UNIVERSIDADE DE VIGO

ANÁLISIS Y APLICACIÓN DE LOS RESULTADOS DE LA INTERCALIBRACIÓN EUROPEA A LOS MÉTODOS DE CLASIFICACIÓN DEL ESTADO ECOLÓGICO DESARROLLADOS PARA AGUAS SUPERFICIALES (RÍOS TEMPORALES Y AGUAS DE TRANSICIÓN) DE LA DEMARCACIÓN HIDROGRÁFICA DE LES ILLES BALEARS

RÍOS TEMPORALES

AGUAS DE TRANSICIÓN

Govern de les Illes Balears

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Análisis y aplicación de los resultados de la intercalibración europea a los métodos de clasificación del estado ecológico desarrollados para aguas superficiales (ríos temporales y aguas de transición) de la Demarcación Hidrográfica de les Illes Balears

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Under the title

"IC report Illes Balears (Spain). Fitting of new national classification methods of ecological status to the results of the complete intercalibration exercise"

This report has for objective to show that the Regional authorities of the Balearic Islands have developed a set of fully compliant Water Framework Directive (WFD) classification methods for the common type rivers and transitional waters that exist in the Balearic Islands, and to fill some of the gaps in the IC of the ecological assessment methods for the Balearic Islands. Through 4 chapters each Biological Quality Element (BQE) is approached.

CHAPTER 1: THE INVMIB (GAP 2)

The method is intercalibrated according to the final IC results, showing that (1) the method is compliant with the WFD normative definitions and (2) that their class boundaries are in line with the results of the IC exercise.

CHAPTER 2: THE DIATMIB (GAP 2)

The method is intercalibrated according to the final IC results, showing that (1) the method is compliant with the WFD normative definitions and (2) that their class boundaries are in line with the results of the IC exercise.

CHAPTER 3: THE MIBIIN (GAP 3)

It is not possible to intercalibrate the method according to the final IC results. We proceed with the description of the method to show its compliance with the WFD normative definitions and justify why the Intercalibration is not attainable. A detailed description of the assessment method and evaluation of its WFD compliance, and a description of the reasons why the IC was not feasible, is provided.

CHAPTER 4: THE PHYTOMIBI (GAP 3)

It is not possible to intercalibrate the method according to the final IC results. We proceed with the description of the method to show its compliance with the WFD normative definitions and justify why the Intercalibration is not attainable. A detailed description of the assessment method and evaluation of its WFD compliance, and a description of the reasons why the IC was not feasible, is provided.

CHAPTER 1

THE INVMIB A NEW CLASSIFICATION METHOD TO ASSESS WITH MACROINVERTEBRATES THE ECOLOGICAL STATUS OF RIVERS IN THE BALEARIC ISLANDS (MED GIG RIVERS)

CONTENTS

1.1. INTRODUCTION

The Mediterranean River GIG finalised the intercalibration (IC) exercise for Macroinvertebrates in the second IC phase, with the participation of 6 Member States (Portugal, France, Spain, Cyprus, Slovenia and Italy), that compared and harmonized their national assessment systems for the common IC types RM1, RM2, RM4 and RM5. All national methods addressed a group of common pressures, mainly general degradation, nutrients and organic matter, and used an IC Option 2 (comparison of assessment methods using a common metric). The intercalibration and harmonization were performed only with spring-summer data, with the exception of Cyprus and Italy. The lack of comparability between MS methods and the insufficient number of reference sites, made impossible the IC of one of the other GIG common types, the RM3.

This report has for objective to show that the Regional authorities of the Balearic Islands have developed a fully compliant Water Framework Directive (WFD) macroinvertebrate classification method (INVMIB) (García et al. 2014) for the common type RM5 that exists in the Balearic Islands, and to fit the assessment system to the completed intercalibration exercise.

The way forward for verifying the WFD compliance with normative definitions, IC feasibility, pressure-impact relationships and comparing boundaries, is described in the CIS Guidance Document n°30: “Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise” (Wilby et al. 2014).

1.2. DESCRIPTION OF NATIONAL ASSESSMENT SYSTEM

The **INVMIB** was developed using macroinvertebrate samples collected by the Illes Balears Government monitoring program in autumn 2005, winter 2006 and 2008, and spring 2005, 2006 and 2008. Here we describe the INVMIB compliance with WFD in relation with its sampling method, taxonomic resolution, calculation, international and national reference criteria, class boundaries establishment and pressure-impact relationships.

1.2.1. Sampling method and taxonomic resolution

Sampling was performed in three campaigns that took place in autumn (November–December), winter (February–March) and spring (April–May).

The sampling was done by wading in the river and taking the samples with a kick-net (500 µm mesh size). A total of 20 “sample units” distributed proportionately to the habitats present in 100 m length were taken (total sampled area of 2.5 m^2) and combined to a single mixed sample. The sampling followed a multi-habitat procedure adapted from EPA (Barbour et al. 1999) and is described in García et al. (2014).

Field samples were preserved with ethanol (70%), transported to the laboratory and stored until their treatment. Invertebrate samples were washed and sorted under tap water in three different fractions (5 mm, 0.5 mm and 0.1 mm), and sorted specimens were counted and identified under 40× magnification (stereo-binocular microscope) to the lowest possible identification level (at least family, and genus/specie if possible). Sub-sampling was made when necessary to obtain a representative fraction of the total community in the finer fractions (Wrona et al. 1982).

1.2.2. Multimetric Calculation

The classification system developed in this study consists of a multimetric index (INVMIB), type-specific for the different Balearic Islands national types: R-B01 (mountain streams), R-B02 (canyon streams) and R-B03 (lowland streams), all them corresponding to the IC common type RM5 for rivers. The criteria followed to select the metrics were: (1) to comply with the normative definitions of the WFD (European Union 2000), (2) to respond to the gradient of pressure, (3) to be able to discern reference values from disturbed sites, and (4) the metrics selected should be non-redundant.

The INVMIB is totally compliant with the WFD, as it is based on the use of individual metrics selected following the requirements of the normative definitions: a) abundance and taxonomic composition of the invertebrate community, b) sensitivity or tolerance of taxa to stressors, and c) taxa biodiversity (see García et al. 2014 for further details). Each individual metric was selected for its potential as indicator, and their response analysed along to pressure gradients. The finally selected metrics per stream type were averaged in the Invertebrate Multimetric Index of the Balearic Islands (INVMIB): INV1MIB (lowlands), INV2MIB (canyons) and INV5MIB (mountains) (Table 1.1). Some of these metrics such as the richness of sensitive taxa (RSS) were included in all the multimetric indices, while other metrics gave reliable results only in one stream type.

Table 1.1. Summary of the metrics composing the INVMIB multimetric for the R-B01 (mountain), R-B02 (canyon) and R-B03 (lowland) stream types of the Balearic Islands. Its expected response to pressure, and the transformations and normalizations necessary to obtain it are also indicated (from García et al. 2014).

Stream type	INVMIB	Meaning	Response to pressure	Transformation	Inversion	Normalisation
Lowlands (INV1MIB)	Bray Curtis	Bray-Curtis Index	-	A fraction of unity	No	Median
	PABST	% of abundance of tolerant taxa	+	A fraction of unity	Yes	
	RSS	Richness of sensitive taxa	-	No	No	
Canyons (INV2MIB)	EPHEMAb	Abundande of Ephemeroptera	-	Log (x+1)	No	Median
	Margalef index	Diversity of Margalef	-	No	No	
	RSS	Richness of sensitive taxa	-	No	No	
Mountains (INV5MIB)	Bray Curtis	Bray-Curtis Index	-	A fraction of unity	No	Median
	PABST	% of abundance of tolerant taxa	+	A fraction of unity	Yes	
	RSS	Richness of sensitive taxa	-	No	No	
	EPT index	EPT % abundance of classes	-	A fraction of unity	No	

1.2.3. Ecological status assessment

The ecological status is defined on the basis of the Ecological Quality Ratio (EQR), calculated by dividing each resulting value of the INVMIB by the median value of the reference data:

EQR_INVMIB = measured INVMIB value / median INVMIB values of references

Then the obtained values ranged from 0 (worst quality) to >1 (best quality), being the ecological status classified by one of five classes (high, good, moderate, poor and bad) shown in Table 1.2.

Table 1.2. Ecological status levels and corresponding national boundaries assignment.

Boundaries	Level of disturbance	Quality class	Colour
		Reference	
> 0.93	Minimal	High	
0.93-0.73	Slight	Good	
0.73-0.50	Moderate	Moderate	
0.50-0.25	Major	Poor	
< 0.25	Severe	Bad	

1.2.4. National reference criteria

The reference sites of the Balearic Islands had to satisfy a series of a priori selection criteria based on the absence of significant pressures in their basins, considering the percentages of artificial and agricultural land uses, absence of sewage effluents, no hydromorphological alterations of the stream bank and absence of significant flow regulation (Pardo et al. 2012).

In a first phase, 8 sites fulfilling the next criteria (Pardo et al. 2010) were identified:

- Land uses in the basin have not been recently intensified.
- There are no artificial surfaces in the catchment.
- % of agriculture < 25% (considering rainfed lands and in absence of irrigated lands).
- Absence of sewage effluents.
- Absence of longitudinal channel alterations.
- Absence of marked discharge reductions in the catchment.
- Absence of flow regulation due to upstream dams.
- Absence of stream bank significant alterations. Connectivity with adjacent trees is maintained.

Another 4 sites were also considered as references regarding its biology, and fulfilled the prior mentioned criteria with slight modifications:

- Artificial surfaces (< 0.4% of catchment area)

- % of agriculture < 35% (natural vegetation and rainfed lands. Absence of irrigation).

In a second phase, pressure levels for artificial and agricultural surfaces were restricted to 0% and ≤ 7% of catchment area respectively, following the conclusions of the review on European reference thresholds by Pardo et al. (2011). A posterior check with water quality levels and invertebrate communities was also performed to assure the absence of these pressures in this area (Pardo et al. 2012). Finally 12 reference sites were identified according to the mentioned reference selection criteria, represented by a total of 65 macroinvertebrate samples (Table 1.3 and Table 1.4).

Table 1.3. List of temporary streams and sites fulfilling the Balearic Island reference criteria.

