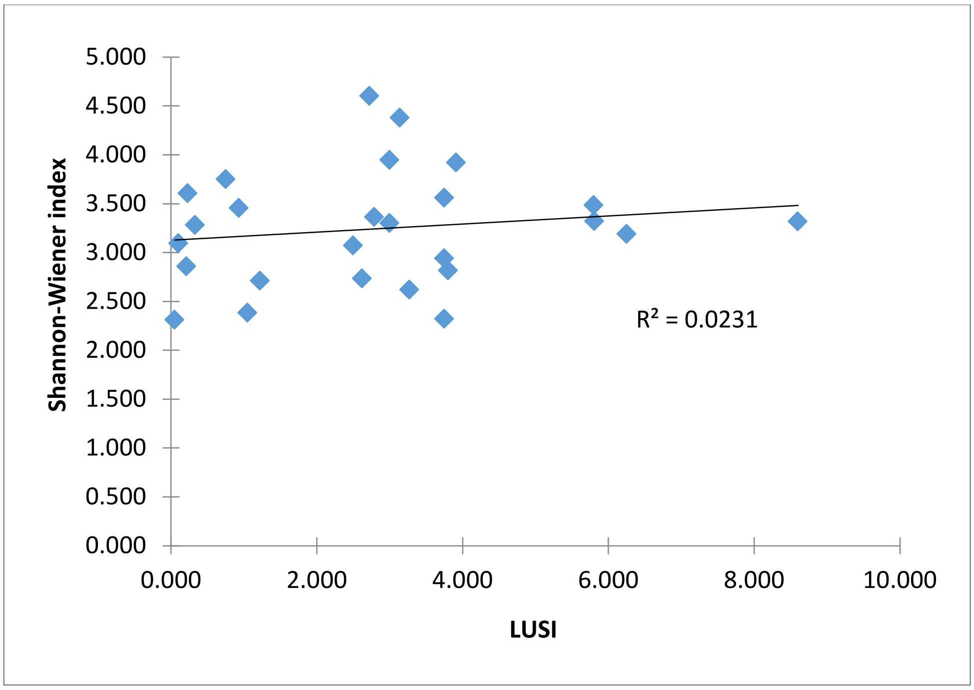

Figure 2.9 Relationship between diversity index and LUS.

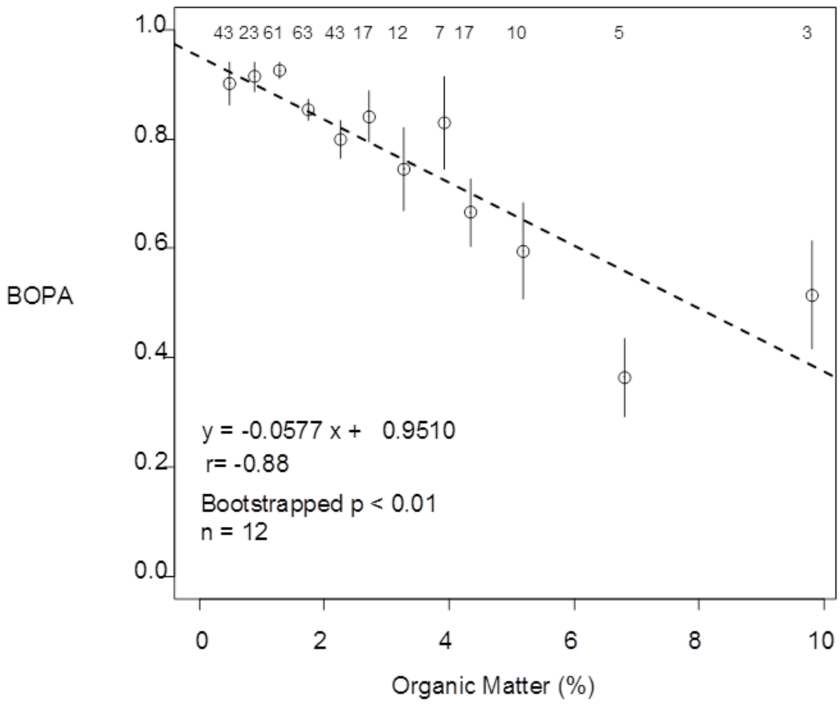

Figure 2.10 *Relationship between BOPA EQRs and organic matter content, averaged by classes of 0.5% OM content. Vertical bars correspond to the EQR standard errors; numbers above points refer to the number of observations used to calculate each averaged value. In order to minimize the effect of the data scattering, organic content values were divided in classes of 0.5 % and the average values of BOPA EQRs, as well as their standard errors, were calculated for each class. A linear correlation analysis was again performed on this new ranged dataset. The significant trend observed in the plot of the original dataset is evident, as highlighted by the high value of r (-0.88)*

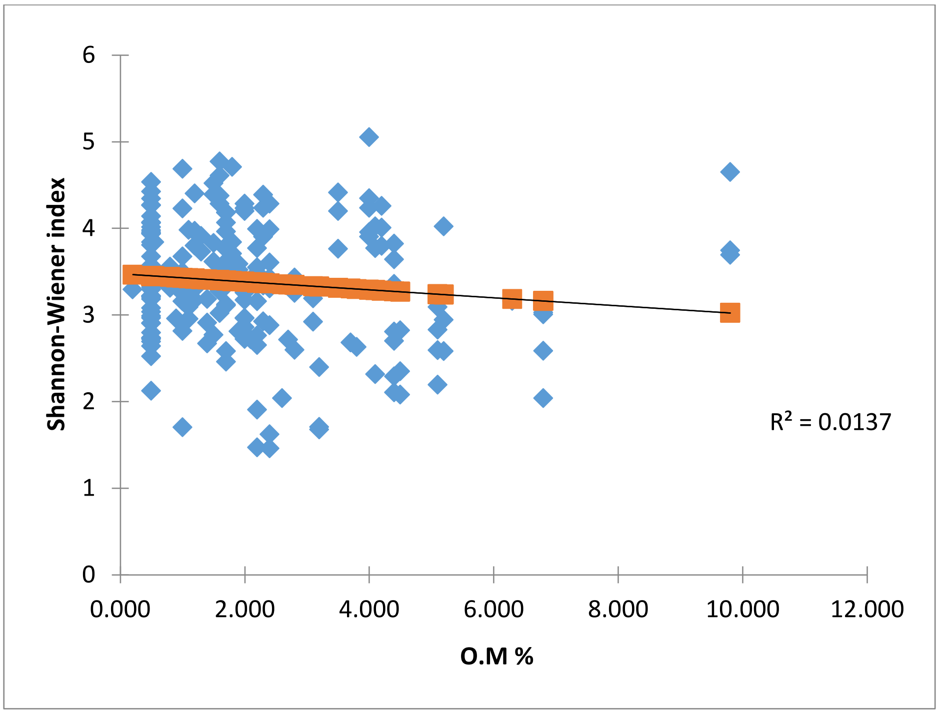

Figure 2.11 Relationship between diversity index and organic matter content

Spain (Catalonia and Balearic islands)

Figure 2.12 to Figure 2.17 show the variation of MEDOCC index and H' along a gradient of stations ordered by increasing pressure-indicator values: organic matter content in sediments (percentage) and LUSI index, and the results of the regression analyses. The results show that MEDOCC index performed better than Diversity index detecting different gradients of pressures and organic matter in the sediment. High values of Shannon-Wiener index were found at the end of the gradient of the organic matter and LUSI index.

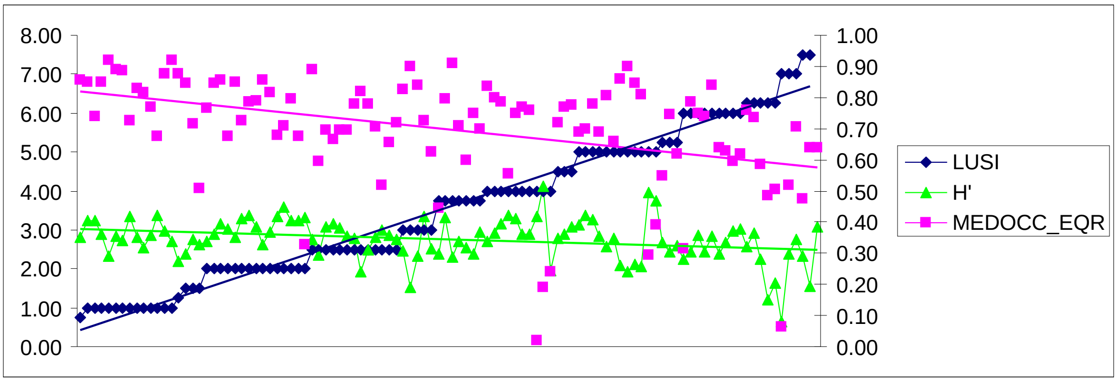

Figure 2.12 Variation of Shannon-Wiener and MEDOCC EQR values along a pressures gradient

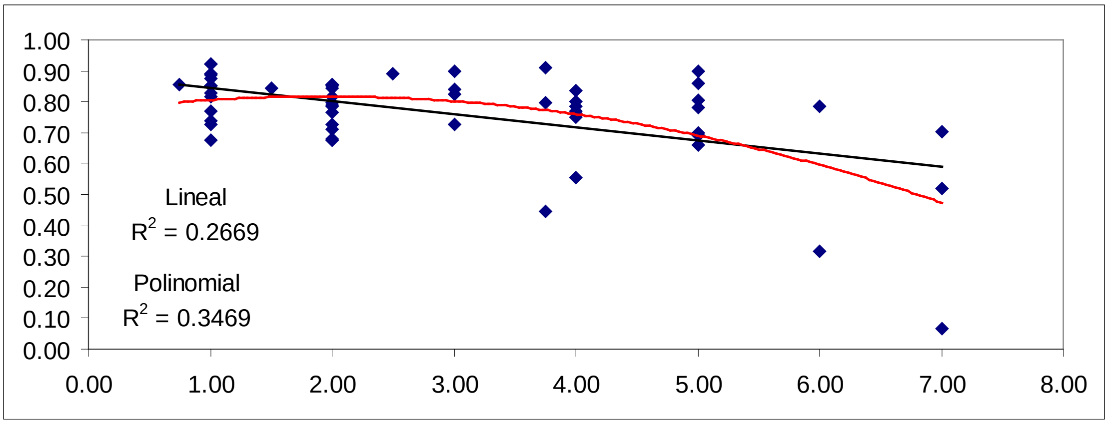

Figure 2.13 Relationship between MEDOCC EQR values and LUSI index

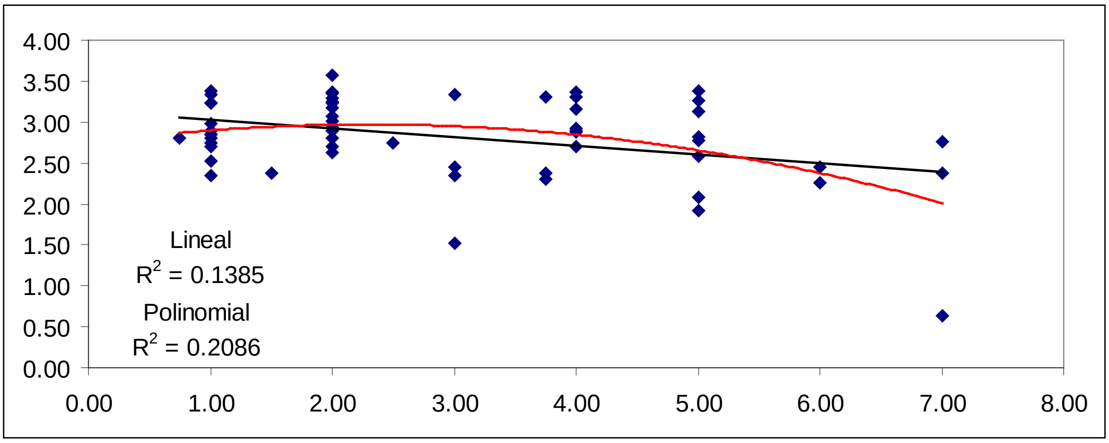

Figure 2.14 Relationship between Shannon-Wiener index and LUSI index

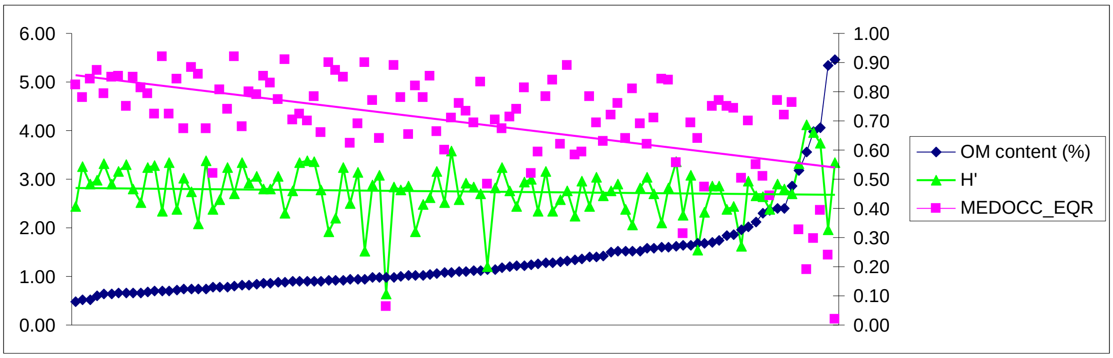

Figure 2.15 Variation of Shannon-Wiener and MEDOCC EQR values along a gradient of organic matter content