Site code	Site name	Island	Type	xUTM	yUTM
AB240	D'Almadra torrent	Majorca	Mountain	483556	4402329
AC19	Comafreda-Guix torrent	Majorca	Canyon	491515	4406292
AC25	Mancor de la Vall headwaters	Majorca	Mountain	485564	4403901
AK28	Matzoc torrent	Majorca	Lowland	533259	4400924
AN260	Coccons	Majorca	Lowland	528499	4398000
B1000	Gorg Blau	Majorca	Mountain	499504	4416150
B2000	Ternelles 3	Majorca	Mountain	499822	4417133
B2001	Ternelles 5	Majorca	Mountain	499754	4416028
G3000	Ses Comer torrent	Majorca	Mountain	492650	4414304
H12	Gorg Blau	Majorca	Canyon	485051	4408100
H220	Lluc	Majorca	Canyon	487000	4409854
K2600	Biniaratx-Camidel L´Ofre	Majorca	Canyon	478464	4402040

Table 1.4. Major environmental features that characterize the Balearic Island stream types. Reference and non reference number of sites and number of samples (in brackets) per type are indicated (from García et al. 2014).

	Stream type		
	Lowlands	**Canyons**	**Mountains**
Reference sites	2 (10)	4 (23)	6 (32)
Non-reference sites	30 (123)	5 (20)	13 (62)
Total	32	9	19
Altitude (m)	6-93	39-333	23-314
Slope (%)	0.2-2.3	2.2-13.9	1.3-10.9
Catchment (km^2)	5.4-151.1	7.0-27.8	6.9-35.1
Water permanence (months)	5-12	5-12	8-12
Island	Majorca	Majorca	Majorca, Minorca & Ibiza

1.2.5. National boundary setting

- Methodology used to set H/G boundary: The H/G was set as the 25th percentile (P25) of the EQR_INVMIB reference values.
- Methodology used to set the remaining boundaries: Below the 25th percentile, the remaining quality class boundaries were defined into equal bands.

The G/M boundary was set as H/G boundary- (P_{25} /4)

The M/P boundary was set as G/M boundary- (P_{25} /4)

The P/B boundary was set as M/P boundary- (P_{25} /4)

A posterior adjustment and confirmation of the boundaries was produced looking at the crossing between the fitted regression lines of paired individual metrics, for its ecological interpretation following the “Guidance on the intercalibration process 2008-2011” (Schmedtje et al. 2009). The obtained class boundaries are illustrated in Table 1.5.

Table 1.5. Officially used class boundaries for the INVMIB method.

Class Boundary	High/Good	Good/Moderate	Moderate/Poor	Poor/Bad
INVMIB	0.93	0.73	0.5	0.25

1.2.6. Pressures-impact relationships

A Multimetric index and per ende the individual metrics have to respond to pressure gradients. The Balearic Island streams (torrents) are mainly affected by pressures related to temporary organic contamination, diffuse source pollution by nutrients and hydromorphological alterations of riverbeds and riverbanks.

For the INVMIB case the pressures-response relationships were evaluated along a general degradation gradient and a gradient of organic or hydromorphological pressure for each stream type. The information of the different pressures was explored through Principal Component Analyses (PCAs) to reduce the information of all environmental variables into different stressor gradients: 1) a general degradation gradient (DEG), that integrates the whole information and variation from different pressures in all stream types, and 2) an organic degradation gradient by stream type (i.e., organic degradation (ORG): oxygen demand, nutrient concentrations, dissolved oxygen, discharge and forestry land use in the basins) (García et al. 2014). Significant correlations among the individual metrics and the INVMIB index, with the degradation and organic gradient obtained from PCAs were found (Table 1.6). In lowland streams, the INV1MIB responded positively to the axis I DEG (i.e.,–agricultural areas and –NO3) and to the axis I ORG (i.e., –NO3 and +O2) (García et al. 2014). In canyon streams, the INV2MIB and also the individual metrics responded positively to the axis IDEG (i.e., –NO3 and +O2) and to the axis II ORG (i.e., +forestal areas and –NO3) (García et al. 2014). While the INV5MIB (mountain streams) and its individual metrics showed the strongest responses to both stressor gradients (García et al. 2014).

Table 1.6. Summary of the Spearman correlation coefficients between the INVMIB and the individual metrics, with the general degradation gradient and the organic degradation gradient. *p < 0.05 (from García et al. 2014).

Stream type	Variables	Axis 1 DEG	Axis 2 DEG	Axis 1 ORG	Axis 2 Org
Lowlands	INV1MIB	0.496*	0.016	0.334*	0.353*
	Bray Curtis index	0.328*	-0.098	0.156	0.254*
	PABST	-0.287*	-0.169	-0.411*	-0.138
	RSS	0.528*	-0.167	0.153	0.410*
Canyons	INV2MIB	0.505*	-0.223	0.202	0.589*
	EPHEMAb	0.361*	-0.132	0.330*	0.376*
	Margalef index	0.303*	-0.268	0.072	0.485*
	RSS	0.531*	-0.081	0.150	0.552*
Mountains	INV5MIB	0.676*	0.113	-0.753*	-0.500*
	Bray Curtis index	0.571*	0.135	-0.637*	-0.298*
	PABST	-0.544*	-0.061	0.550*	0.342*
	RSS	0.546*	0.143	-0.665*	-0.453*
	EPT index	0.616*	0.108	-0.716*	-0.494*

Excepting for the INV2MIB (where there are no strong antrophic pressures), for the rest of INVMIBs the results showed a clear sample segregation along the organic gradient according to the multimetric values, thus confirming the function of the INVMIB as indicator of organic degradation. All INVMIBs also showed a clear response to the general degradation gradient, and when the pressure increase, the macroinvertebrate community is affected and a gradual decline in ecological quality occurs (Fig. 1.1).

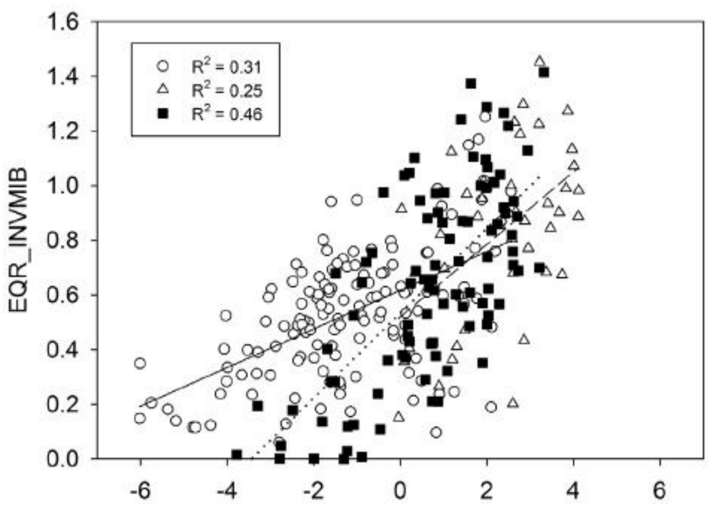

Figure 1.1. INVMIB distribution along the general degradation gradient (Axis1DEG). R-squares are given for each stream type (p < 0.001). Lowland streams (white circles), canyons (white triangles) and mountain streams (dark squares) (from García et al. 2014).

1.3. COMPLIANCE WITH WFD NORMATIVE DEFINITIONS

After the compliance check we concluded that the INVMIB method fulfils all the requirements of the WFD (Table 1.7).

Table 1.7. Assessment of INVMIB compliance with the WFD normative definitions.

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by one of five classes (high, good, moderate, poor and bad).	**Yes**
2. High, good and moderate ecological status are set in line with the WFD's normative definitions (Boundary setting procedure)	**Yes,** see section "National boundary setting".
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.	**Yes,** the INVMIB index is an average of different normalized metrics depending on the stream national type and covering all relevant parameters.
4. Assessment is adapted to intercalibration common types that are defined in line with the typological requirements of the WFD Annex II and approved by WG ECOSTAT	**Yes,** the assessed sites were all assigned to the RM5 common type .
5. The water body is assessed against type-specific near-natural reference conditions	**Yes,** rivers are assessed against existing type-specific near-natural reference conditions.
6. Assessment results are expressed as EQRs	**Yes**
7. Sampling procedure allows for representative information about water body quality/ ecological status in space and time	**Yes,** sampling method complies with the IC criteria. Sampled from 2 to 3 times a year.
8. All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure	**Yes,** the sampling procedure consider both composition and relative abundance.
9. Selected taxonomic level achieves adequate confidence and precision in classification	**Yes,** as minimum family level (requirement for IC) and when possible genus or specie

1.4. IC FEASIBILITY CHECK

1.4.1. Assessment concept

The MED GIG concluded that the IC is feasible as all the methods in the finalized exercise follow similar assessment concepts, based on sampling of different relevant habitats in the reach and including taxa or taxa groups which are sensitive to different kinds of degradation. INVMIB is in line with these assessment concepts. Thus, the method can be accepted and **the intercalibration is feasible.**

1.4.2. Typology agreement

According to system A, the Balearic Islands belong to the Iberic-Macaronesian ecoregion. They are influenced by the Mediterranean climate and the discharge regime is subject of strong seasonal and annual variability (García et al. 2014). The hydrological system of the islands is characterized mainly by the existence of temporary streams called “torrents”, which fit within the characteristics of the IC type RM5. Obligatory descriptors of the WFD (System A: altitude typology, size typology and geology) and other such as slope and bank morphology were used to separate these temporary streams into 3 types (Pardo et al. 2010):

R-B01. Mountain streams (located in the mountains). Intermediate slopes.

R-B02. Canyon streams (deep valley between cliffs carved from the landscape, isolated and with high slopes). These streams have water most of the year, although during summer water is restricted to pools.

R-B03. Lowland streams. These streams are the most abundant type in the Balearic Islands, located at low altitude and close to the most populated areas.

The contrast of the proposed typology with the biological elements supported the definition of three different macroinvertebrate reference communities for the three geomorphologically predefined stream types (R-B01, R-B02 and R-B03) (Fig. 1.2). This approximation is followed in this report, considering three stream types with type-specific macroinvertebrate communities.

Within the Med GIG five common IC river types were considered for intercalibration of macroinvertebrates (Table 1.8). While RM1, RM2 and RM4 types were treated together throughout the IC process, RM5 was treated separately due to its distinct hydrological conditions. The RM3 was not intercalibrated in the MED GIG IC exercise.

In conclusion, for macroinvertebrates the three types established for the Balearic Islands fulfil the typological criteria of the IC common type RM5, and thus **the intercalibration is feasible**.

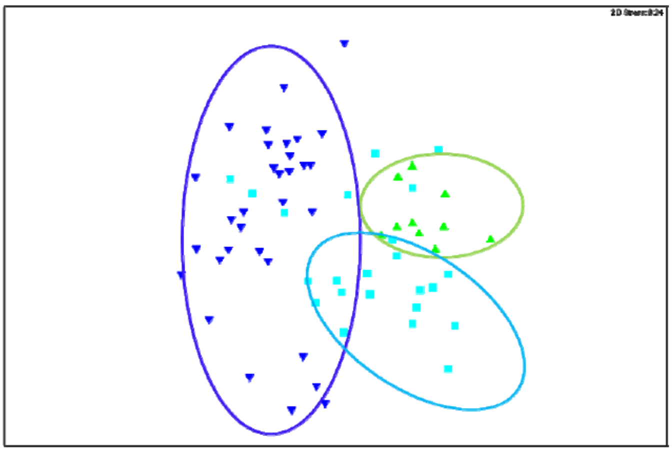

Figure 1.2. Ordination (NMDS) of the macroinvertebrate communities from reference sites of the different stream types defined a priori for the Balearic Islands. Mountain (dark blue triangles), Canyon (light blue squares) and Lowland (green triangles) (from Pardo et al. 2010).

Table 1.8. Common IC river types in the Mediterranean GIG and MS sharing the types.

Common IC Type	Type characteristics	MS sharing IC common type
RM1	Catchment < 100 km^2; mixed geology (except non-siliceous); highly seasonal	SP, FR, IT, PT, SI
RM2	Catchment 100-1000 km^2; mixed geology (except non-siliceous); highly seasonal	SP, IT, PT, SI
RM3	Catchment 1000-10000 km^2; mixed geology (except siliceous); highly seasonal	This type cannot be intercalibrated due to the lack of comparability between MS methods and insufficient number of reference sites.
RM4	Non-siliceous streams; highly seasonal	IT, CY, SP, FR
RM5	Temporary rivers	SP, IT, PT, CY, SI

1.4.3. Pressure addressed

As stated in the subsection *"Pressures-impact relationships"* within *Section 1.2*, the multimetric and individual indices response to pressures were addressed by analysing the pressure gradient according to the streams national types, and evaluating both a general degradation gradient and a gradient of organic pressure. Results indicated that the INVMIB responds well to both pressure gradients, showing that when the pressure increases the macroinvertebrate communities are affected and a gradual decrease in the ecological quality occurs. Thus, in terms of pressure it could be concluded that **the intercalibration is feasible**.

1.4.4. National dataset used

The datasets described in this report were checked against the data acceptance criteria as defined in the MED GIG rivers milestone 6 (Feio 2011) report for macroinvertebrates (Table 1.9).

The national dataset used for the INVMIB development (testing of pressures-response relationships, boundary setting procedure…) comprised a total of 270 samples coming from 60 sites, all them representing the RM5 IC common type. While the national dataset considered for the IC exercise comprises only 109 samples corresponding to winter and spring campaigns, and coming from 43 of the river sites. The existing reference samples within the whole dataset fulfilling IC criteria for benchmarking and corresponding to winter and spring campaigns were considered for IC. As well as a representative and equitable number of non reference sites, covering all the national stream types of the Balearic Islands and all the ecological status classes according to INVMIB. In the MED GIG exercise the data used in the calculations for intercalibration and harmonization were restricted to spring-summer data, with the exception of CY and IT, as for the remaining MS boundaries were defined based on spring-summer data. However, the studies realised in the Temporary streams of the Balearic Islands have concluded that mean annual assessment of the ecological status in the temporary streams related best with the assessment done in late winter and early spring (from February to May) (Pardo et al. 2010). For that in this exercise we have used data corresponding to this period as the optimum to fit the boundaries with results from the complete Mediterranean rivers IC.

Both the typology and the geographical area coverage by the used dataset are considered good enough. For each sample land use data (artificial, agricultural, forest and seminatural areas), physico-chemical data (alkalinity, oxygen, pH, temperature, conductivity, BOD5, orthophosphates, nitrates, nitrites, ammonium, sulphates and cations) and biological data (macroinvertebrate taxalists) are available (all in excel files) (summary in Table 1.10).

Table 1.9. Summary of the data acceptance criteria of the MED GIG rivers for macroinvertebrates.

Data acceptance criteria	Data acceptance checking
Data requirements (obligatory and optional)	Common pressure data, common environmental data, correctly checked typologies and geographical location and biotic data, all properly introduced in harmonized excel files.
The sampling and analytical methodology	All MS sampling methods use a multi-habitat approach. All MS have indicated a response of their indices to pressure using statistical tools.
Level of taxonomic precision required and taxa lists with codes	Family level is required.
The minimum number of sites / samples per intercalibration type	A minimum of 15 benchmark sites by IC type are available.
Sufficient covering of all relevant quality classes per type	All 5 water quality classes are represented

Regarding the sampling and analytical methodology, as mentioned in *Section 1.1* the sampling method is based in a multi-habitat procedure adapted from EPA (Barbour et al. 1999). The taxa were identified to family level as minimum, and to genus or species if possible.

The 5 classes of ecological status are covered within the dataset and the obtained pressure gradients are considered appropriate. The final classification results from the INVMIB indicated that the percentage of samples in the different status classes were 23.7% (Reference), 8.1% (High), 12.8% (Good), 22.4% (Moderate), 20.8% (Poor) and 12.2% (Bad) (García et al. 2014).

In conclusion, for macroinvertebrates the three types established for the Balearic Islands fulfilled the data acceptance criteria of the MED GIG, and thus the dataset could be considered enough and **the intercalibration is feasible**.

Table 1.10. Summary of environmental variables registered within reference and non-reference groups of sites, separated by stream type for the whole dataset (mean ± SD) (from García et al. 2014).

	Reference sites			No-reference sites		
	Lowlands (N = 10)	**Canyons (N = 23)**	**Mountains (N = 32)**	**Lowlands (N = 123)**	**Canyons (N = 20)**	**Mountains (N = 62)**
Conductivity	747.86 ± 29.46	387.44 ± 41.33	753.68 ± 55.5	1573.93 ± 64.12	515.48 ± 36.68	838.07 ± 43.18
pH	7.66 ± 0.12	8.17 ± 0.09	8.04 ± 0.06	7.86 ± 0.03	7.96 ± 0.11	7.79 ± 0.05
Oxygen (mg/l)	9.36 ± 0.56	10.15 ± 0.51	9.65 ± 0.19	8.34 ± 0.28	9.32 ± 0.33	8.22 ± 0.38
N_NO2 (mg/l)	0.32 ± 0.13	0.34 ± 0.13	0.54 ± 0.12	2.96 ± 0.42	0.52 ± 0.1	1.03 ± 0.15
N_NH4 (mg/l)	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.39 ± 0.12	0.02 ± 0.01	0.19 ± 0.1
P_PO4 (mg/l)	0.01 ± 0.01	0.02 ± 0.01	0.02 ±0.0	0.43 ± 0.09	0.02 ± 0.0	0.27 ± 0.09
Chlorophyll a (mg/m2)	5.92 ± 2.23	9.01 ±2.44	7.24 ± 1.25	20.55 ± 1.92	9.58 ± 1.48	19.05 ± 2.48
AFDM (g/m2)	6.18 ± 1.07	9.88 ±2.14	7.73 ± 1.83	15.4 ± 1.36	15.28 ± 3.01	13.49 ± 1.42
Artificial areas (%)	0.0 ± 0.0	0.66 ± 0.25	0.0 ± 0.0	1.61 ± 0.17	1.32 ± 0.34	2.34 ± 0.27
Agricultural areas (%)	18.01 ± 3.78	6.92 ± 2.49	6.33 ± 0.45	53.2 ± 1.72	24.95 ± 1.14	27.38 ± 0.8
Forest and seminatural areas (%)	81.99 ± 3.78	92.42 ± 2.74	93.67 ± 0.45	45.14 ± 1.76	73.73 ± 1.41	70.18 ± 0.87
HQA (Habitat Quality Assessment)	36.0 ± 2.67	38.94 ± 0.41	45.8 ± 1.8	34.78 ± 0.92	32.69 ± 2.31	38.19 ± 1.3
HMS (Habitat ModificationScore)	2.0 ± 0.67	12.0 ± 3.9	15.33 ± 3.7	34.61 ± 2.7	21.65 ± 4.17	31.6 ± 2.98

1.4.5. Conclusions

It is concluded that it is feasible applying the fit-in procedure to INVMIB, fitting the method to the results of the completed MED GIG river macroinvertebrates IC exercise.

1.5. IC PROCEDURE

Following the diagram for the selection of the fitting procedure (in CIS Guidance nº30), case A1 (Option 2) was identified as the option to carry out the intercalibration exercise. Thus the procedure for this case from the manual (Willby et al. 2014) is applied for fitting the INVMIB method to the River MED GIG results for the RM5 common type.

1.5.1. Requirements

a. Full details of the common metric (e.g. species scores and metric weights).

In the MED GIG it was concluded that the Intercalibration Common Metric index, ICMi (according to Buffagni et al. 2006) was the best option to use. The ICMi is a multimetric composed of six macroinvertebrate metrics: average score per taxon (ASPT), log 10 (sel_EPTD+1), 1-GOLD, total number of taxa Families, number of EPT taxa (Families) and the Shannon-Wiener diversity index. The multimetric is calculated by adding the 6 weighed metrics (the weight for each metric is in Table 1.11; Buffagni et al. 2005). Normalization of the metrics before adding it is needed, and achieved by dividing the observed values by the median value of each metric in the reference sites of the national dataset.

Table 1.11. Weight given to each of the individual metrics composing the ICMi.

Metric	Weight
ASPT	0.033
log10(selEPTD+1)	0.266
1-GOLD	0.067
nº Families	0.167
EPT	0.083
Shannon-Wiener H'	0.083

Finally the EQR_ICMi is calculated by dividing the observed ICMi values obtained, by the median value of the reference sites.

b. A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated.

A total of 109 samples were available covering the 5 classes of ecological status (see subsection *"National dataset used"* in *Section 1.4*).

c. Accompanying pressure data in the same format as that used in the completed exercise.

In subsection *"National dataset used"* within *Section 1.4*, pressure data for the whole dataset are available. In Table 1.12 are indicated some pressure ranges considering only the 109 winter and spring samples included in this IC exercise.

Table 1.12. Range of values of some physico-chemical variables at those sites and samples included in the intercalibration.

		Winter		Spring	
	n	Minimum	Maximum	Minimum	Maximum
Conductivity (µS/cm)	109	214.5	1875.0	245.0	2703.0
pH	109	6.7	8.5	6.1	8.5
O_2 (%)	109	13.3	135.1	1.5	170.0
T (ºC)	109	7.2	17.7	12.9	25.2
N_NO_3 (mg/L)	109	0.005	4.802	0.005	12.764
N_NH_4 (mg/L)	109	0.001	0.390	0.001	7.799
P_PO_4 (mg/L)	109	0.001	2.232	0.001	5.876
SO4 (mg/L)	109	1.02	155.32	3.33	182.74
Cl^- (mg/L)	109	13.57	638.17	18.40	423.38
Ca^{2+} (mg/L)	109	15.50	177.00	6.50	243.43
Mg^{2+}(mg/L)	109	2.79	78.7	3.84	66.44
Na^+ (mg/L)	109	4.50	514.20	6.56	318.00
K^+ (mg/L)	109	0.38	39.90	0.39	50.33

d. Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites (e.g. human population density, extent of agricultural land in catchment, nutrient concentrations etc.).