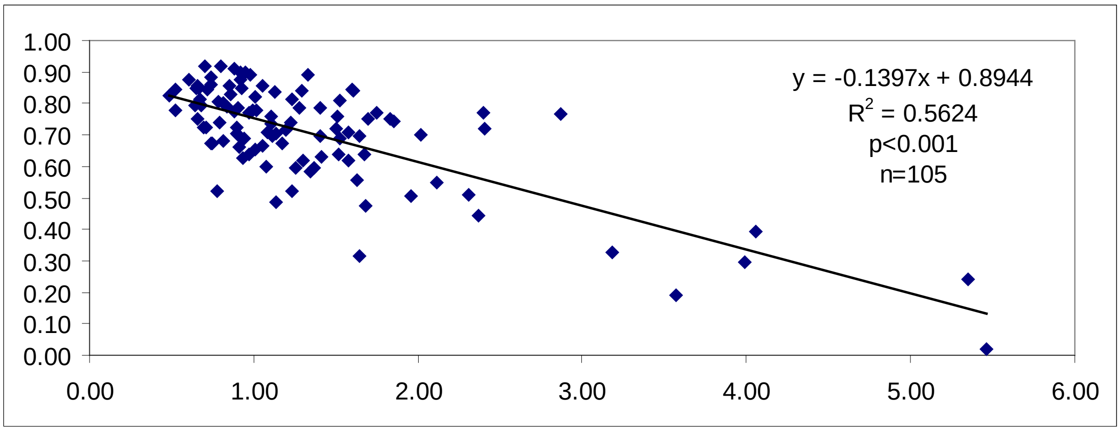

Figure 2.16 Relationship between MEDOCC EQR values and organic matter content

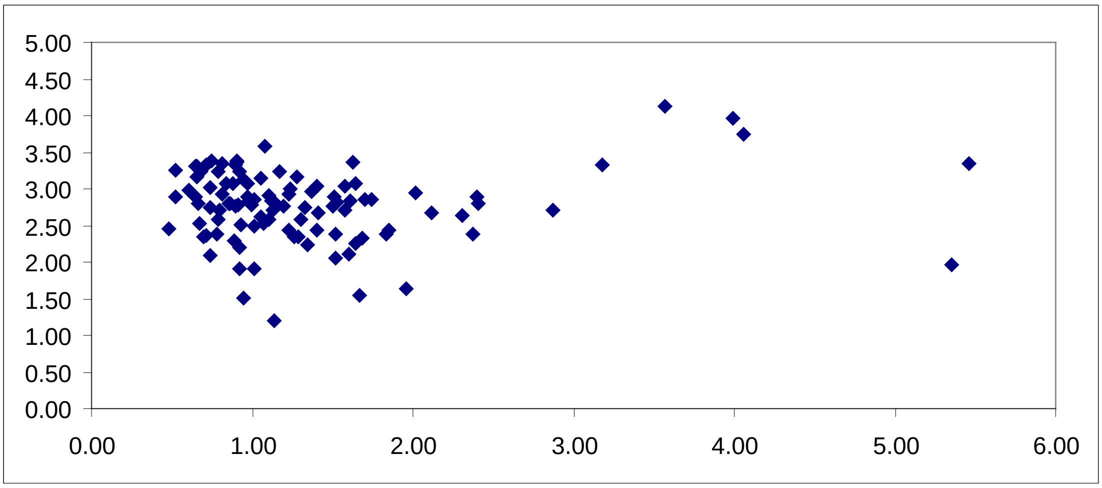

Figure 2.17 Relationship between MEDOCC EQR values and organic matter content

France

The values of the LUSI index in the French water bodies are not related neither with AMBI nor Shannon Wiener index. Due to this fact, in order to analyse the performance of the AMBI method and the diversity index related to anthropogenic disturbance, only the organic matter content (and not the LUSI index) was considered as an indicator of anthropogenic disturbance.

Figure 2.18 shows the stations ordered by an increased gradient of organic matter. Results indicate low values of AMBI EQR at the end of the gradient. However, high values of the Shannon are observed in stations with high levels of organic matter in the sediment. Regression analyses (Figure 2.19 and Figure 2.20) confirm these results.

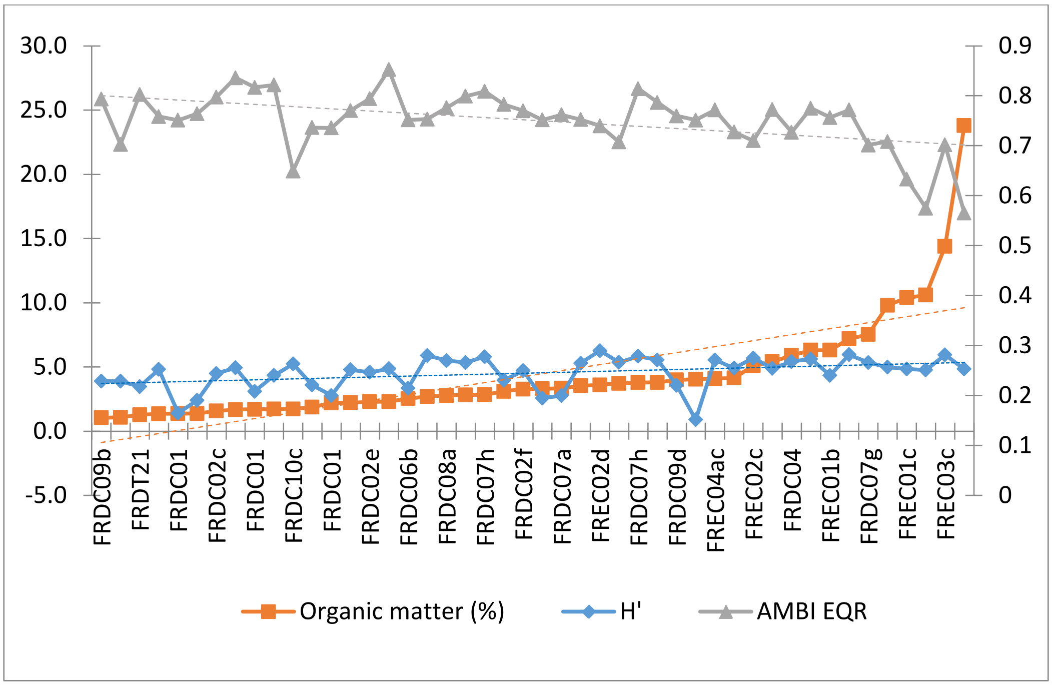

Figure 2.18 *Variation of Shannon-Wiener and MEDOCC EQR values along a gradient of organic matter content*

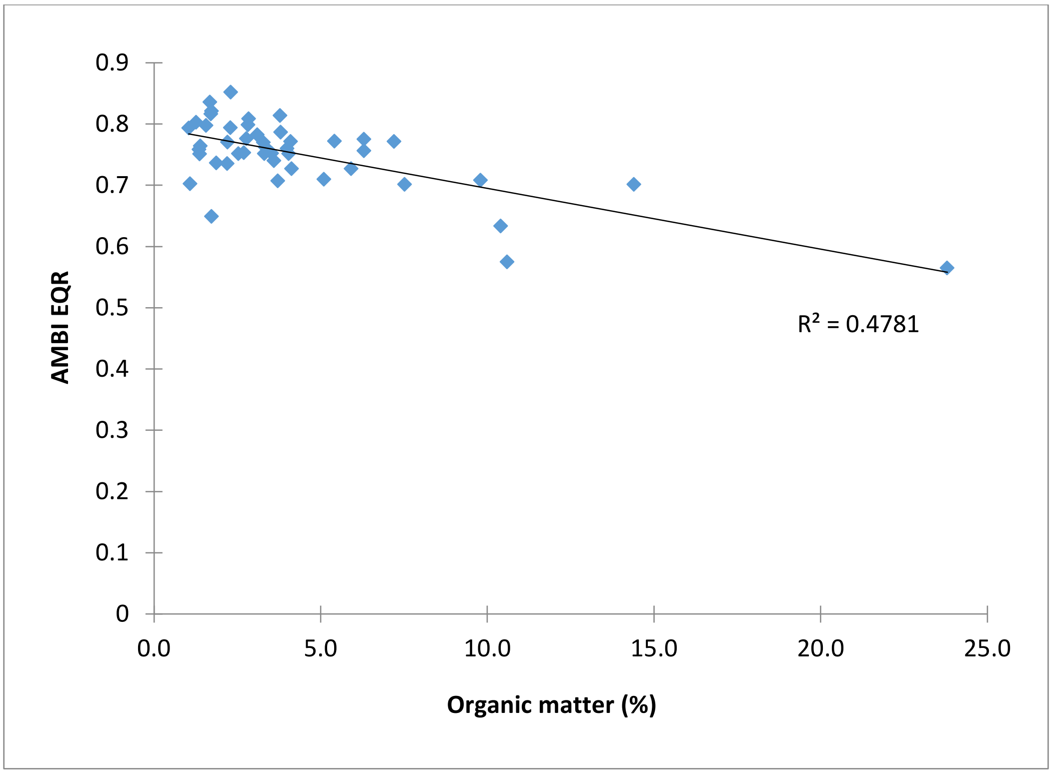

Figure 2.19 *Relationship between AMBI EQR values and the organic matter content*

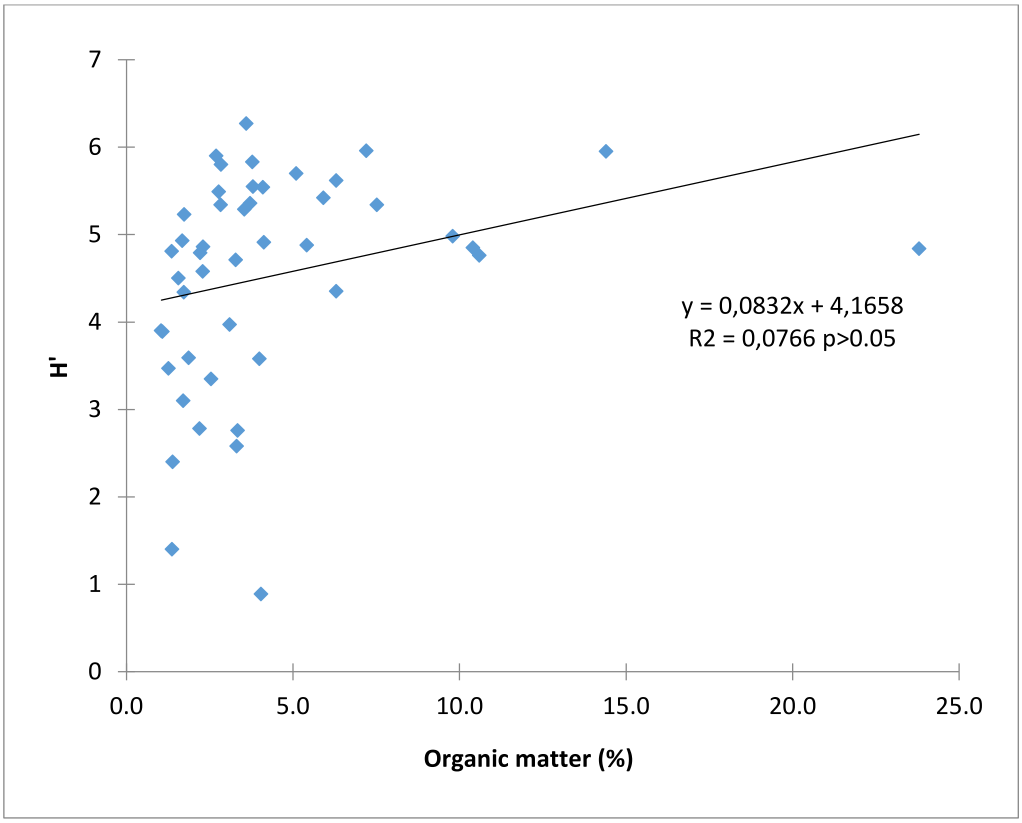

Figure 2.20 Relationship between diversity index and the organic matter content

3. Results IC feasibility checking

3.1. Typology

Common IC type: Entire Mediterranean Sea, no subdivision

Typologies are not relevant in the MEDGIG ecosystem as it was concluded in MEDGIG CW benthic invertebrate working group, and therefore they are not relevant in MEDGIG IC procedure for macro-invertebrates element. This justification has been made using the results after the application of an ordination analysis on the data provided by Spain (all the regions) Slovenia, Greece, Cyprus and Italy.

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 (). 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 all BQEs, as Mediterranean ecosystem is quite homogeneous in comparison to Northern Seas (some ecological differences do exist but within the Mediterranean scale).

Table 3.1 Main water body types of coastal waters within the Mediterranean Sea

Type	Name of Type	Substratum[1]	Depth[2]
CW - M1	Rocky shallow coast	Rocky	Shallow
CW - M2	Rocky deep coast	Rocky	Deep
CW - M3	Sedimentary shallow coast	Sedimentary	Shallow
CW - M4	Sedimentary deep coast	Sedimentary	Deep

A Detrended Correspondence Analysis (DCA) was carried out using the data sets from Spain (all regions), Slovenia, and Greece, for the current Intercalibration Phase, including also the Coastal types set for the Mediterranean Sea: M1, M2, M3 and M4 (Figure 3.11 to Figure 3.17). As presented below, in most of the cases the types do not differentiate from one another and there is a high percentage of mixed types ordination of stations. Typologies for the Mediterranean do not reflect any actual differences in the structure and composition of benthic communities. It seems that in the Mediterranean ecoregion a diverse mosaic of habitats-substrates-communities may exist in every bathymetrical zone and that a given habitat, substrate and community type may be found in various depths.

In the case of Italy, a nMDS analysis was applied on the most recent National data set referred to time period 2008-2009 that is coming both from Adriatic and Thyrrenian sea (111 stations in total) and from the four different geo-morphological types (M1/M2/M3/M4), and also taking into account the different water column density. No significant differences among stations and among different typologies were found.