In the IC exercise the MED GIG established the Reference thresholds (Table 1.13) conditioning the benchmarks acceptance, for each pressure variable and for all IC types. Thus providing a common tolerance level for all the types, with the exception of RM5 where different ranges for water oxygenation were established for low water periods.

Table 1.13. Thresholds established for each pressure variable and used by the MEDGIG for IC.

Pressure variables	RM1+RM2+RM4	RM5
Channelization (classes 1-4)		
Bank alteration (classes 1-4)		
Connectivity (classes 1-4)		
Local hábitat alteration (classes 1-4)	≤ 2	
Stream flow (classes 1-4)		
Upstream dams influence (classes 1-4)		
Hydropeaking (classes 1-4)		
Riparian vegetation (classes 1-4)		
DO (mg/L)[1]	6.39-13.70	
O_2 (%)	73.72-127.92	60.34-127.92
$N\text{-}NH_4^+$ (mg/L)	≤ 0.09	
$N\text{-}NO_3^-$ (mg/L)	≤ 1.15	
P-Total (mg/L)	≤ 0.07	
$P\text{-}PO_4^{3-}$ (mg/L)	≤ 0.06	
% Artificial areas (catchm)	≤ 1	
% Intensive agriculture (catchm)	≤ 11	
% Extensive agriculture (catchm)	≤ 32	
% Semi-natural areas (catchm)	≥ 68	
% Urbanisation (reach)[2]	≤ 1	
% Land use (reach)[2]	≤ 20	
% Agriculture (reach)[2]	≤ 20	

[1] for macrophytes only, instead of O_2 (%)

[2] for diatioms only, instead of land use in the catchment

e. Details of exactly how the benchmarking was undertaken in the completed exercise (e.g. creation of a common metric EQR by dividing the observed value by the median common metric value of a set of national reference or benchmark sites). If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method.

The given benchmark criteria were applied by each MS to identify benchmark sites within each national dataset. Median values of individual metrics of the national benchmarks datasets were used for calculation of the common metric index, and median values of the resultant ICMi for the same national benchmarks were used for the calculation of the common metric EQR. Linear regression was established between the EQR values of the national method and the common metric (ICMi) so that the national boundaries could be translated to ICMi using the equation.

f. Values of the global mean view of the HG and GM boundaries on the common metric scale for Member States who participated in the completed exercise.

Mean H/G (relevant for RM5): 0.990 (original); 0.975 (harmonized)

Mean G/M (relevant for RM5): 0.722 (original); 0.722 (harmonized)

1.5.2. Process

1. Calculate the common metric (ICM) on the national dataset.

The common metric used in the IC exercise (ICMi) was calculated according to the formula:

ICMi = (0.033 * N_ASPT) + (0.266 * N_Log10(selEPTD+1)) + (0.067 * N_1-GOLD) + (0.167 * N_nºFam) + (0.083 * N_EPT) + (0.083 * N_H‘)

For each of the 3 Balearic Islands types, we used type specific reference values for the individual metrics that represent the median value of each metric in the reference sites of the national database (in this case the reference sites complaining reference selection criteria and corresponding to samples collected during winter and spring campaigns, that were the ones subject of this IC exercise). ICMi results are shown in Table 1.14.

Table 1.14. List of national winter and spring IC sites with its INVMIB and ICMi values, as well as the corresponding EQRs. The sample code indicated in the table is a combination of the site code + campaign (pri = spring; inv = winter) + year.

	National assessment		IC metrics	
Sample code	INVMIB	EQR_INVMIB	ICMi	EQR_ICMi
AK28INV06	3.438	1.149	1.019	1.028
AK28INV08	3.026	1.011	1.141	1.150
AK28PRI06	3.499	1.169	0.980	0.988
AN260INV06	2.764	0.924	1.003	1.012
AN260INV08	3.747	1.252	0.948	0.956
AN260PRI08	2.837	0.948	0.634	0.639
AC19INV06	3.066	1.010	0.964	0.927
AC19INV08	3.314	1.091	1.094	1.052
H12INV06	3.850	1.268	1.035	0.995
H12INV08	3.964	1.306	1.045	1.005
H12PRI08	3.038	1.001	0.901	0.866
K2600INV06	3.052	1.005	1.051	1.010
K2600INV08	2.843	0.936	1.069	1.027
K2600PRI08	3.678	1.211	1.104	1.061
H220INV08	3.036	1.000	0.900	0.866
H220PRI08	2.875	0.947	0.795	0.764
AC25INV06	4.058	0.990	1.164	1.208
AC25PRI05	3.432	0.837	0.858	0.890
AC25PRI08	4.478	1.092	1.097	1.138
B1000_PRI08	3.682	0.898	0.974	1.010
B1000INV06	4.499	1.097	1.088	1.129
B1000INV08	4.521	1.103	1.048	1.087

Table 1.14. Continued.

	National assessment		IC metrics	
Sample code	**INVMIB**	**EQR_INVMIB**	**ICMi**	**EQR_ICMi**
B2000INV06	4.997	1.219	0.828	0.860
B2000INV08	5.267	1.285	1.098	1.140
B2000PRI05	5.065	1.235	1.015	1.053
B2000PRI08	5.177	1.262	1.000	1.038
B2001INV06	4.100	1.000	0.911	0.945
B2001INV08	4.389	1.071	0.932	0.967
B2001PRI05	3.547	0.865	0.683	0.709
B2001PRI08	3.562	0.869	0.954	0.990
G3000INV06	4.485	1.094	0.867	0.899
G3000INV08	5.813	1.418	0.948	0.984
G3000PRI05	3.324	0.811	0.717	0.744
G3000PRI06	4.241	1.034	1.028	1.066
AN260PRI06	2.961	0.989	0.615	0.620
H12PRI06	4.460	1.469	1.120	1.076
H12PRI05	3.794	1.250	1.028	0.989
K26INV06	3.113	1.025	1.025	0.986
J13INV06	2.930	0.965	0.924	0.888
L3000PRI08	2.876	0.947	0.888	0.853
K31PRI08	5.641	1.376	0.987	1.025
K31PRI06	4.313	1.052	1.034	1.073
B2001PRI06	4.237	1.033	1.153	1.196
AC25PRI06	4.154	1.013	1.084	1.125
B1000PRI06	4.018	0.980	1.007	1.045
B2000PRI06	3.980	0.971	0.977	1.013
K31INV08	3.953	0.964	0.900	0.934
K31INV06	3.873	0.945	0.884	0.917
AC25INV08	3.860	0.941	1.022	1.060
AF700INV08	2.599	0.868	0.896	0.903
F460INV06	2.398	0.801	0.712	0.717
AL3200INV06	2.309	0.771	0.605	0.610
B213INV06	2.293	0.766	0.848	0.855
Q520INV08	2.280	0.762	0.971	0.979
AF700PRI06	2.254	0.753	0.849	0.856
Q520PRI08	2.188	0.731	0.951	0.959
K2600PRI06	2.769	0.912	0.899	0.864
J13INV08	2.641	0.870	0.890	0.855
H220INV06	2.536	0.835	0.641	0.616
J13PRI05	2.455	0.809	0.754	0.725
K2101INV06	3.576	0.872	1.001	1.039
K2100INV06	3.559	0.868	0.831	0.862
K3100INV06	3.166	0.772	0.897	0.931
K2101PRI06	3.083	0.752	0.965	1.001
R516PRI08	2.126	0.710	0.796	0.802
F460PRI08	1.775	0.593	0.863	0.870
B216INV08	1.758	0.587	0.794	0.801
J560INV06	1.645	0.550	0.852	0.859

Table 1.14. Continued.

	National assessment		IC metrics	
Sample code	**INVMIB**	**EQR_INVMIB**	**ICMi**	**EQR_ICMi**
AN271PRI08	1.533	0.512	0.839	0.846
L3000INV08	2.107	0.694	1.061	1.021
J13_PRI08	2.061	0.679	0.642	0.617
K3100INV08	2.946	0.718	0.798	0.828
K23INV06	2.685	0.655	0.661	0.686
K2101PRI05	2.632	0.642	0.597	0.620
N79INV08	2.587	0.631	0.773	0.802
K2101INV08	2.530	0.617	0.887	0.920
Y288PRI08	2.494	0.608	0.939	0.974
K23INV08	2.331	0.568	0.798	0.828
V3190INV06	2.185	0.533	0.865	0.897
AB500PRI05	1.498	0.500	0.506	0.510
F459INV06	1.463	0.489	0.595	0.600
U470PRI06	1.413	0.472	0.616	0.621
B216PRI06	1.309	0.437	0.674	0.679
B213PRI06	1.119	0.374	0.351	0.354
AJ364PRI08	1.045	0.349	0.378	0.381
AB500INV08	0.918	0.307	0.375	0.378
Y274PRI05	1.380	0.455	0.543	0.522
Y274INV08	1.242	0.409	0.653	0.628
Y274PRI06	1.064	0.351	0.445	0.428
K2100PRI06	1.736	0.423	0.638	0.662
N79PRI06	1.606	0.392	0.603	0.625
Y289PRI08	1.501	0.366	0.406	0.421
V3190PRI05	1.174	0.286	0.459	0.476
N79PRI05	1.137	0.277	0.540	0.560
F459PRI05	0.711	0.237	0.502	0.506
F464INV06	0.703	0.235	0.406	0.409
AB500PRI06	0.615	0.205	0.393	0.396
AG254PRI06	0.543	0.181	0.395	0.398
AJ364PRI06	0.416	0.139	0.496	0.500
AC223PRI05	0.345	0.115	0.354	0.357
F464PRI06	0.345	0.115	0.307	0.310
Y274PRI08	0.581	0.191	0.369	0.355
L3001PRI06	0.438	0.144	0.291	0.280
Y289INV06	0.962	0.235	0.414	0.429
V319INV06	0.485	0.118	0.347	0.360
Y286PRI06	0.196	0.048	0.283	0.294
Y286INV08	0.112	0.027	0.217	0.225
Y286PRI05	0.008	0.002	0.210	0.218
Y286PRI08	0.000	0.000	0.196	0.203

2. Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.

The MED GIG provides numbers of benchmark samples instead of benchmark sites and we followed the same approach. After checking the MED GIG requirements for identifying benchmarking, a total of 34 reference samples (from the total of 54 available in the whole dataset for the winter and spring campaigns) were accepted as references and used in this IC exercise (Table 1.15).

Table 1.15. Balearic Islands winter and spring samples fulfilling criteria for benchmarking.