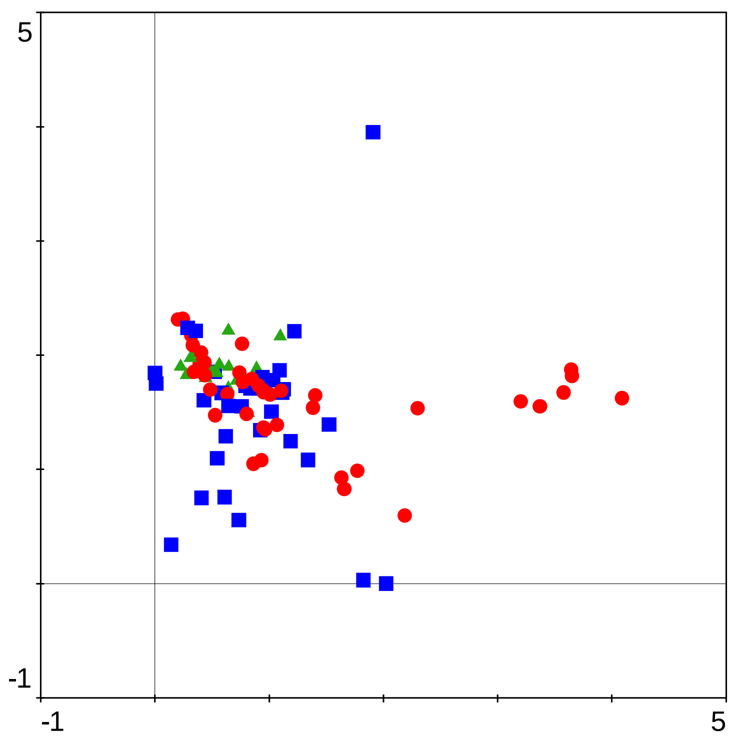

Figure 3.1 Balearic Islands (Spain)

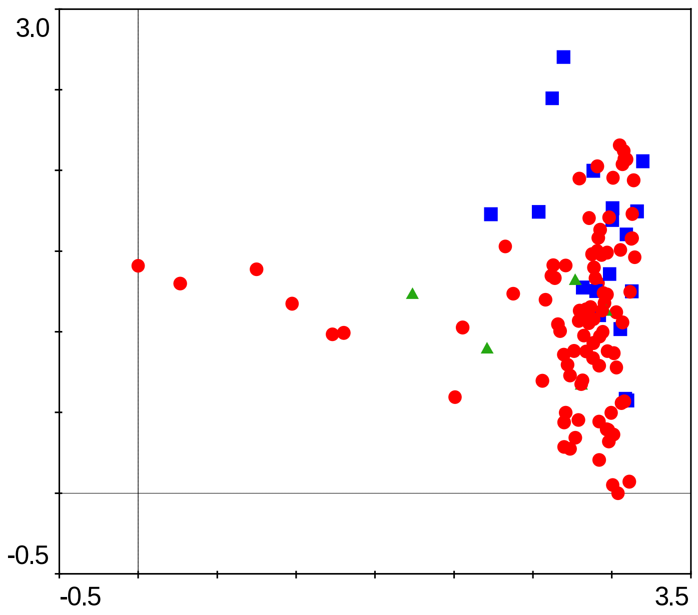

Figure 3.2 Catalonia (Spain) Square: M2; circle: M3; triangle: M4

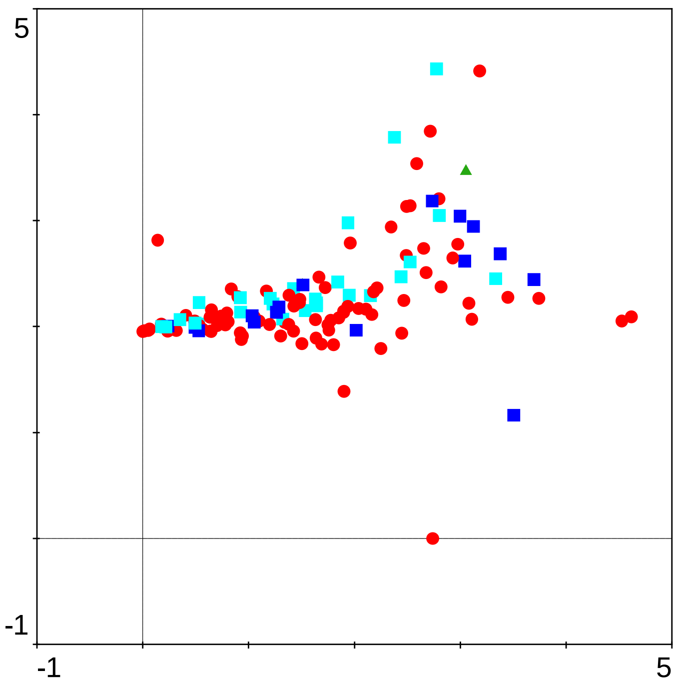

Figure 3.3 Valencia region (Spain) - Square blue light: M1; square blue strong M2; circle: M3; triangle: M4

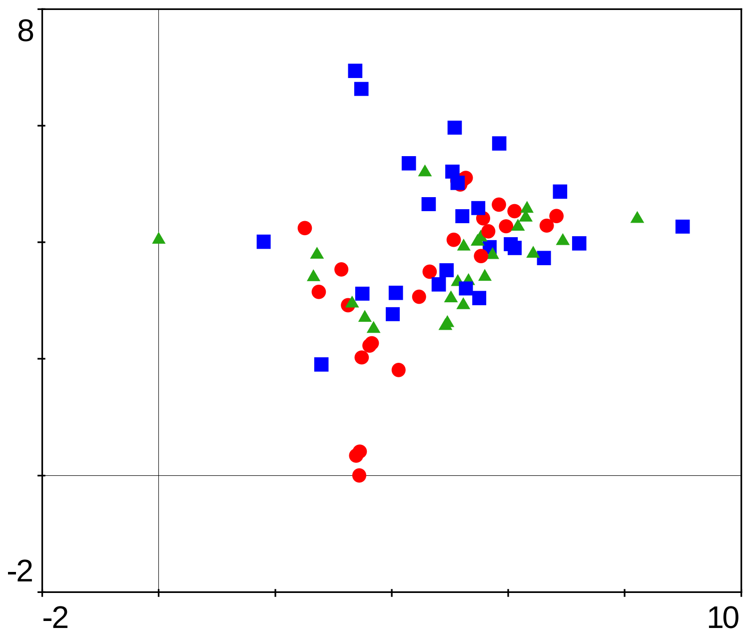

Figure 3.4 Murcia and Andalusia regions (Spain) - Square: M2; circle: M3; triangle: M4

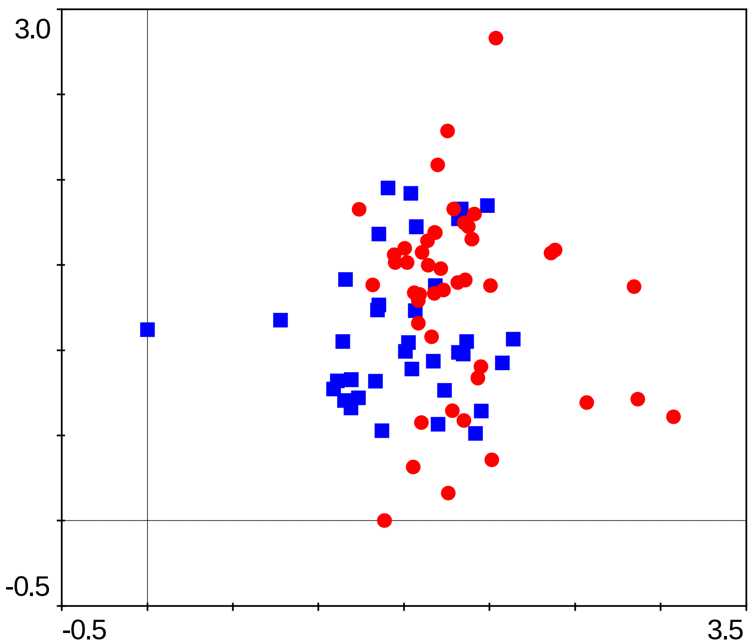

Figure 3.5 Slovenia - Square: M1; circle: M3

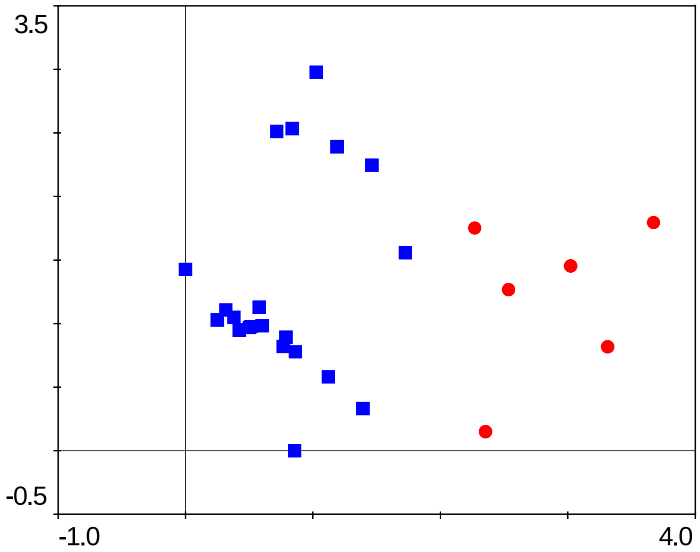

Figure 3.6 Greece - Square: M2; circle: M3

The situation from Greece is not so clear as shallow muds from M3 type Thessaloniki gulf (G/M) stations do not mix with M2 types including deep muddy sands from S. Evvoikos gulf (G/H) or deep muddy stations from Saronikos gulf (G/M). However, some M3 stations are closer to deep muddy G/M sites and some are closer to deep sand H/G sites.

It seems that the community composition in terms of species composition and abundance distribution is not relevant neither with types as defined in the original typology neither exclusively with ecological status. A mosaic of habitats-communities exist in different depths or substrates as demonstrated in Simboura *et al.*, 2005. For example the coastal terrigenous mud community can be found in deeper (ex. Saronikos gulf) as well as in shallower zones (Thessaloniki gulf) and also sandy communities can be found in sedimentary shallow gulfs (ex. st. DA3 in Thessaloniki gulf) as well as in deeper rocky coasts as in S. Evvoikos gulf. Community similarities maybe governed by other factors as well as local particularities of the substrate and land based influences as in Thessaloniki gulf.

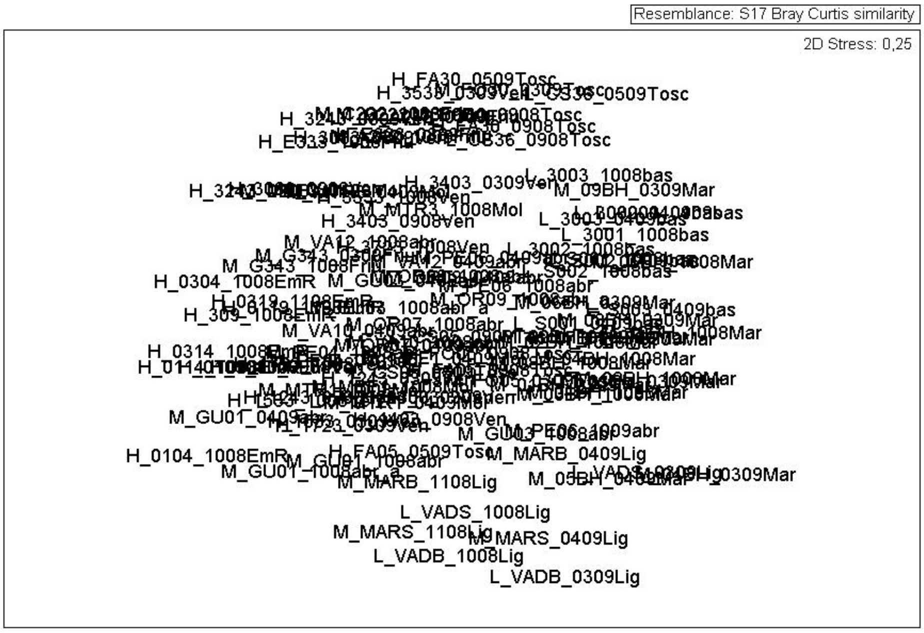

Figure 3.7 Italy: M1, M2, M3 and M4 Typology are represented by the station points in the ordination above.

3.2. Pressures addressed

The intercalibration is feasible in term of pressures addressed by the methods

Description of the pressures addressed by the MS assessment methods:

All methods show a significant response to the pressures, as it was tested with the LUSI index. The LUSI index was constructed by Flo et al. (2011) and modified by Romero (2011), and it has been successfully applied in the MED GIG phytoplankton group in order to ascertain the relationships between anthropogenic pressures and biological indicators.

Figure 3.8 to Figure 3.12 show the significant relationship between the different methods and the LUSI index. The linear regression analysis has been applied on the common data set, and taking into account the EQR values of the methods obtained in the different MS water bodies (EQRs calculated as the average values obtained in the stations of each MS water bodies), because the LUSI index is calculated at the water body level, as it is stated by the authors of the pressure index.

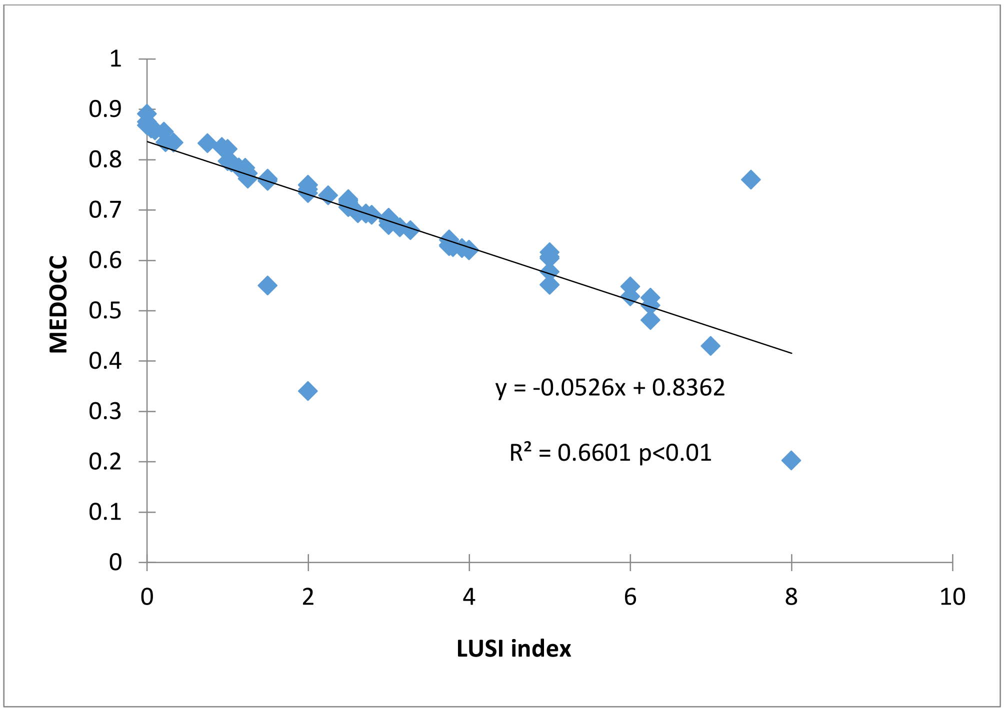

Figure 3.8 Relationship between the Pressure index and the MEDOCC EQR values

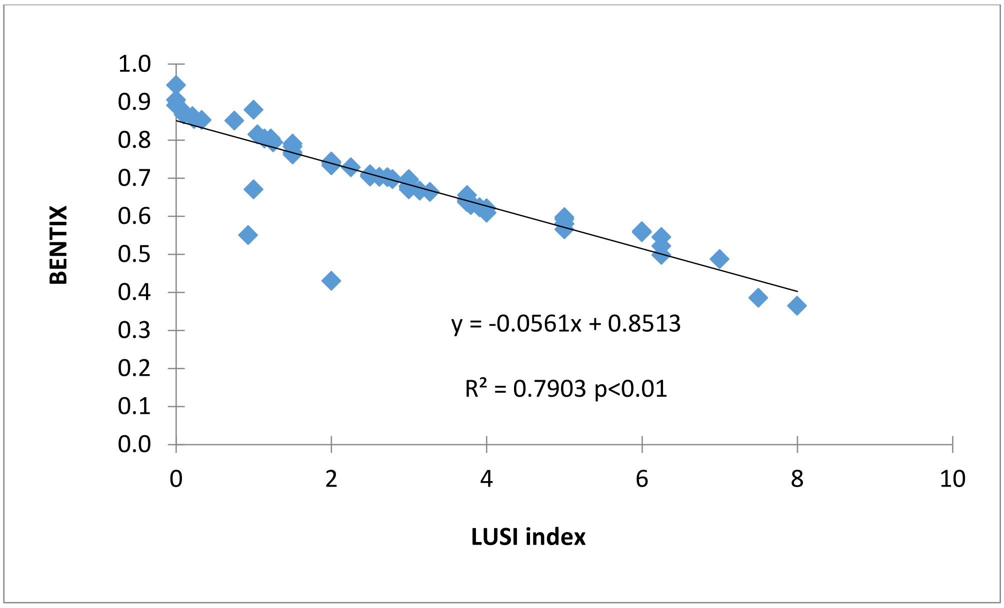

Figure 3.9 Relationship between the Pressure index and the BENTIX EQR values.

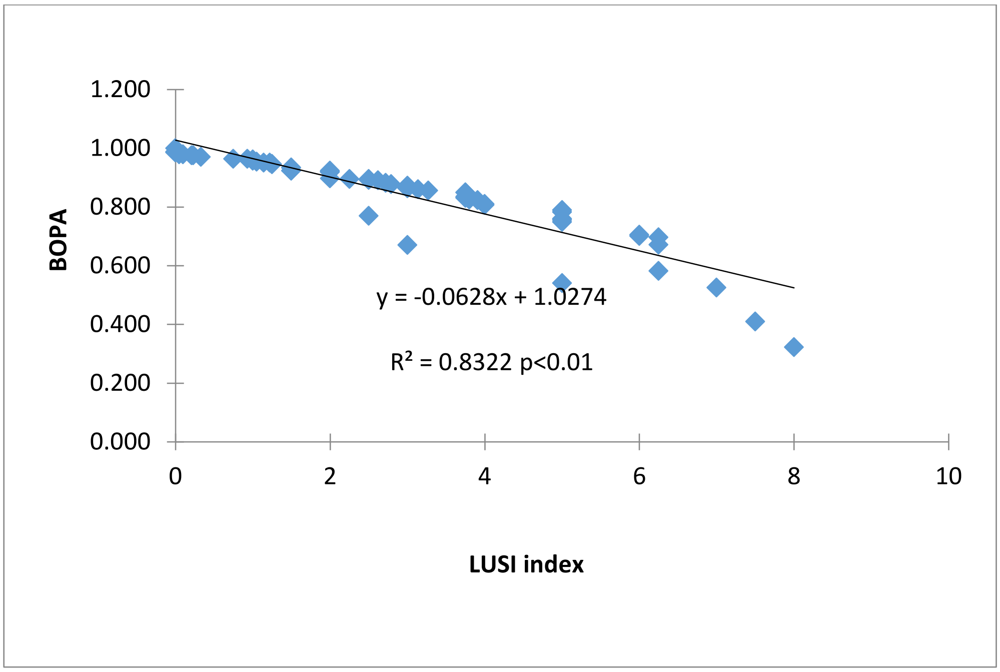

Figure 3.10 Relationship between the Pressure index and the BOPA EQR values

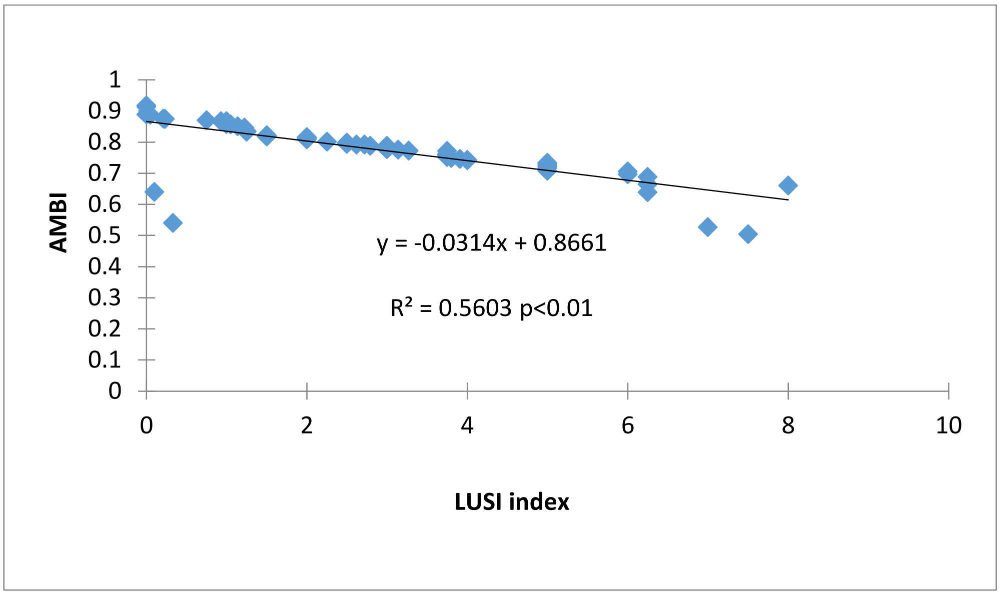

Figure 3.11 Relationship between the Pressure index and the AMBI EQR values

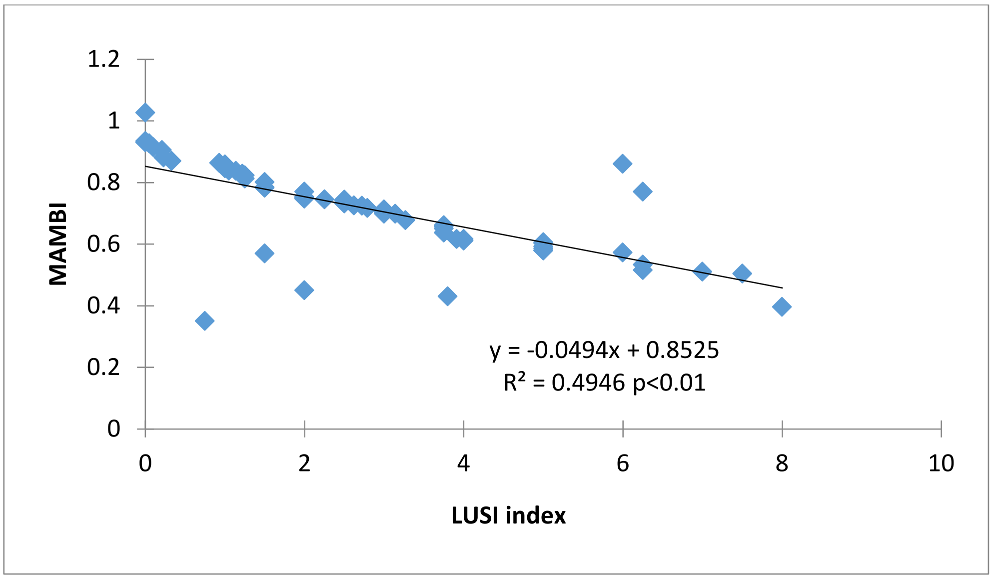

Figure 3.12 Relationship between the Pressure index and the MAMBI EQR values

Besides the exploration of the relationship between the LUSI index and the national methods on the common dataset, the Italian data set has been used to describe the response of the biotic indices to different pressure indicators testing the type of the relationship and the weight of each regressor. The Italian data set was used for it, because it includes the highest number of data from different pressures.

The Italian data set consists of 118 station points collected in the framework of the National monitoring program in 2008-2009, from which 47 stations were selected based on the strength of taxonomic lists and geographical representativeness. Seven sampling stations belong to the High class, 21 to the Good class and 19 to the Moderate class. (Figure 3.13)

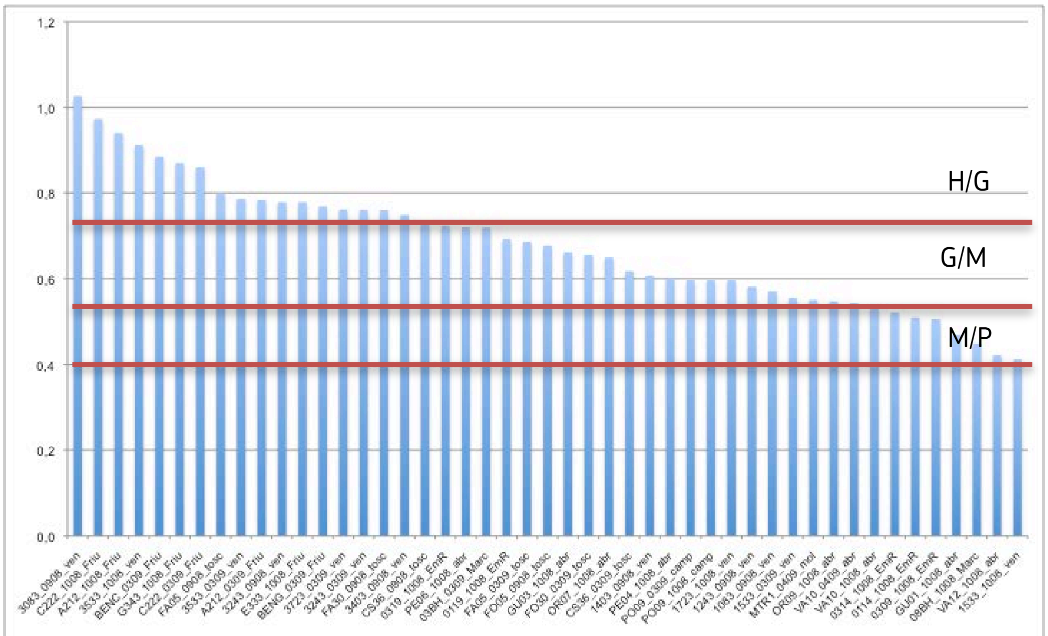

Figure 3.13 Distribution of the EQR values in the Italian data-set

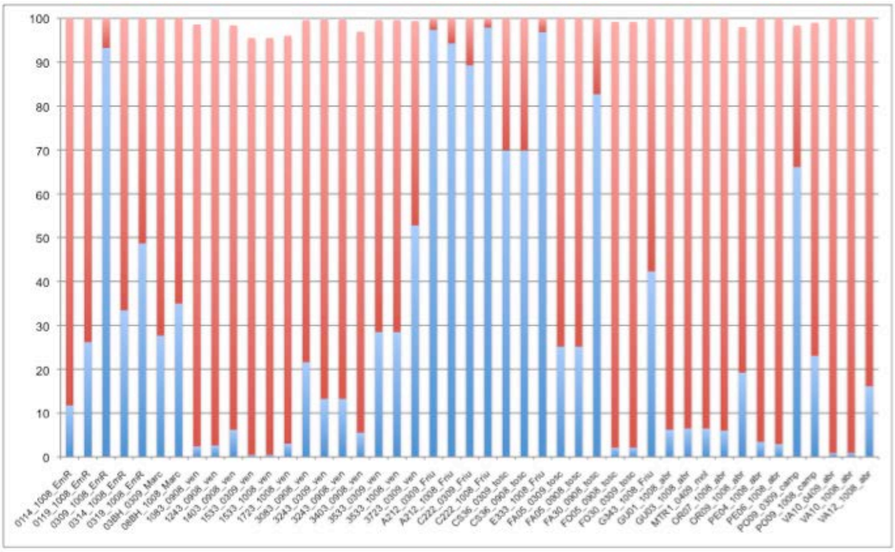

Figure 3.14 Sediment composition of the sampling stations (blue=mud; red=sand)

The distribution of the sediment size (Figure 3.14) shows that the most of the sampling stations are composed by sandy sediment. This kind of coastal biotope is characterized by benthic communities naturally subject to environmental stresses showing high resilience (Sarà, 1985). This biocenosis is able to counteract changes resulting from both natural and human activities, surviving even in unfavorable conditions, and restoring the ecological balance (Odum, 1988). This is probably the reason why the quality gradient does not include poor and bad classes. In order to detect which environmental stressors insist on the investigated community and to verify the correlation with our classification method (M-Ambi), a multiple linear regression analysis has been performed.

To verify the national methods functional relationship with pressure indicators, we adopt a function of a linear kind (a linear model or multiple linear regression - LM) where *Y* is the dependent variable, or response variable, tested against one or more independent variables (X_1, X_2 ..X_n), called explanatory variables or regressors. The choice of the multiple linear regression (instead of the single linear regression) is because, in general the effects of different human induced pressures on a community are combined due to the ecological complexity of the benthic ecosystem.

The LM is formulated as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + \varepsilon$$

where β_0 is the known term (the intercept), $\beta_1 \ldots \beta_n$ are the regression coefficients and ε represents the error, to say the difference between the sample measurements of *Y* and the estimated *Y* values by the model. Together with the error variance (i.e. the variance of the residuals), the intercept and the regression coefficients are therefore the parameters of the model to be evaluated, starting from the sample observations.

The choice of the independent variables, i.e. the pressures and status indicators considered in the LM, depend on the results of a Stepwise regression technique previously applied to the whole pressures dataset. The iterative process of the Stepwise Regression (backward), clearly stops when all the regr. coeff. not significantly different from zero (i.e. not influencing the response-variable), have been eliminated. With respect to the initial model, several pressure/status indicators were eliminated, as not significant regressors, namely: % Corine artificial surfaces, industrial and urban Equivalent Population, marketed pesticides and TRIX Index, TOC%.

Moreover the LM procedure adopted allows to identify anomalous sampling stations (outliers) and leave them out from the analysis (in practice 8 sampling stations have been eliminated). The pressure and status variables have been previously standardized.