Sample code	Site name	Sampling year	INVMIB	EQR INVMIB	ICMi	EQR ICMi
AK28INV06	Matzoc torrent	2006	3.438	1.149	1.019	1.028
AK28INV08	Matzoc torrent	2008	3.026	1.011	1.141	1.150
AK28PRI06	Matzoc torrent	2006	3.499	1.169	0.980	0.988
AN260INV06	Coccons	2006	2.764	0.924	1.003	1.012
AN260INV08	Coccons	2008	3.747	1.252	0.948	0.956
AN260PRI08	Coccons	2008	2.837	0.948	0.634	0.639
AC19INV06	Comafreda-Guix torrent	2006	3.066	1.010	0.964	0.927
AC19INV08	Comafreda-Guix torrent	2008	3.314	1.091	1.094	1.052
H12INV06	Gorg Blau	2006	3.850	1.268	1.035	0.995
H12INV08	Gorg Blau	2008	3.964	1.306	1.045	1.005
H12PRI08	Gorg Blau	2008	3.038	1.001	0.901	0.866
K2600INV06	Biniaratx-Camidel L´Ofre	2006	3.052	1.005	1.051	1.010
K2600INV08	Biniaratx-Camidel L´Ofre	2008	2.843	0.936	1.069	1.027
K2600PRI08	Biniaratx-Camidel L´Ofre	2008	3.678	1.211	1.104	1.061
H220INV08	Lluc	2008	3.036	1.000	0.900	0.866
H220PRI08	Lluc	2008	2.875	0.947	0.795	0.764
AC25INV06	Mancor de la Vall headwaters	2006	4.058	0.990	1.164	1.208
AC25PRI05	Mancor de la Vall headwaters	2005	3.432	0.837	0.858	0.890
AC25PRI08	Mancor de la Vall headwaters	2008	4.478	1.092	1.097	1.138
B1000_PRI08	Gorg Blau	2008	3.682	0.898	0.974	1.010
B1000INV06	Gorg Blau	2006	4.499	1.097	1.088	1.129
B1000INV08	Gorg Blau	2008	4.521	1.103	1.048	1.087
B2000INV06	Ternelles 3	2006	4.997	1.219	0.828	0.860
B2000INV08	Ternelles 3	2008	5.267	1.285	1.098	1.140
B2000PRI05	Ternelles 3	2005	5.065	1.235	1.015	1.053
B2000PRI08	Ternelles 3	2008	5.177	1.262	1.000	1.038
B2001INV06	Ternelles 5	2006	4.100	1.000	0.911	0.945
B2001INV08	Ternelles 5	2008	4.389	1.071	0.932	0.967
B2001PRI05	Ternelles 5	2005	3.547	0.865	0.683	0.709
B2001PRI08	Ternelles 5	2008	3.562	0.869	0.954	0.990
G3000INV06	Ses Comer torrent	2006	4.485	1.094	0.867	0.899
G3000INV08	Ses Comer torrent	2008	5.813	1.418	0.948	0.984
G3000PRI05	Ses Comer torrent	2005	3.324	0.811	0.717	0.744
G3000PRI06	Ses Comer torrent	2006	4.241	1.034	1.028	1.066

3. Standardise the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise. If benchmark standardisation was concluded not to be required in the completed exercise the mean CM value of the joining method's benchmark sites must lie inside the range of mean values of the benchmark sites of the methods already intercalibrated for this conclusion to remain applicable. If the joining method's benchmark sites lie outside of this range the joining method must benchmark standardise its sites relative to the global mean CM value of the benchmark sites included in the completed exercise. These scenarios are illustrated in Table 1 and 2.

The MED GIG report didn't provide a range of mean CM values of benchmark sites of the methods already intercalibrated for all the MS. For determining the need of standardisation in this IC exercise, the mentioned range is inferred from two diagrams for the ICMi values from all benchmark sites provided in the MED GIG report for the RM5 common type (Fig. 1.3).

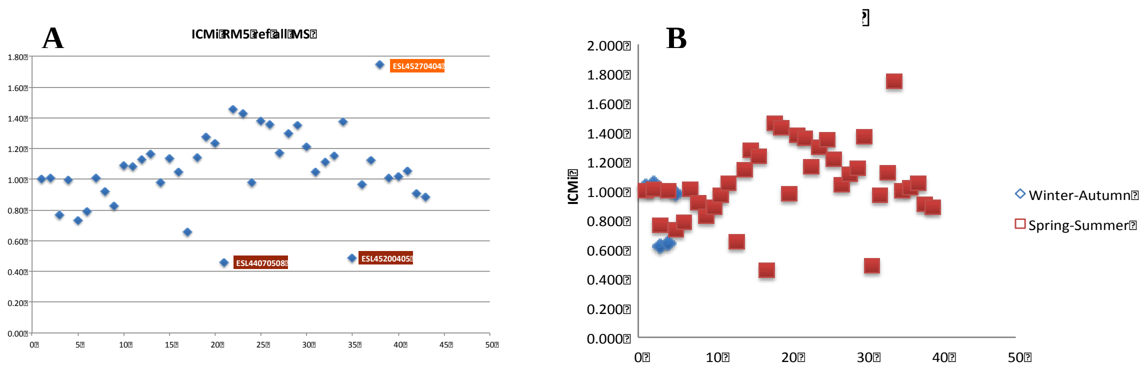

Figure 1.3. A) Distribution of ICMi values of benchmark sites from the IC river type RM5 for all MS; B) Comparison between winter-autumn and spring-summer ICMi benchmark values for RM5 type (from MED GIG report).

The mean of the CM values from the joining method benchmark sites is 0.977 (range 0.639–1.208), while the range from the other MS of the finalized IC exercise is approximately 0.63–1.78. It could be concluded that both the mean value and the whole range of the common metric values from de Balearic Islands data object of this IC exercise, fall within the approximated range of the CM values from the MED GIG. Thus, as stated in the CIS Guidance Document nº30, no standardisation is required.

4. Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x). A specialist case is that when a joining method relies exclusively on the common metric developed in the completed exercise for its classification rather than devising an original method (then being more like Option 1). In such cases a regression would be meaningless as y is directly dependent on x. The goal for an MS choosing to use the CM as the basis for their method is simple – after any benchmarking their boundaries must simply lie within one quarter of class of the global mean view.

The regression between the EQR values from the national assessment method (EQR_INVMIB) and those obtained from the intercalibration common metric (EQR_ICMi) is highly significant, for the 3 Balearic Islands types combined with $R^2 = 0.7531$ (Figure 1.4).

EQR_INVMIB and EQR_ICMi were also strongly correlated (Pearson test: $R = 0.868$, $P<0.001$) (The requirement during the IC exercise was $R>0.5$).

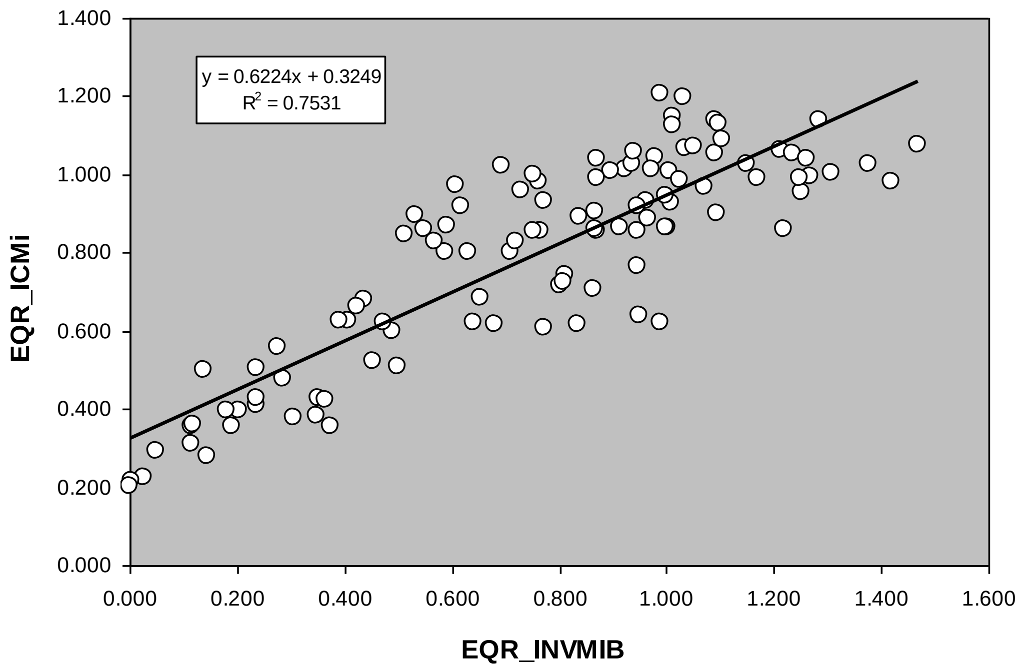

Figure 1.4. OLS regression to establish the relationship between the Balearic Islands joining method and the intercalibration common metric (EQR_ICMi), for the IC river type RM5.

5. Predict the position of the national class boundaries (MP, GM, HG and reference) on the CM_bm scale.

National class boundaries predicted on the ICM scale using the relationship established from the OLS regression, are provided in Table 1.16.

Table 1.16. Positions of the class boundaries according to the INVMIB method and predicted positions after translation to the ICM scale.

Boundary	EQR_INVMIB	Boundaries on ICM scale
High/Good	0.93	0.904
Good/Moderate	0.73	0.779
Moderate/Poor	0.50	0.636
Poor/Bad	0.25	0.481

6. Apply the comparability criteria as summarised in Chapter 6 of the manual.

The global mean views of the H/G and G/M boundaries for IC river type RM5 in the MED GIG were extracted from the final table of harmonized class boundaries:

H/G boundary global mean view of ICM = 0.975

G/M boundary global mean view of ICM = 0.722

As the national **G/M boundary** on ICM scale lies above the global mean view, the amount of this deviation was calculated and expressed as a proportion of the class width (CW) of the Moderate (national) status on the same scale. The obtained deviation was >0.25. According to the manual, in that case the G/M boundary can be lowered until the deviation between the national boundary on the ICM scale and the global view on the same scale is ≤0.25 class widths (but there is no obligation to make this adjustment). However if the deviation is >0.5, then an adjustment is strongly recommended. In the Balearic Islands method case the deviation for the G/M boundary is <0.5, it is still quite high (0.400) (Table 1.17). Thus the G/M boundary was finally lowered to achieve a deviation ≤0.25. This was done by lowering the G/M boundary of the national EQR_INVMIB from 0.73 to 0.68 (Table 1.17).

Table 1.17. Summary of boundary values, class widths and boundary bias obtained for the G/M boundary before and after adjustment.

	Original G/M Boundary	Adjusted G/M Boundary
G/M Boundary of the EQR_INVMIB	0.73	0.68
G/M Boundary on ICM scale	0.779	0.748
Global mean view of the G/M boundary	0.722	0.722
Deviation	0.057	0.026
Class width good status	0.124	0.156
Class width moderate status	0.143	0.112
Deviation as proportion of moderate status CW	0.400 (>**0.25**)	0.233 (<**0.25**)

The national **H/G boundary** on ICM scale lies below the global mean view. Thus, the amount of this deviation was calculated and expressed as a proportion of the class width (CW) of the High (national) status on the same scale. The obtained deviation was <0.25 (Table 1.18). According to the manual, in that case the H/G boundary meets the comparability criteria and no adjustment is needed.

Table 1.18. Summary of boundary values, class widths and boundary bias obtained for the H/G boundary.

	Original H/G Boundary
H/G Boundary of the EQR_INVMIB	0.93
H/G Boundary on ICM scale	0.904
Global mean view of the H/G boundary	0.975
Deviation	-0.071
Class width high status	0.304
Class width good status	0.124
Deviation as proportion of good status CW	-0.234 (<0.25)

1.6. CONCLUSIONS ON THE BALEARIC ISLANDS RM5 TYPE IC

The INVMIB assessment method for the temporary streams (common IC type RM5) of the Balearic Islands (Spain) was compared with the finalized IC exercise of the Mediterranean Rivers GIG following the procedure described in the IC guidance nº30. The INVMIB is a fully compliant WFD assessment method as demonstrate in this report. According Wilby et al. (2014), the INVMIB is considered a comparable assessment method with the existing intercalibrated methods. The relationship of the INVMIB method with the common intercalibration common metric was very good R = 0.868. The H/G boundary on the common metric scale was within the accepted class boundary. The G/M boundary was above the boundary on the common metric scale, we adjusted it by lowering the class boundary for the INVMIB and consequently its EQR. After the adjustment, the deviations of the national view on the class boundaries expressed as proportions of the corresponding class widths were 0.233 (G/M) and -0.234 (H/G). It is recommended to submit the method to the ECOSTAT group for official approval.

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CHAPTER 2

THE DIATMIB A NEW CLASSIFICATION METHOD TO ASSESS WITH PHYTOBENTHOS THE ECOLOGICAL STATUS OF RIVERS IN THE BALEARIC ISLANDS (MED GIG RIVERS)

CONTENTS

2.1. INTRODUCTION

The Mediterranean River GIG finalised the intercalibration (IC) exercise for Phytobenthos during the second IC phase, with the participation of 6 Member States (Cyprus, France, Italy, Portugal, Slovenia and Spain), that compared and harmonized their national assessment systems for the common IC types RM1, RM2, RM4 and RM5. All national methods addressed a group of common pressures, mainly nutrients and organic matter, and used an IC Option 2 (comparison of assessment methods using a common metric). The intercalibration and harmonization were performed only with spring-summer data, with the exception of Cyprus and Italy. The lack of comparability between MS methods and the insufficient number of reference sites, made impossible the IC of one of the GIG common types, the RM3.

This report has for objective to show that the Regional authorities of the Balearic Islands have developed a fully compliant Water Framework Directive (WFD) phytobenthos classification method (DIATMIB) (Delgado et al. 2012) for the common type RM5 that exists in the Balearic Islands, and to fit the assessment system to the completed intercalibration exercise.

The way forward for verifying the WFD compliance with normative definitions, IC feasibility, pressure-impact relationships and comparing boundaries, is described in the CIS Guidance Document n°30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise" (Wilby *et al.* 2014).

2.2. DESCRIPTION OF NATIONAL ASSESSMENT SYSTEM

The **DIATMIB** was developed using diatom samples as a proxy of Phytobenthos, collected by the Illes Balears Government monitoring program in autumn 2005, winter 2006 and 2008, and spring 2005, 2006 and 2008. Here we describe the DIATMIB compliance with WFD in relation with its sampling method, taxonomic resolution, calculation, international and national reference criteria, class boundaries establishment and pressure-impact relationships.

2.2.1. Sampling method and taxonomic resolution

The sampling design, sample treatment procedure and the study of the diatom communities were based on the instructions of the European standards (CEN TC230 N68, 2003; EN 13946 2003; EN 14407 2004).

Sampling was performed in three campaigns that took place in autumn (November–December), winter (February–March) and spring (April–May).

Two kinds of periphyton samples were scrubbed from hard substrata: some for analysis of photosynthetic pigment concentration (chlorophyll -Chl a-) and periphytic biomass, and the others for the quantitative and qualitative analysis of the composition of the benthonic diatom communities.

Epilithic diatoms were taken from natural substrata (minimum 5 stones) by scraping with a small toothbrush, and fixed with formaldehyde (4%v) until their analysis in the laboratory. The samples were digested to remove all organic material and carbonates with hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl-). Clean diatom suspension was mounted on permanent microscopic slides prepared with Naphrax®. Diatoms were identified to the lowest taxonomic level possible and a minimum of 400 diatom valves were counted in each slide.

Sampling for quantitative analysis of periphytic biomass and chlorophyll was performed by scraping known areas in the surface of submerged stones. Three rocks were randomly chosen and their upper surface divided in two halves, providing six-replicated samples (20–60 cm^2). Three samples were for the estimation of the chlorophyll (Chl a) and other three to estimate periphyton biomass as ash-free dry mass (AFDM). Samples of periphyton were taken by scraping with a toothbrush, rinsed with distilled water, stored in ice and kept in darkness at 4 ◦C until their analysis in the laboratory. The samples were filtered through Whatman GF/C glass-fiber filters, and the Chl a extracted with acetone (90%) at 4ºC for 48 h and kept in darkness. Its concentration was measured by spectrophotometer, and corrected for degradation products using the equations given by Lorenzen (1967). Samples for AFDM were filtered in glass-fiber filters (pre-ashed and weighed), dried to constant mass at 105 ºC for 24 h, and reweighed. Then, they were placed in a muffle furnace at 505 ºC for 1.5 h to estimate the AFDM (difference between initial and final ash masses).

2.2.2. Multimetric Calculation

The classification system developed in this study consists of a multimetric index (DIATMIB), common to all the Balearic Islands national types: R-B01 (mountain streams), R-B02 (canyon streams) and R-B03 (lowland streams), all them corresponding to the IC common type RM5.

The criteria followed in the development and selection of metrics were: (1) to comply with the normative definitions of the WFD (European Union 2000), (2) to respond to the gradient of pressure, (3) to be able to discern reference values from disturbed sites, and (4) the metrics selected should be non-redundant.

The DIATMIB is a method totally compliant with the WFD normative definitions. It is based on the use of metrics describing diatom composition (metrics of sensitive and tolerant taxa, PABSS and PABST, respectively) and abundance (Chl a values as a surrogate for algal biomass) (Table

2.1), and it shows an ecological response at the community level that can be easily calculated (see Delgado et al. 2012 for further details). Each individual metric was selected for its potential as indicator, and their response analysed along general degradation pressure gradients. The finally selected metrics were averaged in the Diatom Multimetric Index of the Balearic Islands (DIATMIB) (Table 2.1):

$$\boxed{\text{DIATMIB} = \text{NPABSS} + \text{NPABST} + \text{NChl a/max}}$$

where PABSS = relative abundance of sensitive taxa; PABST = relative abundance of tolerant taxa; and Chl a/max = values of periphytic chlorophyll a divided by the maximum of the data series.

Table 2.1. Summary of the metrics composing the DIATMIB multimetric for the R-B01 (mountain), R-B02 (canyon) and R-B03 (lowland) stream types of the Balearic Islands. Its expected response to pressure, and the transformations and normalizations necessary to obtain it are also indicated (based in Pardo et al. 2010).

Type	Metric	Response to pressure	Transformation	Reverse	Normalisation
R-B01, R-B02 and R-B03	Chl a	+	yes	yes	Median ref.
	PABSS	-	no	no	Median ref.
	PABST	+	no	yes	Median ref.

2.2.3. Ecological status assessment

The ecological status is defined on the basis of the Ecological Quality Ratio (EQR), calculated by dividing each resulting value of the DIATMIB by the median value of the reference data:

$$\boxed{\mathit{EQR\_DIATMIB} = \text{measured DIATMIB value / median DIATMIB value of references}}$$

Then the obtained values ranged from 0 (worst quality) to >1 (best quality), being the ecological status classified by one of five classes (high, good, moderate, poor and bad) shown in Table 2.2.

Table 2.2. Ecological status levels and corresponding national boundaries assignment.

Boundaries	Level of disturbance	Quality class	Colour
		Reference	
> 0.93	Minimal	High	
0.93-0.73	Slight	Good	
0.73-0.50	Moderate	Moderate	
0.50-0.25	Major	Poor	
< 0.25	Severe	Bad	

2.2.4. National reference criteria

The reference sites of the Balearic Islands had to satisfy a series of a priori selection criteria based on the absence of significant pressures in their basins, considering the percentages of artificial and agricultural land uses, absence of sewage effluents, no hydromorphological alterations of the stream bank and absence of significant flow regulation (Pardo et al. 2012).

In a first phase 8 sites fulfilling the next criteria (Pardo et al. 2010) were identified:

- Land uses in the basin have not been recently intensified.
- There are no artificial surfaces in the catchment.
- % of agriculture <25% (considering rainfed lands and in absence of irrigated lands).
- Absence of sewage effluents.
- Absence of longitudinal channel alterations.
- Absence of marked discharge reductions in the catchment.
- Absence of flow regulation due to upstream dams.
- Absence of stream bank significant alterations. Connectivity with adjacent trees is maintained.

Another 4 sites were also considered as references regarding its biology, and fulfilled the prior mentioned criteria with slight modifications:

- Artificial surfaces (<0.4% of catchment area)
- % of agriculture <35% (natural vegetation and rainfed lands. Absence of irrigation).

In a second phase, pressure levels for artificial and agricultural surfaces were restricted to 0% and ≤ 7% of catchment area respectively, following the conclusions of the review on European reference thresholds by Pardo et al. (2011). A posterior check with water quality levels and invertebrate communities was also performed to assure the absence of these pressures in this area (Pardo et al. 2012). Finally 12 reference sites were identified according to the mentioned reference selection criteria, represented by a total of 58 diatom samples (Table 2.3; see Delgado et al. 2012 for further details).

Table 2.3. List of temporary streams and sites fulfilling the Balearic Island reference criteria.