Therefore, the adopted linear models were the following:

MAMBI

lm(formula = MAMBI~agric+LoadP_s+stab+Fe+Hg+Zn,data=mambi file)

where the M-AMBI Index was tested against: % of Corine agricultural areas (*agric*), the load of phosphorous released per Km^2 of agricultural land (*LoadP_s*), the stability of the water column that represents the fresh water inputs (*stab*) and Iron, Mercury and Zinc sediment content (*Fe*, *Hg*, *Zn*). The output provided by the fitted model is shown in the following table.

Regressors	Regression Coefficients	Std. Error	t value	Pr(>|t|)
(Intercept)	0.6655	0.01165	57.108	< 2e-16"***"
Agric	-0.08754	0.01455	-6.015	0.000000824"***"
LoadP_s	-0.03858	0.01466	-2.632	0.01266"*"
Stab	0.05506	0.01587	3.471	0.00143"**"
Fe	0.07797	0.0154	5.065	0.0000142"***"
Hg	-0.04072	0.01657	-2.457	0.01927"*"
Zn	-0.05228	0.01825	-2.864	0.00712"**"

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

Residual standard error: 0.07462 on 34 degrees of freedom. Multiple R-squared: 0.7378, Adjusted R-squared: 0.6915, F-statistic: 15.95 on 6 and 34 DF, p-value: 1.266e-08

The output of the LM presented it shows some important results:

- The type of relationships (direct or inverse, depending on the sign of the regr. coeff.).
- The weight of the regressors (i.e. the % value of the regr. coeff. on the total sum).
- The significance and the related Probability.
- The value of the R^2 (0.75), to be meant as the %amount of the variability of the response variable (i.e. the Index M-AMBI) explained by the six chosen regressors in the LM.

The regression coefficients reported represent the "weight" of each regressor in terms of importance in defining the LM. The percentage of agricultural land (Agric %) is the most important pressure indicator being related to loads of nutrient exchanged in the coastal ecosystem conveyed by agricultural activities. The other significant regressors in order of importance are Iron concentration in sediment, inputs of fresh water represented by the stability of the water column, zinc and mercury concentration in sediment, and finally the specific load of phosphorous.

The TOC% in sediment was not significant in defining the LM, since the TOC % values in the sampling stations forming the Italian data set hardly exceeds the 2%. It is well known that while an overabundance of organic matter in sediments can cause reductions in species richness, abundance, and biomass due to oxygen depletion and buildup of toxic byproducts below threshold values, it represents an important source of food for benthic fauna. Hyland et al. (2005) suggest that TOC seems to be toxic to benthic fauna at concentrations over 3.5% due to the deoxygenating effect of organic matter. At TOC concentrations below 1%, benthic assemblages did not show any evident alterations (Hyland et al., 2005). Also according to the Pearson and Rosenberg model (1978), abundance and diversity show different patterns. For example, there may be a co-existence of species with varying life-history strategies and levels of tolerance to stress throughout the intermediate TOC range. As TOC increases heartier opportunistic species are able to maintain high abundances even though other more sensitive species may be dropping off. In Magni et al. (2009) they have found the highest abundances at a high TOC range (>2.5–3%), while benthic diversity had declined. On the other hand, diversity measures showed a maximum in the lowest TOC range (<1%), a gradual decline over the intermediate TOC range (from 1% to 2.8%), and a minimum in the highest TOC range (>2.8%)

In any case, the effectiveness of using M-AMBI in detecting organic content pollution effects in Mediterranean environments is witnessed for example by specific study on pollution by organic load by sea cages aquaculture (Tomassetti et al., 2009; Borja et al., 2009). In Tomassetti et al., (2009) paper both AMBI and M-AMBI seems to be a good indicator of benthic stress, in terms of response to organic enrichment of sediment, because stations under the cages especially showed an increase in AMBI directly related to the biomass reared in the cage and consequently organic enrichment. M-AMBI in particular, by using reference conditions taken from the data used to calculate the index, showed a better resolution to discriminate the stations related to different magnitude of organic enrichment.

Back to the LM (2) the hypotheses tests applied on the residuals, insure us that their distributions are random, that serial autocorrelation is not significantly different from zero and consequently there are reasonably no other possible source of variation and or factors not considered or forgotten, in the LM. Diagrams reported in Figure 3.15 are referred to the goodness of the M-AMBI linear model, showing the approximation to normality of the residuals.

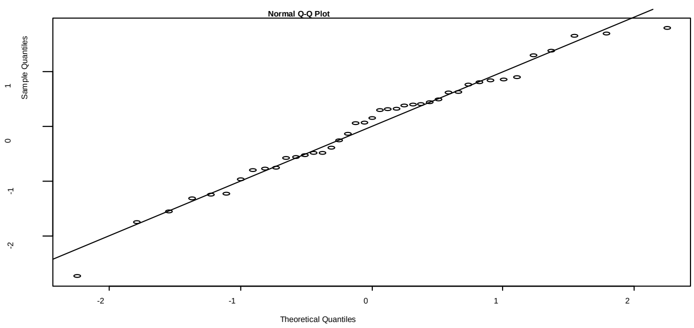

Figure 3.15 Linear Model for M-AMBI: diagraam showing the approximation to normality of the residuals (Graphical elaborations from R Stats Package)

In Figure 3.16 the response variable (M-AMBI) is plotted against each of the regressors considered in the adopted LM: the functional relationships are biased by the effects of the other independent variables.

The "partial (or component) residual plot" (Figure 3.17) technique allows to plot each regressor against the corresponding value of the response-variable, having eliminated the combined effects of the other regressors. [b1X1+ residuals= Y-(b0+b2X2+….+bnXn)].

The graphical outputs reported in Figure 3.16 and Figure 3.17, show the results of this technique to our data and can give a clear explanation why so low correlation values are obtained, when considering the single relationships. Partial residual plots provide also a proof that the log-transformation previously adopted for the pressure data gives rise to a linear relationship fully acceptable.

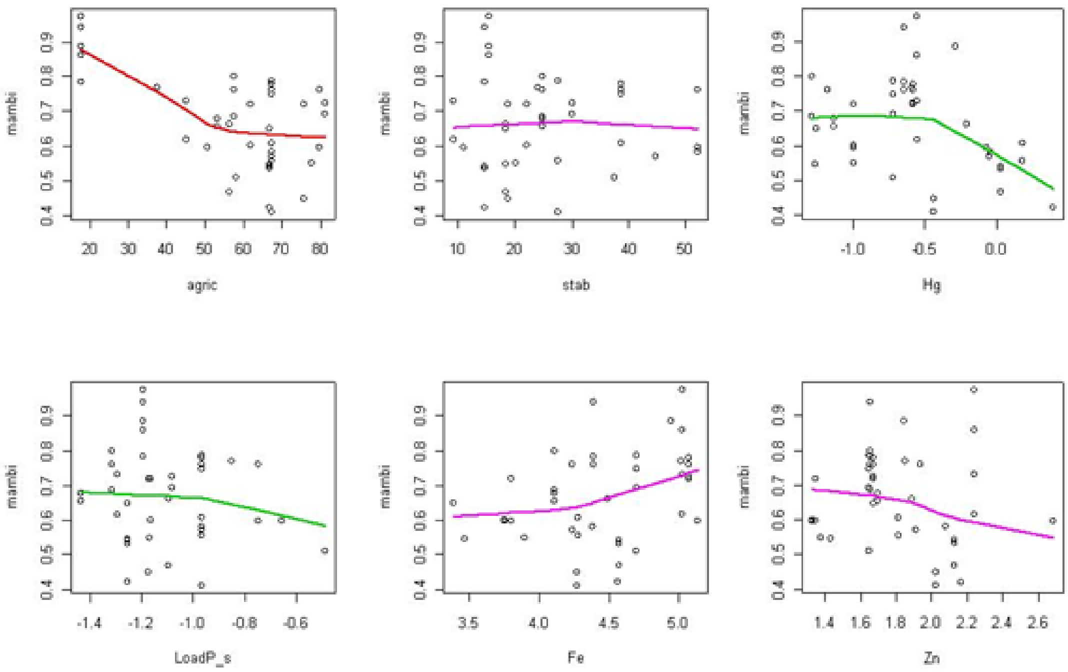

Figure 3.16 Scatter plots of the response variable M-AMBI against each of the regressors biased by the effect of the other independent variables

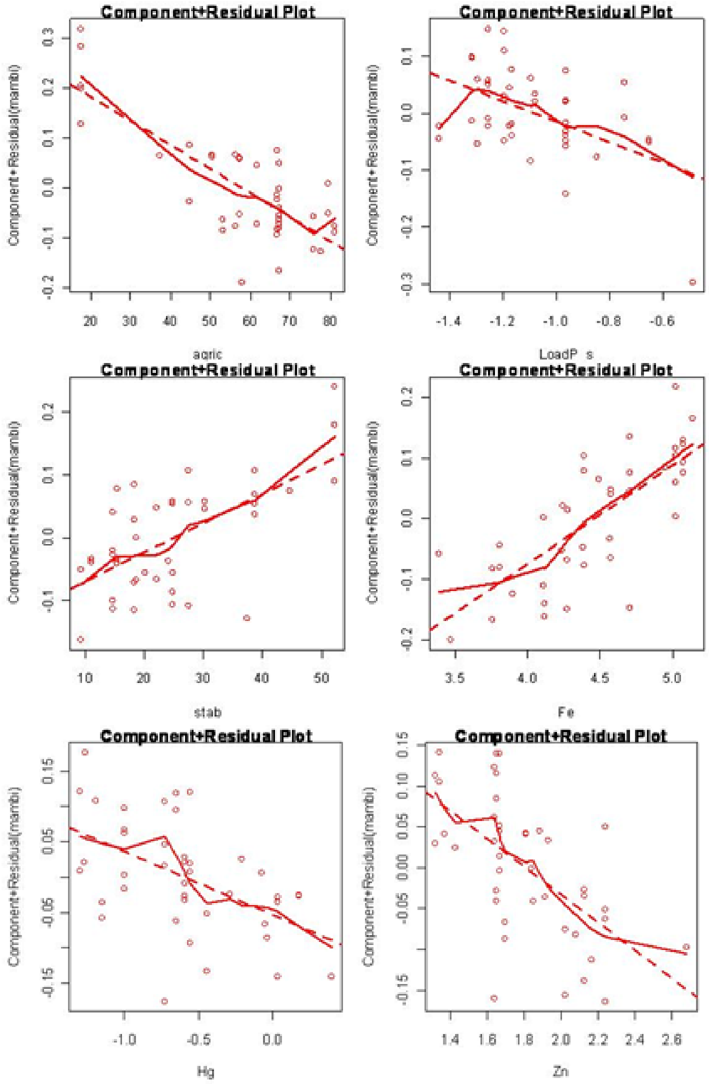

Figure 3.17 *Partial residual plots of the same response variable against each regressor, without the effects of the other independent variables*

In conclusion, we have identified the stressors that most affect the response of macrozoobenthos described by the M-AMBI index. Based on the available data we can say that in general this biocoenosis is influenced by the contributions of continental organic matter either directly by the contributions of phosphorus or by the contributions due to agricultural activity. It was not possible to show a direct effect of total organic carbon in sediment due to the low percentage in the sediment investigated.

AMBI multiple linear model: lm (Ambi ~ agric + artif + Nload_spec + fitos + TOC + TRIX + Zn)

Results: Estimate Std. Error t value Pr(>|t|)

(Intercept)	2.4736165	0.2190068	11.295	3.19E-13	***
Agric%	-0.0016215	0.0007069	-2.294	0.027921	*
Artif%	-0.0086873	0.0020494	-4.239	0.000156	***
Nload_s ton/km2	0.0865987	0.0159029	5.445	4.16E-06	***
Fitos commercialized	-0.1960691	0.031309	-6.262	3.49E-07	***
TOC%	-0.0449874	0.0086777	-5.184	9.19E-06	***
TRIX	-0.1117357	0.0180107	-6.204	4.16E-07	***
Zn_mg/kg	-0.0004966	0.0001256	-3.955	0.000356	***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
Residual standard error: 0.05841 on 35 degrees of freedom					
Multiple R-squared: 0.709, Adjusted R-squared: 0.6508					
F-statistic: 12.18 on 7 and 35 DF, p-value: 9.088e-08					

BENTIX multiple linear model: lm(Bentix ~ fitos + TOC + TRIX + Cr + Fe)

Results: Estimate Std. Error t value Pr(>|t|)

(Intercept)	1.20E+00	1.11E-01	10.845	1.01E-11	***
Fitos commercialized	-2.65E-02	1.49E-02	-1.781	0.085431	.
TOC%	-2.98E-02	7.25E-03	-4.105	0.0003	***
TRIX	-9.47E-02	1.40E-02	-6.777	1.94E-07	***
Cr mg/kg	-1.32E-03	1.99E-04	-6.641	2.79E-07	***
Fe mg/kg	-4.95E-07	2.02E-07	-2.459	0.020161	*
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
Residual standard error: 0.04601 on 29 degrees of freedom					
Multiple R-squared: 0.7324, Adjusted R-squared: 0.6863					
F-statistic: 15.87 on 5 and 29 DF, p-value: 1.524e-07					

MEDOCC multiple linear model: lm(Medocc ~ artif + noncolt + Nload_spec + fitos + TRIX + Zn)

Results: Estimate Std. Error t value Pr(>|t|)

(Intercept)	2.7484695	0.2638693	10.416	2.89E-12	***
Artif%	-0.0110604	0.0022012	-5.025	1.49E-05	***
Noncolt%	0.001831	0.0008747	2.093	0.043637	*
Nload_s_ton/km2	0.1167451	0.0195522	5.971	8.43E-07	***
Fitos commercialized	-0.2779923	0.0388909	-7.148	2.46E-08	***
TRIX	-0.1342429	0.0217576	-6.17	4.61E-07	***
Zn mg/kg	-0.0006202	0.0001543	-4.02	0.000294	***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
Residual standard error: 0.07165 on 35 degrees of freedom					
Multiple R-squared: 0.6783, Adjusted R-squared: 0.6232					
F-statistic: 12.3 on 6 and 35 DF, p-value: 2.097e-07					

BOPA multiple linear model (in this case the analyses have applied on BOPA values and not on EQRs values): lm(BOPA~agric+Load Ps+TOC+Va+ Ni+Cd)

Results: Estimate Std. Error t value Pr(>|t|)

(Intercept)	**0.0565018**	**0.0201236**	**2.808**	**0.008986**	**
Agric	-0.0009259	0.0002170	-4.267	0.000204	***
Load_Ps	0.3739267	0.0565552	6.612	3.59e-07	***
TOC	0,0154048	0.0030023	5.131	1.94e-05	***
Cd	-0.2087723	0.0687069	-3.039	0.005105	**
Ni	-0.0003518	0.0001452	-2.423	0.022113	*
Va	0.0003949	0.0001759	2.245	0.032821	*
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
Residual standard error: 0.01959on 28 degrees of freedom					
Multiple R-Squared: 0.7338 Adjusted R-squared: 0.6768					
F-statistic: 12.87 on 6 and 28 DF p-value: 6.077e-07					

Results show that the multiple linear model calculated on IT dataset (LM) is confident in all the cases and describes the relations among the different pressure indicators and the different biotic indices used to classify marine water bodies by soft-bottom macroinvertebrates in France, Greece, Cyprus and Spain.