Site code	Site name	Island	Type	xUTM	yUTM
AB240	D'Almadra torrent	Majorca	Mountain	483556	4402329
AC19	Comafreda-Guix torrent	Majorca	Canyon	491515	4406292
AC25	Mancor de la Vall headwaters	Majorca	Mountain	485564	4403901
AK28	Matzoc torrent	Majorca	Lowland	533259	4400924
AN260	Coccons	Majorca	Lowland	528499	4398000
B1000	Gorg Blau	Majorca	Mountain	499504	4416150
B2000	Ternelles 3	Majorca	Mountain	499822	4417133
B2001	Ternelles 5	Majorca	Mountain	499754	4416028
G3000	Ses Comer torrent	Majorca	Mountain	492650	4414304
H12	Gorg Blau	Majorca	Canyon	485051	4408100
H220	Lluc	Majorca	Canyon	487000	4409854
K2600	Biniaratx-Camidel L´Ofre	Majorca	Canyon	478464	4402040

2.2.5. National boundary setting

- Methodology used to set H/G boundary: The H/G was set as the 25th percentile (P_{25}) of the EQR_DIATMIB reference values.
- Methodology used to set the remaining boundaries. Below the 25th percentile, the remaining quality class boundaries were defined into equal bands.

The G/M boundary was set as H/G boundary- (P_{25} /4)

The M/P boundary was set as G/M boundary- (P_{25} /4)

The P/B boundary was set as M/P boundary- (P_{25} /4)

A posterior adjustment and confirmation of the boundaries was produced looking at the crossing between the fitted regression lines of paired individual metrics, for its ecological interpretation following the "Guidance on the intercalibration process 2008-2011" (Schmedtje et al. 2009).

The obtained class boundaries are illustrated in Table 2.4.

Table 2.4. Officially used class boundaries for the DIATMIB method.

Class Boundary	High/Good	Good/Moderate	Moderate/Poor	Poor/Bad
DIATMIB	0.93	0.73	0.5	0.25

2.2.6. Pressures-impact relationships

A Multimetric index and per ende the individual metrics have to respond to pressure gradients. The Balearic Island streams (torrents) are mainly affected by pressures related to temporary organic contamination, diffuse source pollution by nutrients and hydromorphological alterations of riverbeds and riverbanks.

For the DIATMIB case the pressures-response relationships were evaluated along a general degradation gradient, covering all the pressures affecting the study sites. This general gradient was obtained through a Principal Component Analysis (PCA) that allowed the integration of the individual variables in a multiple variable, reflecting the pressure gradient defined by the same. The first axis (Axis1DEG) was mainly related to DIN, silica and % agriculture in opposition to O_2 (%) (Pardo et al. 2010). A significant correlation among the individual metrics and the DIATMIB index with the degradation gradient obtained from PCA was found (Table 2.5).

Table 2.5. Summary of the Spearman correlation coefficients between the DIATMIB and the individual metrics, and the general degradation gradient. ** *P*<0.01.

		Chl a	PABSS	PABST	DIATMIB
Axis1DEG	Correlation coef.	-.306**	.579**	-.538**	.590**
	p	.000	.000	.000	.000

Results also showed sample segregation along the pressure/degradation gradient according to the DIATMIB values and the localities ecological condition, thus confirming the function of the DIATMIB multimetric index as indicator. The multimetric response to the general degradation gradient (Axis1DEG) implies that when pressure increase, the diatom community is affected and a gradual decline in ecological quality occurs (Fig. 2.1).

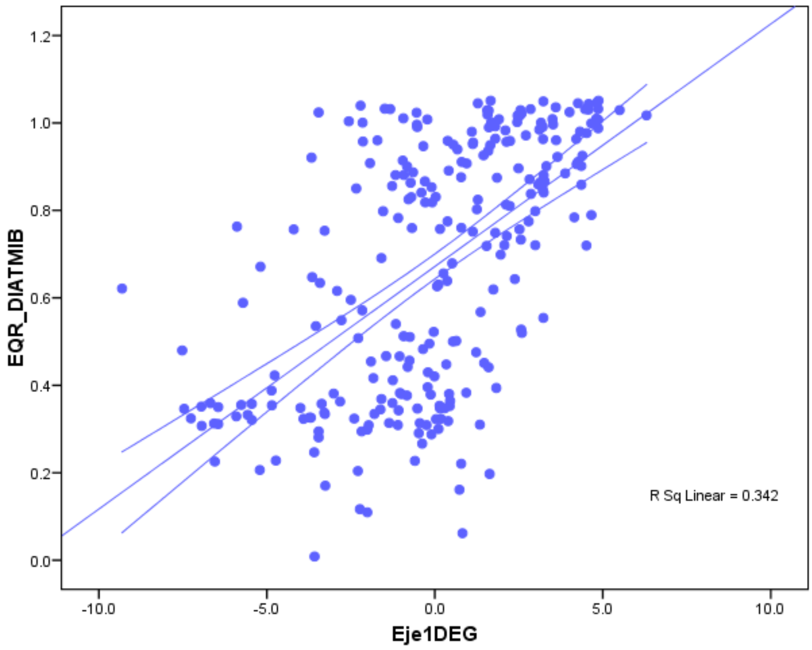

Figure 2.1. DIATMIB distribution along the general degradation gradient (Axis1DEG) (from Pardo et al. 2010).

In Table 2.6, there is a summary of the Spearman correlation coefficients between the values of the DIATMIB multimetric or the individual biological metrics, and some independent physico-chemical pressure variables that are indicators of organic/nutrient degradation.

Table 2.6. Spearman correlations among individual metrics and the multimetric DIATMIB with some physico-chemical variables (n = 255). * *P*<0.05; ***P*<0.01 (from Delgado et al. 2012).

	P_PO_4 (mg/L)	N_NO_3 (mg/L)	N_H_4 (mg/L)	SiO_2 (mg/L)	AFDM (g/m^2)	CE (µS/cm)
Achnanthidium minutissimum	0.120	- 0.072	- 0.030	- 0.054	- 0.076	- 0.175*
Nitzschia frustulum	0.168	- 0.135	0.031	0.074	0.007	0.163
Diversity	0.097	0.191**	0.096	0.126*	0.146*	0.172**
Evenness	0.165**	0.235**	0.157*	0.186**	0.158*	0.221**
Bray Curtis	- 0.555**	- 0.268**	- 0.453**	- 0.287**	- 0.278	- 0.525**
A3SPDOM	- 0.385**	- 0.120	- 0.399**	- 0.224**	- 0.193**	- 0.276**
A6SPDOM	0.423**	0.348**	0.319**	0.309**	0.092	0.448**
IPS	- 0.565**	- 0.352**	- 0.498**	- 0.349**	- 0.290**	- 0.586**
PABSS	- 0.583**	- 0.335**	- 0.442**	- 0.333**	- 0.225**	- 0.617**
PABST	0.552**	0.322**	0.445**	0.299**	0.225**	0.562**
Chl a/max	0.300**	0.295**	0.172**	0.212**	0.471**	0.229**
DIATMIB	- 0.593**	- 0.388**	- 0.435**	- 0.340**	- 0.323**	- 0.591**

DIATMIB showed the highest correlations with phosphates and ammonium (Table 2.6). This fact reveals its response to nutrients and organic pollution, in line with the results obtained in the MED GIG exercise for de ICM index.

2.3. COMPLIANCE WITH WFD NORMATIVE DEFINITIONS

After the compliance check we concluded that the DIATMIB method fulfils all the requirements of the WFD (Table 2.7).

Table 2.7. Assessment of DIATMIB compliance with the WFD normative definitions.

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by 1 of 5 classes (high, good, moderate, poor and bad).	**Yes**
2. High, good and moderate ecological status are set in line with the WFD's normative definitions (Boundary setting procedure)	**Yes,** see section "National boundary setting".
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.	**Yes,** the DIATMIB index is an average of 3 normalized metrics, covering all relevant parameters. Both relative abundance and taxonomic composition are considered. And also the ratio between sensitive and tolerant taxa.
4. Assessment is adapted to intercalibration common types that are defined in line with the typological requirements of the WFD Annex II and approved by WG ECOSTAT	**Yes,** the assessed sites were all assigned to the RM5 common type .
5. The water body is assessed against type-specific near-natural reference conditions	**Yes,** rivers are assessed against existing type-specific near-natural reference conditions.
6. Assessment results are expressed as EQRs	**Yes**
7. Sampling procedure allows for representative information about water body quality/ ecological status in space and time	**Yes,** sampling method follows EU standards and complies with the IC criteria. Sampled from 2 to 3 times a year.
8. All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure	**Yes,** the sampling procedure consider both composition and relative abundance. Diatom communities were used as proxies for phytobenthos.
9. Selected taxonomic level achieves adequate confidence and precision in classification	**Yes,** species level.

2.4. IC FEASIBILITY CHECK

2.4.1. Assessment concept

The MED GIG concluded that the IC is feasible as all the methods in the finalized exercise follow similar assessment concepts based on indicator species. The methods are based basically in diatom metrics, calculated from species data through relative abundances. DIATMIB is in line with these assessment concepts. Thus, the method can be accepted and **the intercalibration is feasible.**

2.4.2. Typology agreement

According to system A, the Balearic Islands belong to the Iberic-Macaronesian ecoregion. They are influenced by the Mediterranean climate and the discharge regime is subject of strong seasonal and annual variability (García et al. 2014). The hydrological system of the islands is characterized mainly by the existence of temporary streams called “torrents”, which fit within the characteristics of the IC type RM5. Obligatory descriptors of the WFD (System A: altitude typology, size typology and geology) and other such as slope and bank morphology were used to separate these temporary streams into 3 types (Pardo et al. 2010):

R-B01. Mountain streams (located in the mountains). Intermediate slopes.

R-B02. Canyon streams (deep valley between cliffs carved from the landscape, isolated and with high slopes). These streams have water most of the year, although during summer water is restricted to pools.

R-B03. Lowland streams. These streams are the most abundant type in the Balearic Islands, located at low altitude and close to the most populated areas.

The contrast of the proposed typology with the biological elements supported the definition of a unique diatom reference community for the three geomorphologically predefined stream types (R-B01, R-B02 and R-B03) (Fig. 2.2), which is common to all the torrents of the Balearic Islands. This approximation is followed in this IC report, considering a single stream type with a type-specific diatom community.