As in the case of MAMBI, the type of relation (direct/inverse) linking pressure indicators (regressors) and response variables (indices), are ecological coherent: e.g. most of the pressure indicators that describe the anthropogenic use of the territory are inversely (except in the case of the BOPA that it is directly because the analyses have applied on BOPA values, and not on EQRs values) or proportional to the Indices considered.

LMs show R2 significant coefficients for all the national methods (R2>0.6).

Table 3.2 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
Slovenia	M-AMBI	EU, GD	Pressures considered were land usage (urbanisation, industry, agriculture) and sea usage (mariculture, ports, waste water discharges on sea). Only estimates were made. AT 7 stations OM content was measured, but the contents and gradient were to low (max of 0.6%) to be used for examining the relationship.	Biological data and pressure evaluation for 39 samples	Correlation on basic data (0.70; $p<0.01$) and log-transformed data (0.56; $p<0.01$)
Spain	MEDOCC	EU, GD	Pressures were urban sewage discharge, urban land use and harbours (as defined in the IMPRESS document). All these pressures are measured as a gradient of LUSI index, organic matter content in sediments, nutrients in the water column; also PPCI (Industrial and Commercial Harbours) and PUU (Urban Land use) are provided..	Biological data, pressure evaluation, and organic matter content in sediments from 100 stations; nutrients from 51 stations; copper concentration, PPCI and PUU from 57 stations	Linear regression for all pressures ($p<0.01$)
Spain	BOPA	EU, GD	Pressures were urban land use, sewage discharge, harbours, and fish farming cages. All these pressures are measured as a gradient of LUSI index and organic matter content in sediments.	Biological data, pressure evaluation, and organic matter content from 307 sites	Linear regression ($p<0.01$)
Greece	BENTIX	EU, GD	Pressures were urban land use, sewage discharge, harbours. All these pressures are measured as a gradient of LUSI index, and organic carbon content (OC).	Biological data, pressure evaluation, and OC from 85 stations	Linear regression ($p<0.01$)
Cyprus	BENTIX	EU, GD	Pressures were urban land use, industry, agriculture and fish farm cages (organic loading and nutrients). All these pressures are measured as a gradient of LUSI index, and % organic matter in sediments	Biological data, pressure evaluation from 10 stations	Linear regression for all pressures ($p<0.01$)
Italy	M-AMBI	EU, GD	Pressures were land use, sewage discharge, harbours.etc. : All these pressures are measured as a gradient of LUSI index, ARTIFICIAL (%);Load_N (ton/y);Load_P (ton/y);Pesticed (kg);TOC (%);TRIX;Stability (cycles/h)	Biological data , pressure evaluation and pressures indicators data from 35 sites	Linear and Multiple Linear regression (see Annex Italy)

Member State	Method/Metrics tested	Pressure	Pressure indicators	Amount of data	Strength of relationship
France	AMBI		Pressures were urban sewage discharge, and rivers sources. These pressures are measured as a gradient of organic matter content in sediments.	Biological data, pressure evaluation, and organic matter content from 43 sites	Linear regression ($p<0.05$)
Malta	-	-	-	-	-
Croatia	-	-	-	-	-

3.3. Assessment concept

Method	Assessment concept
MEDOCC, BENTIX, AMBI, BOPA	Method focused on soft bottom macroinvertebrates, based on the abundance of sensitive/tolerant species faced with the increased or decreased disturbance.
M-AMBI	Method focused on soft bottom macroinvertebrates, based on the abundance of sensitive/tolerant species faced with the increased or decreased disturbance, and in the values of specific richness and Shannon Wiener index.

The Intercalibration is feasible in terms of assessment. The five methods proposed by all the countries participating in this intercalibration exercise, follow a very similar philosophy.

AMBI, MEDOCC, BENTIX, BOPA, and MAMBI are methods focused on soft bottom macroinvertebrates, and based on the abundance of sensitive/tolerant species faced with the increased or decreased disturbance. Besides that, the MAMBI method also includes the diversity parameter.

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	696
Number of Member States	6
Repackage/disaggregation of samples/WB results?	Data points represent the result of different samples taken at one site
Gradient of ecological quality	Fully covered
Coverage per ecological quality class	The data set covers all the relevant classes. France:High-19; Good-23:Moderate or Worse-4 Greece: High-8; Good-29:Moderate or Worse-71 Cyprus: High-5; Good-3:Moderate or Worse-2 Italy: High-7; Good-21:Moderate or Worse-19 Slovenia: High-4; Good-14:Moderate or Worse-9 Spain (Catalonia, Balearic Islands): High-54; Good-43:Moderate or Worse-9 Spain(Valencia, Andalusia and Murcia regions): High-141; Good-59:Moderate or Worse-151

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
Greece	108 samples	108 samples	108 samples
Cyprus	10 stations	10 stations	10 stations
Italy	46 stations	46 stations	46 stations
Slovenia	53 samples	7 samples	39 samples
Spain (Catalonia and Balearic islands)	105 stations	105 stations	105 stations
Spain (Valencia, Andalusia and Murcia)	340 samples	340 samples	340 samples
France	45 samples	45 samples	45 samples

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)	All the data are from soft sediment habitats, as the national methods are for use in those habitats.
The sampling and analytical methodology	Three or two replicates were randomly collected using a benthic grab (van Veen grab, Ponar grab or Box corer). The minimum size of organism sampled was 1 mm (mesh size of sieving net) in Slovenia, Greece, and Italy, and 0.5 mm in Cyprus and Spain (all regions).
Level of taxonomic precision required and taxa lists with codes	Organisms were identified to species level, or to the lowest reliable taxonomic level.
The minimum number of sites / samples per intercalibration type	
Sufficient covering of all relevant quality classes per type	Data covers all relevant classes

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).

Because no truly unimpacted reference conditions exist in the Mediterranean Sea, CW benthic group selected benchmark sites, defined as locations situated in low impacted areas in basis on the following requirements:

- no harbours
- no beach regeneration
- no urban sewages
- no industrial sewages
- no fish farms

- no desalination plants
- no thermal industries
- no influence of agriculture activities
- >3 Km as a distance to the closer city with more than 1000 inhabitants.

Several sites were identified as alternative benchmark sites for each Member State:

Spain (Valencia, Murcia and Andalusia regions):

Valencia	Station	Coordinates UTM (Europ1950)
2	FB09	31 T 267000 4453500
4	FB31	31 T 248753 4435733
9	FB01	30 S 744960 4324184
11	FB61	31 S 259061 4292557

Murcia	Coordinates UTM (Europ1950)	
	X	Y
710032	624309	4140088
710045	694943	41774478
710046	698292	4177460
31MAV	654721	4159696

Andalusia	Lon_(DD ETRS89)	Lat_DD ETRS89)
61C0010	-5,4748	36,0477
61C0225	-3,7775	36,7347
61C002509	-5,4243	36,0956
61C003007	-5,4224	36,1416

Spain (Catalonia and Balearic islands)

	Coordinates UTM (DATUM50)	
	X	Y
Cala Deià	469370	4401725
Badia de Alcúdia-Coll Baix	516277	4412989
Canyet	498742	4622934
Llorell-Porto Pi	491910	4617217

Greece

	Station	X	Y
Bay of Nikopolis (Kalamitsi)	D3	5248756,99817	1827702,18760
Bay of Nikopolis (Kalamitsi)	D4	5249252,06612	1829556,99550
Makronissos	E11	5564324,11104	1733123,48682
Bay of Methoni	M14	5370978,79636	1606928,49565
Bay of Nikopolis (Kalamitsi)	E1	5251387,48071	1824299,72182
Bay of Nikopolis (Kalamitsi)	E8	5248657,50954	1837988,87207

Cyprus

Station	Lon_WGS84	Lat_WGS84
CY_25-C3-S1	34.083933°	34.970267°

France

Zone	Station	NORD WGS84	EST WGS84
West-Rhone	FRDC02a	320452	4315330
East Rhone	FRDC07h	634197	4307495
Corsica	FREC03eg	900900	4146398

Italy

Station	WB	NORD WGS84	EST WGS84
H_3533_1008Ven	IT05ME2_1	5020496	764729,392
H_1533_0309Ven	IT05CE1_2	5020667,233	759921,9301
H_3243_0908Ven	IT05CE1_1	5044268,84	790564,173
H_3723_1008Ven	IT05ME2_2	4999073,751	769873,7039
H_E333_1008Friu	IT06CACE18	5077017,421	855868,1889
M_C222_1008Friu	IT06CACA32	5071081,35	866145,378
M_F005_0908Tosc	ITR000OM009AC	4724175,41	664344,984

Slovenia

Station	Coordinates UTM (EUROP1950)	
	X	Y
VT2P1	5050639.057	5399863.142
VT2P2	5050816.504	5399540.86
VT2P4	5050746.491	5398942.151
VT4P9	5044876.131	5391458.726

Validation of the selection of the alternative benchmark with biological data: In each benchmark it was established the EQR values and it was checked in the biological data matrix (abundance and composition data) that the communities are typical of reference conditions.

4.4. Benchmark standardization

Benchmark standardization serves to homogenize the EQR results of common datasets where needed, minimising typological and methodological differences between the Member states which may otherwise influence the comparability of their classifications.

Spain, Greece, Cyprus and France (parallel intercalibration 1):

The benchmark standardization was not necessary because there are no subtypes within the common type.

The subtraction option was used for the EQRs normalisation, as the pressure responses behave in a parallel way. It was done has been done automatically with the data-excel sheet provided to the intercalibration group: IC_Opt3_sub v1.24.

Italy and Slovenia (parallel intercalibration 2):

Italy and Slovenia are taking subtypes into account within the common type, by taking different values for the M-AMBI at the reference conditions. The explanation below clarifies the regional differences why it is legitimate to do so, by comparing Italian and Slovenian data in a similar pressure environment.

The hypothesis to explain different values of reference conditions and boundaries for the M-AMBI in Italy and Slovenia, is that the observed difference between IT and SLO communities could come from:

a. real natural differences
b. methodological differences (e.g. taxonomy)

To eliminate taxonomical differences station from FVG are in Italy and Slovenia were chosen, where taxonomical approach is very similar/the same.

nMDS (B-C. similarity) showed clear distinction between FVG and SLO data (similarity ≤ 20%), demonstrating natural differences in communities.

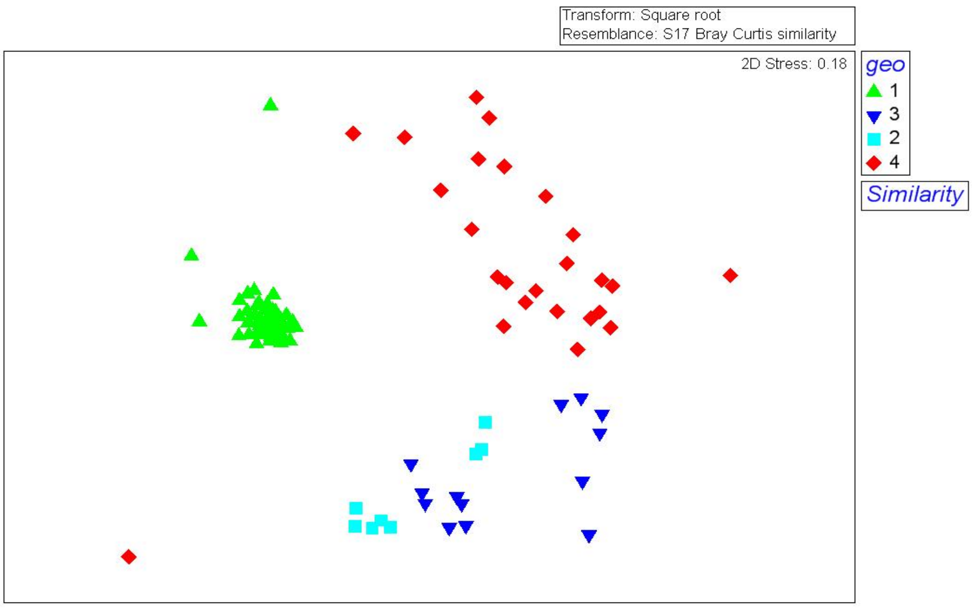

Figure 4.1 1: SLO – 2: FVG – 3: Veneto – 4: Others

With Simper (Similarity percentage) procedure we obtained the list of species that cause most distinctness between the FVG and the SLO dataset.

Groups 1 & 2 Average dissimilarity = 93.09

Species	Group 1_SLO	Group 2 IT_FVG	Av.Diss	Diss/SD	Contrib %	Cum. %
	Av.Abund	Av.Abund				
Maldane sarsi	0	**15.88**	5.01	4.5	5.38	5.38
Pseudopolydora antennata	0	**10.75**	3.24	1.23	3.48	8.86
Lumbrineris latreilli	**3.16**	**14.1**	3.22	1.87	3.46	12.32
Thoralus cranchii	0.07	**10.54**	3.13	2.15	3.37	15.69
Ophiura albida	0	**10.05**	3.01	1.81	3.23	18.92
Tellina (Moerella) donacina	0	**6.85**	1.96	1.01	2.11	21.03
Antalis inaequicostata	2.16	**7.76**	1.89	2.21	2.03	23.05

It was observed that the SLO and the IT-FVG communities were mostly characterized by species living in mixed-muddy sediment with wide ecological distribution. Nevertheless the SLO community is defined by shallower elements than the IT_FVG one. In fact, species like the polychaeta *Maldane sarsi* and the echinodermata *Ophiura albida* typical of deeper habitats, strongly characterizing IT_FVG community, are not indeed, present in SLO species list.

Actually depths of sampling sites were different: for FVG about 20 m, for SLO 7-10 m, which could explain the differences observed. IT complete data-set shows weak regional differences (Figure 4.2) and depth differences are not detectable.

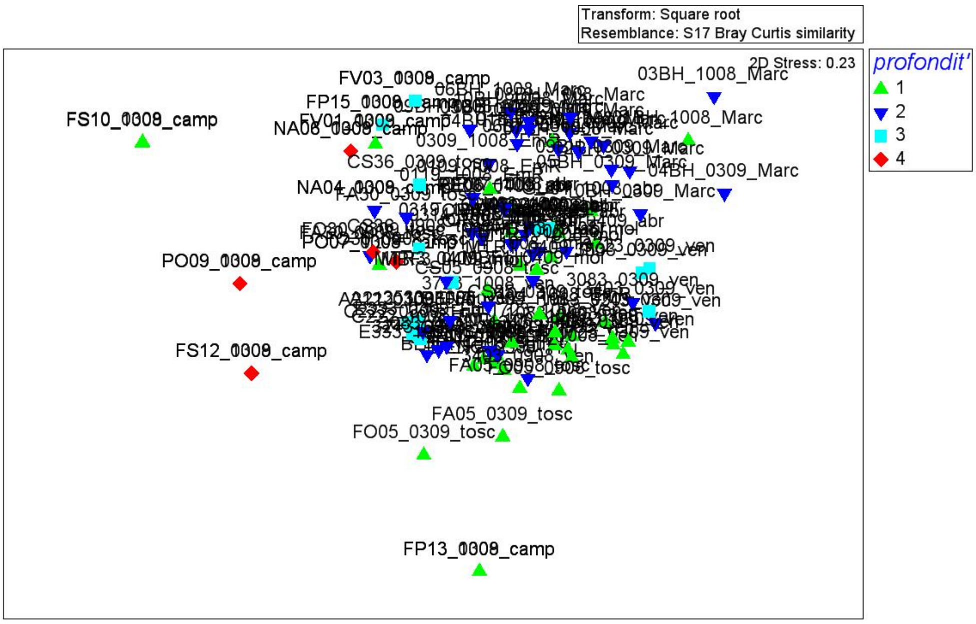

Figure 4.2 1: very shallow (2-6m); 2: shallow (7-14m); 3: deep (15-25m); 4: very deep (>26m)

Analysing the parameters composing the M-AMBI in the Italian and Slovenian benchmark sites, and comparing them with Italy and Slovenia values set for reference conditions, we can see that the existing difference is due to the particular natural differences charactering the Slovenian habitat sampled. Slovenia was then treated as sub typology. This justifies that Italy and Slovenia adopt different Reference Conditions using the same classification method.

Data from 7 Benchmark sites from Italy and 4 sites from Slovenia, which are under a comparable pressure level, were chosen to define the correction factor, representing natural difference (depth, habitat), and were used to calculate the sEQR with "division".

5. Comparison of methods and boundaries

5.1. IC option and common metrics

Firstly, benthic group selected the Option 3a. but the regression results showed no relationship between the M-AMBI and the Pseudo-common metric (R^2=0.167 p>0.05) suggesting that the M-AMBI is not comparable with the rest of methods.

Then, we thought that due to habitat specification, or sample size, maybe it was not possible to use the diversity metric (included on the M-AMBI method) on the datasets of all the intercalibrating member states, and therefore we needed to shift to an Option 2 approach. So, we also applied the Option 2, using a common metric (AMBI index) but M-AMBI was the one method that failed the requirements to continue with the IC procedure (R^2=0.14;p>0.05). Then, we selected another common metric (percentage of sensitive species), but on the basis of these results, the M-AMBI was again the one method that failed the requirements to continue with the IC procedure, showing a non-significant relationships with the common metric (R^2=0.17; p>0.05), and a low Pearson coefficient (r=0.41).

On the basis of the above mentioned results, the CW benthic group decided that France, Spain (all regions), Greece and Cyprus should follow the option 3a (as BQE sampling and data processing are generally similar, so that national assessment methods can reasonably be applied to the data of the other Member States) using as the pseudo-common metric the benchmark standardised average of all national EQRs per sample excluding the method to be compared against. On the other hand, Italy and Slovenia should follow the revised Option 3a adapted for two Member States.

5.2. Results of the regression comparison

Parallel Intercalibration 1

Cyprus, France, Greece and Spain with BENTIX, AMBI, MEDOCC and BOPA

The correlation coefficient (r) and the probability (p) for the correlation with the pseudo common metric (with an Ordinary Least Square regression), of national methods EQRs (non standardised values[1]) from Spain (all regions), Greece, Cyprus, and France are shown in Table 5.1 and Figure 5.1 to Figure 5.4.

[1]Standardisation was not neccesary because there are not subtypes.

Table 5.1 Results of the regression analysis

Member State/Method	R^2	r	P
France-AMBI	0.775	0.880	P<0.001
Spain (Catalonia and Balearic Islands)-MEDOCC	0.816	0.903	P<0.001
Spain (Murcia, Valencia and Andalusia regions)-BOPA	0.651	0.807	P<0.001
Greece and Cyprus-BENTIX	0.435	0.660	P<0.001

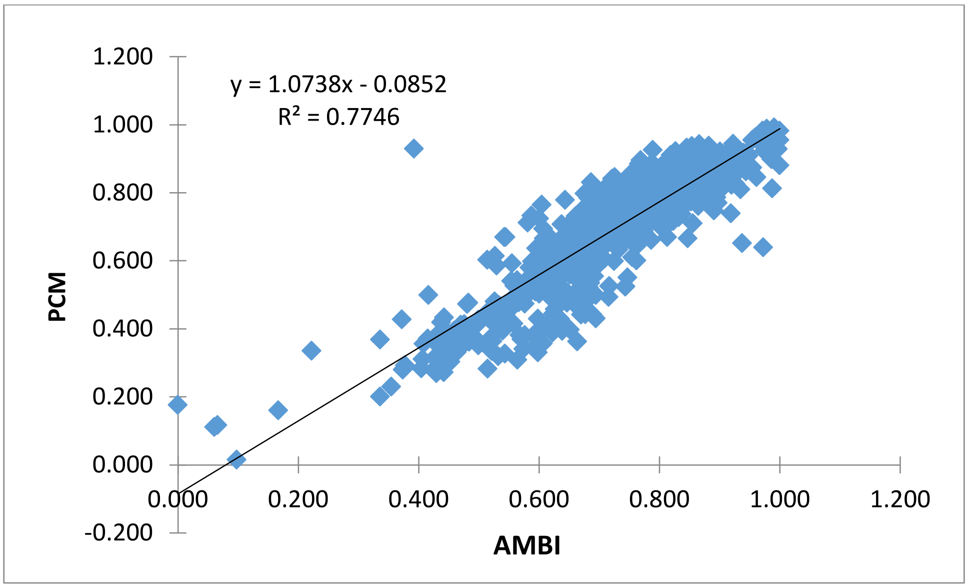

Figure 5.1 Relationship between the Pseudo-Common metric and the AMBI nsEQR values

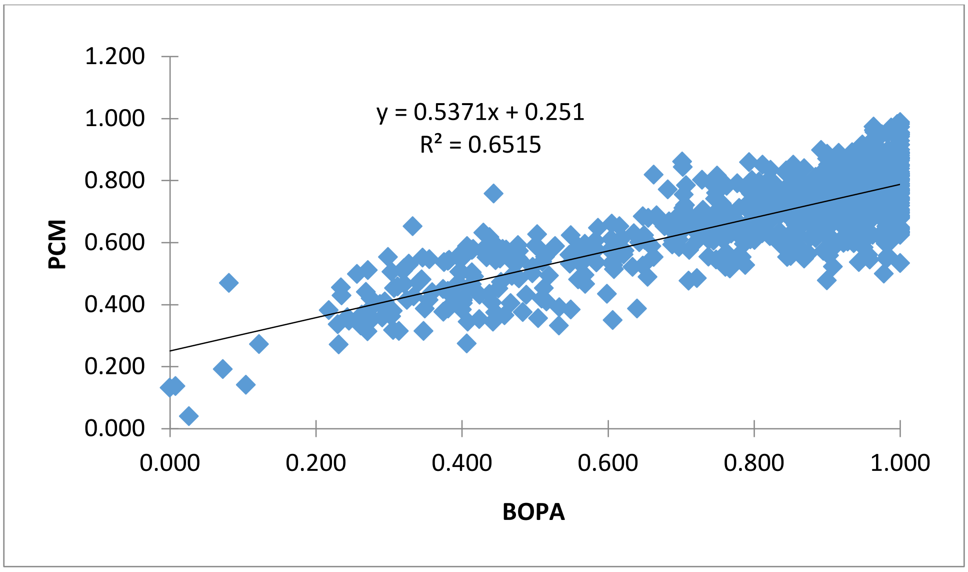

Figure 5.2 Relationship between the Pseudo-Common metric and the BOPA nsEQR values

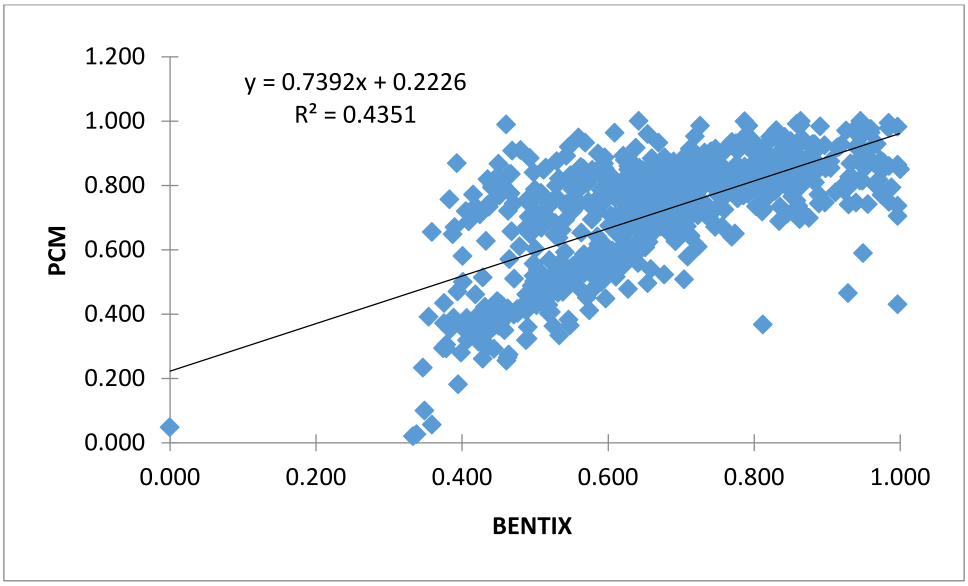

Figure 5.3 Relationship between the Pseudo-Common metric and the BENTIX nsEQR values

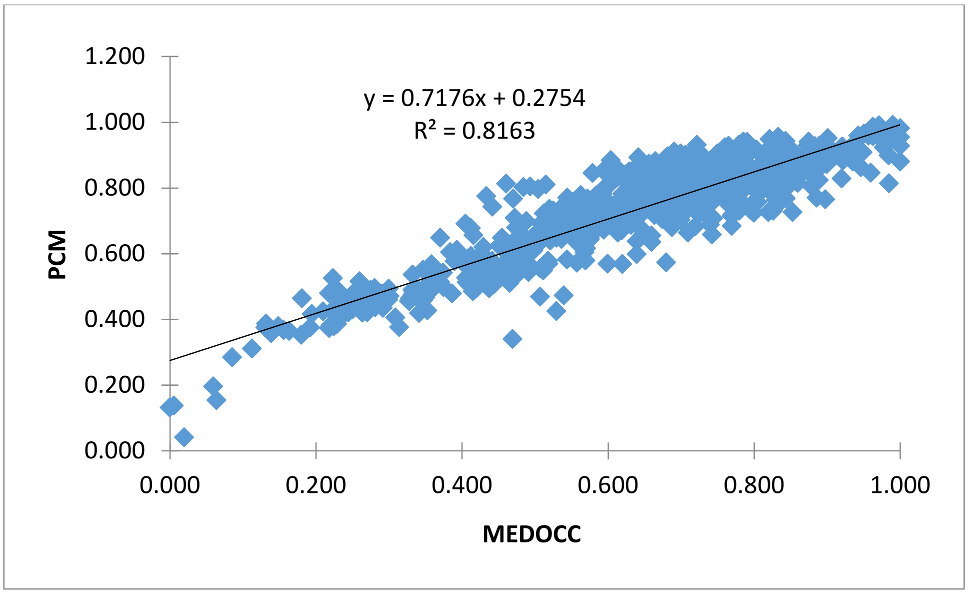

Figure 5.4 Relationship between the Pseudo-Common metric and the MEDOCC nsEQR values

All methods present a good correlation with the Pseudo-Common Metric, 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.

The assumptions of normally distributed error and variance (homoscedasticity) of model residuals is met.

The Pseudo Common metric represent all methods (r^2>0.5, absolutely r^2>0.3).

Observed minimum r^2 is at least half of the observed maximum r^2.

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

Parallel intercalibration 2

Italy and Slovenia with M-AMBI

Italy and Slovenia are using the same method, so the following results are the correlation coefficient and the probability of the regression analysis for Italian and Slovenian MAMBI standardised EQRs. The relation is highly significant, and the slope of the regression lies between 0.5 and 1.5.

Member State/Method	R^2	p
Italy and Slovenia-M-AMBI	0.966	P<0.001

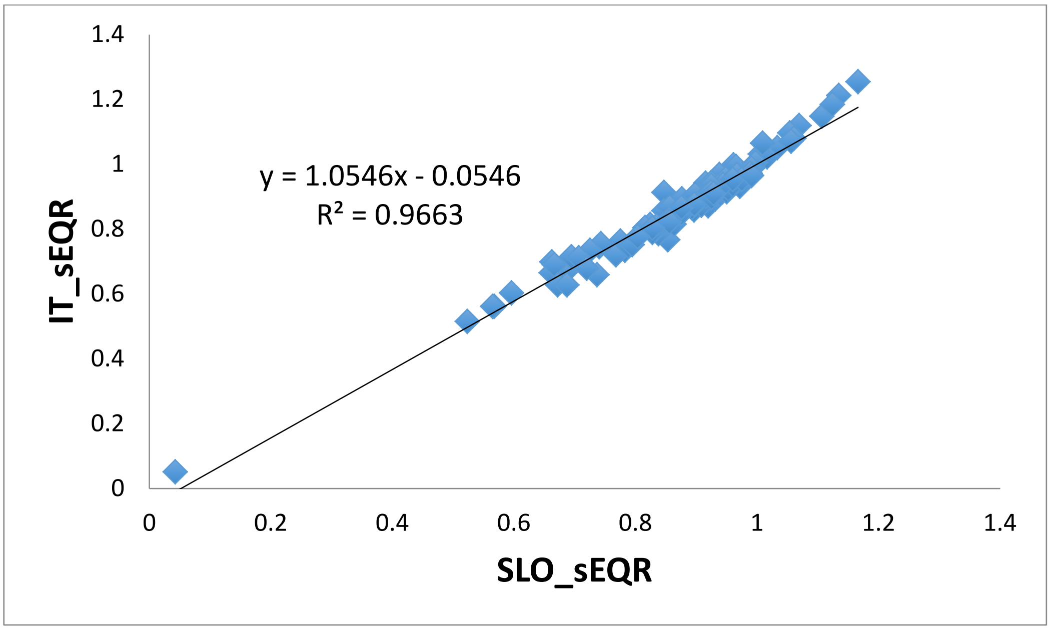

Figure 5.5 Relationship between the MAMBI sEQR values in Italy and Slovenia

5.3. Comparability criteria

Parallel intercalibration 1

Cyprus, France, Greece and Spain with BENTIX, AMBI, MEDOCC and BOPA

Assessing level of boundary bias

The Option 3a was applied using the excel sheet IC_Opt3_sub v1.24.

Spain, France, Greece and Cyprus also made parallel calculations in order to confirm the results obtained by the application of the excel sheet IC_Opt3_sub v1.24. These calculations were made in accordance to the Annex V of the Intercalibration Guidance and the work of Willby and Birk (2010).

The boundary comparison and harmonisation following the steps defined in the step 7 of the Annex V of the IC guidance:

- The lower (and upper) acceptable class boundary was defined by subtracting (adding) the permitted boundary deviation of 0.25 class in the respective class equivalents of the Member State from (to) the global mean or median boundary.
- The regression model between the (pseudo-)common metric and the national EQR was inverted, so it is straightforward to determine where national class boundaries should be positioned in order to secure an acceptable level of bias in the boundary comparison.
- The adjusted boundary was translated to the benchmarked national scale, and inverted the formula of the regression previously established: EQR of the Member State for the boundary = $(y_{harmonized}-c)/m$.

In the case of the comparison of national methods of Spain (all regions), Greece, Cyprus, and France, for the BOPA and AMBI methods, the boundary bias was >0.25 class equivalents, so boundary adjustment was needed. So, BOPA H/G and AMBI G/M were adjusted (Figure 5.6 and Figure 5.7).

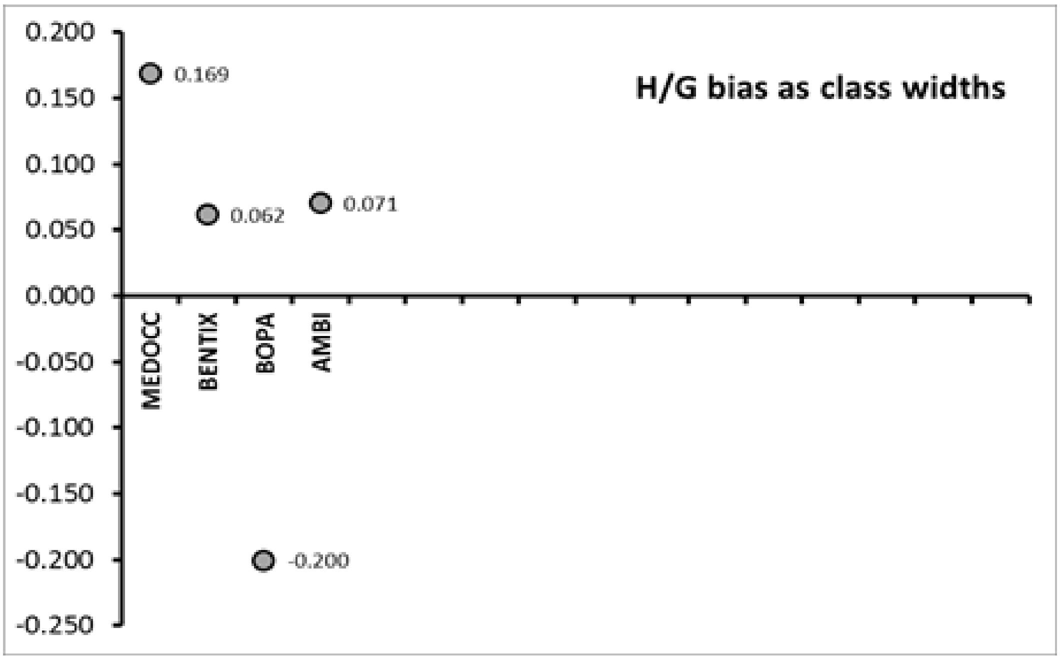

Figure 5.6 Comparison of the methods: GM boundary biases (GM- Good-Moderate class boundary)

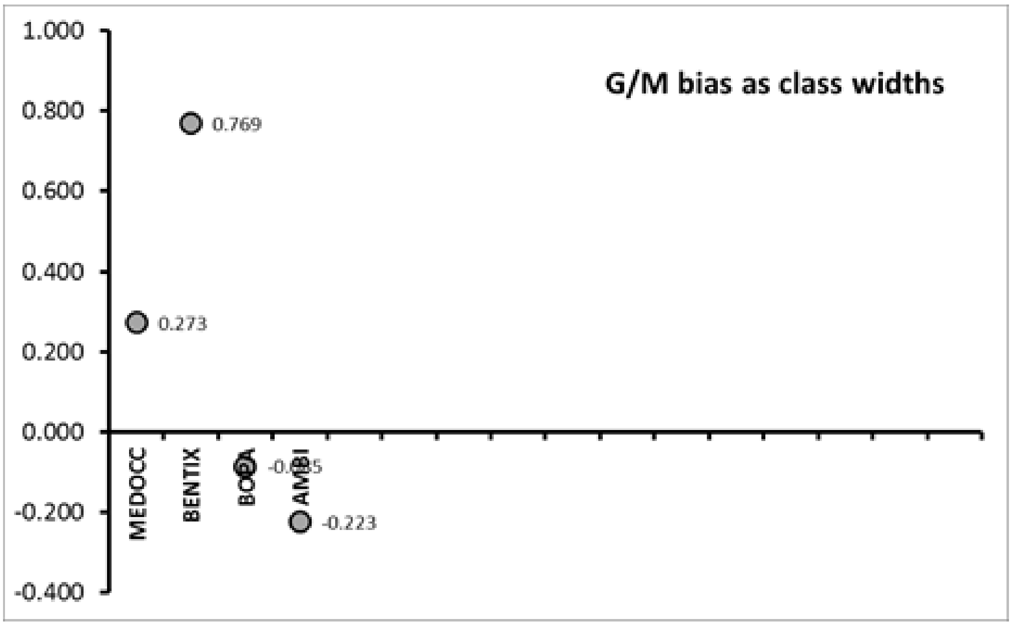

Figure 5.7 Comparison of the methods: HG boundary biases (HG-High Good class boundary)

Class Agreement:

Regarding class agreement criteria defined in the Annex V, the absolute average class difference for all the methods was <0.5 class, so they complied with the class agreement criteria. Also the Kappa analyses indicated an acceptable agreement (>0.4) between AMBI, MEDOCC, BOPA and BENTIX.

Parallel intercalibration 2:

Italy and Slovenia with M-AMBI

Assessing level of boundary bias

The comparison was done using the excel sheet Revised_Option3_TwoMS_Final.No adjustment was necessary.

5.4. Class agreement check between all the methods of parallel IC 1 and parallel IC 2

On the basis of the obtained results, showing in general terms, a similar response of all the indices to anthropogenic pressures, we analyzed the agreement between ecological quality assessments obtained with the no diversity and diversity indices, through a Kappa analysis (Cohen, 1960; Landis and Koch, 1977). The Kappa test indicates whether the level of agreement between all indices is statistically significant ($p < 0.05$).

Kappa values indicated a low or poor agreement between the indices (0.29). Meanwhile, Kappa analyses indicated an acceptable agreement (>0.4) between AMBI, MEDOCC, BOPA and BENTIX. When the M-AMBI index is included in the analysis, the agreement is low (0.29). This result is coherent with the results obtained in the IC exercise, and it is suggested that the diversity parameter is the main responsible of the low relation between M-AMBI and the rest of the methods.

The existence of two IC groups is understood by the MED GIG experts as a normal result, as it can be reported in numerous scientific works. Many studies have shown the different response of the diversity to the disturbance, being in some cases inverse to the pressure level, as it is expected, but in other cases showing high values at the end of the disturbance gradient, depending on the area, habitat, pressures, etc. So, the results showed in the present IC exercise are in accordance to those obtained in other studies, showing the expected behavior of the diversity against the pressures gradient in some areas (as it occurs in the stations of Italy and Slovenia), or the uselessness of the diversity in the establishment of the ecological status in other zones (as it occurs in the stations of France, Spain, Greece and Cyprus).

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		**Benthic invertebrate fauna**	
Results coastal waters: Ecological quality ratios of national classification systems The following results apply to soft sediments only			
Member State	**National classification systems intercalibrated**	**Ecological Quality Ratios**	
		High-Good boundary	**Good-Moderate boundary**
Methods including diversity parameter			
Italy	M-AMBI -	0.81	0.61
Slovenia	M-AMBI -	0.83	0.62
Methods not including diversity parameter			
Cyprus	Bentix -	0.75	0.58
France	AMBI -	0.83	0.58
Greece	Bentix -	0.75	0.58
Spain (Murcia-Valencia-Andalusia regions)	BOPA -	0.95	0.54
Spain (Catalonia-Balearic islands)	MEDOCC index -	0.73	0.47

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

Croatia and Malta have not participated in the intercalibration exercise, being necessary in the future, the proposal of national methods and their posterior intercalibration.

7. Ecological characteristics

7.1. Description of reference or alternative benchmark communities

Description of the biological communities at reference sites or at the alternative benchmark, considering potential biogeographical differences:

In general terms, the benchmark communities are represented by a high abundance of sensitive species, and the almost absence of opportunistic species. The high status stated by the different national methods is defined as follows:

Greece and Cyprus (BENTIX):

The fauna is composed by mostly sensitive species (over 75%). The benthic fauna is usually very diverse and evenly distributed with no one species naturally dominating over 10%.

France (AMBI):

Sensitive species included in the Ecological Groups I and II represent over 60% of abundance, and opportunistic species (Ecological Groups IV and V) are almost absent.

Italy and Slovenia (M-AMBI):

Sensitive species included in the Ecological Groups (EG) I and II represent over 60% of abundance, and opportunistic species (Ecological Groups IV and V) are almost absent. There is a high taxa richness and diversity.

Spain - Catalonia and Balearic islands (MEDOCC):

Most species belong to EGI (sensitive species) and EGII (indifferent species). The fauna is composed of only sensitive (EGI: 90%) and indifferent species (EGII: 10%) in Catalonia; and sensitive (80%), indifferent (15%), and tolerant (5%) species in the Balearic Islands.

Spain - Murcia, Valencia and Andalusia regions (BOPA):

The fauna is only composed by sensitive species (amphipod group excepting *Jassa* genus) and there are no opportunistic polychaetes.

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).

Communities representing borderline conditions between good and moderate status are characterised by a decrease of the sensitive species and an increase of tolerant and opportunistic species.

The G/M boundary stated by the different national methods is defined as follows:

Greece and Cyprus (BENTIX):

At the G/M boundary, the percentage of tolerant species becomes over 60% (roughly 2/3 of the fauna) and the sensitive taxa less than 40% (1/3 of the fauna).

France (AMBI):

At the G/M boundary, sensitive taxa and opportunist species are present (10%), but tolerant species (EGIII) are the dominant group.

Italy and Slovenia (M-AMBI):

At the G/M boundary, sensitive taxa and opportunist species are present (10%), but tolerant species (EGIII) are the dominant group, 'H diversity is lower than about 3.5 and the Richness value is lower than about 20 species.

Spain - Catalonia and Balearic islands (MEDOCC):

The tolerant ecological group (EGIII) accounts for 50%, but sensitive taxa (EGI) are also present (10%)

Spain - Murcia, Valencia and Andalusia regions (BOPA):

At the G/M boundary, polychaetes frequency is 0.625 and amphipods frequency is approximately 0.325.

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Annex

A. Land Uses Simplified Index (LUSI)

Land Uses Simplified Index (LUSI) is a specific combination of pressures that influences a Water Body.

The selected pressures are related to main characteristics and uses of land that could have an influence on phytoplankton growth:

- Urban
- Industrial
- Agricultural (only irrigated land)
- Rivers (Typology based on salinity is used).

Each pressure has been categorized in two or three categories and each category has a score.

For urban, agricultural (irrigated) and industrial pressures, categories have been created depending on the % of surface used for this activity (Catalan land uses study of 1997). An area comprised between the coast line and 1,5 km inland and between the limits of each water body has been taken into account to associate a category of each pressure to each water body.

For river pressure, categories have been created depending on salinity, thus each water body has been assigned a category depending on its typology.

Categories and scores of each pressure are:

Urban	Agricultural (irrigated)	Industrial	River (Typology)	Score
	<10%	<10%	Type III	0
<33%	10 a 40%	>10%	Type II	1
33 a 66 %	>40%		Type I	2
>66%				3

For other significant pressures, different aspects have been taken into account. These are:

- Rivers, channels… that significantly affect, Score = 1
- Harbours that significantly affect, Score = 1
- Influence of adjacent water bodies that significantly affect, Score = 1.

For each water body all scores are summed. Afterwards, a correction is applied to the sum in order to take into account the degree of confinement that could emphasize or diminish the effect of these pressures on the water body. Depending on the shape of the coastal line the sum is multiplied by the correction number:

Confinement	Correction number
Concave	1.25
Convex	0.75
Straight	1.00

Finally LUSI is obtained as follows:

LUSI=
(Score urban + score agricultural + score industrial + score typology) * Correction number