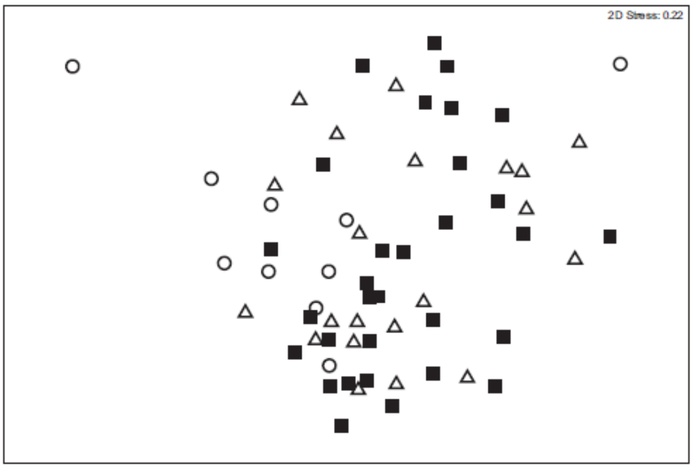

Figure 2.2. Ordination (NMDS) of the diatom community from the reference sites of the different stream types defined a priori for the Balearic Islands. Mountain (dark squares), Canyons (white triangles) and Lowlands (white circles) (from Delgado et al. 2012).

Within the Med GIG five common IC river types were considered for intercalibration of phytobenthos (Table 2.8). While RM1, RM2 and RM4 types were treated together throughout the IC process, RM5 was treated separately due to its distinct hydrological conditions. The RM3 was not intercalibrated in the MED GIG IC exercise.

Table 2.8. Common IC river types in the Mediterranean GIG and MS sharing the types.

Common IC Type	Type characteristics	MS sharing IC common type
RM1	Catchment < 100 km^2; mixed geology (except non-siliceous); highly seasonal	SP, FR, IT, PT, SI
RM2	Catchment 100-1000 km^2; mixed geology (except non-siliceous); highly seasonal	SP, IT, PT, SI
RM3	Catchment 1000-10000 km^2; mixed geology (except siliceous); highly seasonal	This type cannot be intercalibrated due to the lack of comparability between MS methods and insufficient number of reference sites.
RM4	Non-siliceous streams; highly seasonal	IT, CY, SP, FR
RM5	Temporary rivers	SP, IT, PT, CY, SI

In conclusion, for diatoms the three types established for the Balearic Islands (unified in a single type for IC purposes, according to the similarity of its reference diatom communities) fulfil the typological criteria of the IC common type RM5, and thus **the intercalibration is feasible**.

2.4.3. Pressure addressed

As previously stated, the DIATMIB clearly respond to a general degradation pressure gradient (first axis of the PCA), showing that when the pressure increases the diatom community is affected and a gradual decrease in the ecological quality occurs. By the way, the pressure-impact relationships results also show that the DIATMIB responds well to nutrients, especially to phosphates and ammonium, fact that is in line with the MED GIG results. Thus, in terms of pressure it could be concluded that **the intercalibration is feasible**.

2.4.4. National dataset used

The datasets described in this report were checked against the data acceptance criteria as defined in the MED GIG rivers milestone 6 report for phytobenthos (Table 2.9).

The national dataset used for the DIATMIB development (testing of pressures-response relationships, boundary setting procedure…) comprised a total of 255 samples coming from 60 sites, all them representing the RM5 IC common type. While the national dataset considered for the IC exercise comprises only 102 samples corresponding to winter and spring campaigns and coming from 48 river sites. The existing reference samples within the whole dataset fulfilling IC criteria for benchmarking and corresponding to winter and spring campaigns, where considered for IC. As well as a representative and equitable number of no reference sites covering all the ecological status classes according to DIATMIB. In the MED GIG exercise the data used in the calculations for intercalibration and harmonization were restricted to spring-summer data, with the exception of CY and IT, as for the remaining MS boundaries were defined based on spring-summer data. However, the studies realised in the Temporary streams of the Balearic Islands have concluded that mean annual assessment of the ecological status in the temporary streams related best with the assessment done in late winter and early spring (February and May) (Pardo et al. 2010). For that in this exercise we have used data corresponding to this period as the optimum to fit the boundaries with results from the complete Mediterranean rivers IC.

Both the typology and the geographical area coverage by the used dataset are considered good enough. For each sample land use data (artificial, agricultural, forest and seminatural areas), physico-chemical data (alkalinity, oxygen, pH, temperature, conductivity, BOD5, orthophosphates, nitrates, nitrites, ammonium, sulphates and cations) and biological data (diatom taxalists) are available. A summary of some selected variables registered within reference and non reference groups of sites is available in Delgado et al. 2012 for the whole dataset.

Table 2.9. Summary of the data acceptance criteria of the MED GIG rivers for phytobenthos.

Data acceptance criteria	Data acceptance checking
Data requirements (obligatory and optional)	Appropriate geographical location and river typology, as well as biological, environmental and pressure data. These data were organized under the same agreed frame.
The sampling and analytical methodology	All Member States delivered documents containing the diatoms sampling and analytical methodology. All MS (except SI) sample epilithic communities. All MS treat and study diatom samples in a similar way: identification followed by a count of at least 400 valves.
Level of taxonomic precision required and taxa lists with codes	All MS delivered taxa lists with similar taxonomic precision (species level) and with common codes; taxonomic and nomenclatural harmonization of diatom assemblages took place during this exercise.
The minimum number of sites / samples per intercalibration type	Minimum of 15 sites per IC type are available
Sufficient covering of all relevant quality classes per type	All 5 water quality classes are represented, but class 5 corresponding to Bad may be under-represented for most types.

The 5 classes of ecological status are covered within the dataset and the obtained pressure gradient is considered appropriate. The final classification results from the DIATMIB multimetric for the whole dataset indicated that the percentage of samples in the different status classes were 22.7% (Reference), 11.4% (High), 18.1% (Good), 12.5% (Moderate), 29.8% (Poor) and 5.5% (Bad) (Delgado et al. 2012). The under-representation for the Bad class was also revealed in the MED GIG exercise for most common types.

Regarding the sampling and analytical methodology, the diatom samples were collected from epilithon and the minimum count of valves was 400, while the diatom taxa were identified to the species or variety level.

In conclusion, for diatoms the three types established for the Balearic Islands (unified in a single type for IC purposes according to the similarity of its reference diatom communities) fulfilled the data acceptance criteria of the MED GIG, and thus the dataset could be considered enough and **the intercalibration is feasible**.

2.4.5. Conclusions

It is concluded that it is feasible applying the fit-in procedure to DIATMIB, fitting the method to the results of the completed MED GIG river phytobenthos IC exercise.

2.5. IC PROCEDURE

Following the diagram for the selection of the fitting procedure (in CIS Guidance nº30), case A1 (Option 2) was identified as the option to carry out the intercalibration exercise. Thus the procedure for this case from the manual (Wilby et al. 2014) is applied for fitting the DIATMIB method to the River MED GIG results for the RM5 common type.

2.5.1. Requirements

a. Full details of the common metric (e.g. species scores and metric weights).

In the MED GIG it was concluded that the Intercalibration Common Metric, ICM (according to Kelly et al. 2009) was the best option to use. The ICM is a multimetric index composed of two diatom metrics:

- IPS (Coste in CEMAGREF 1982): this metric measures ‘general water quality’, with low values corresponding to high pressure levels and, therefore, low EQRs.
- TI (Rott *et al.* 1999): a trophic index which needs to be adjusted so that high values represent high EQR values.

$$\boxed{ICM = (EQR_{IPS} + EQR_{TI})/2}$$

where

EQR_{IPS} = Observed value / reference value*

* (reference value = median IPS value of reference sites for a national dataset)

QR_{TI} = (4-observed value) / (4-reference value*)

*(reference value = median TI value of reference sites for a national dataset)

b. A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated.

A total of 102 samples were available covering the 5 classes of ecological status (see subsection *"National dataset used"* in *Section 2.4*).

c. Accompanying pressure data in the same format as that used in the completed exercise.

In subsection *“National dataset used”* within *Section 2.4*, pressure data for the whole dataset are available. In Table 2.10 are indicated some pressure ranges considering only the 102 winter and spring samples included in this IC exercise.

Table 2.10. Range of values of some physico-chemical variables at those sites and samples included in the intercalibration.

		Winter		Spring	
	n	Minimum	Maximum	Minimum	Maximum
Conductivity (µS/cm)	102	214.5	3425.0	245.0	3084.0
pH	102	6.6	8.5	6.1	8.6
O_2 (%)	102	13.3	136.5	1.5	170.0
T (ºC)	102	7.2	18.5	13.3	26.7
N_NO_3 (mg/L)	102	0.005	20.089	0.005	14.305
N_NH_4 (mg/L)	102	0.001	0.390	0.001	6.088
P_PO_4 (mg/L)	102	0.001	2.232	0.001	5.876
SO4 (mg/L)	102	1.02	510.51	3.33	285.65
Cl^- (mg/L)	102	12.59	638.17	17.80	846.46
Ca^{2+} (mg/L)	102	15.50	154.00	14.35	243.43
Mg^{2+}(mg/L)	102	2.79	78.70	3.84	66.44
Na^+ (mg/L)	102	4.50	514.20	6.56	327.10
K^+ (mg/L)	102	0.38	39.90	0.39	50.33

The environmental information is sufficient to demonstrate the existence of a response to individual pressures and to a general degradation gradient, in line with MED GIG results.

d. Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites (e.g. human population density, extent of agricultural land in catchment, nutrient concentrations etc.).

In the IC exercise the MED GIG established the Reference thresholds (Table 2.11) conditioning the benchmarks acceptance, for each pressure variable and for all IC types. Thus providing a common tolerance level for all the types, with the exception of RM5 where different ranges for water oxygenation were established for low water periods.

Table 2.11. Thresholds established for each pressure variable and used by the MEDGIG for IC.

Pressure variables	RM1+RM2+RM4	RM5
General morphology (Classes 1-3)		
General hydrology (Classes 1-3)	≤ 2	
Riparian vegetation (Classes 1-3)		
DO (mg/L)[1]	6.39-13.70	
O_2 (%)	73.72-127.92	60.34-127.92
N-$NH4^+$ (mg/L)	≤ 0.09	
N-$NO3^-$ (mg/L)	≤ 1.15	
P-Total (mg/L)	≤ 0.07	
P-$PO4^{3-}$(mg/L)	≤ 0.06	
% Artificial areas (catchm)	≤ 1	
% Intensive agriculture (catchm)	≤ 11	
% Extensive agriculture (catchm)	≤ 32	
% Semi-natural areas (catchm)	≥ 68	
% Urbanisation (reach)[2]	≤ 1	
% Land use (reach)[2]	≤ 20	
% Agriculture (reach)[2]	≤ 20	

[1] for macrophytes only, instead of O_2 (%)
[2] for diatoms only, instead of land use in the catchment

e. Details of exactly how the benchmarking was undertaken in the completed exercise (e.g. creation of a common metric EQR by dividing the observed value by the median common metric value of a set of national reference or benchmark sites). If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method.

Given benchmark criteria were applied by each MS to identify benchmark sites within each national dataset. Median values of IPS and TI of the national benchmarks datasets were used for calculation of the common metric EQR. When the number of available benchmarks for a MS is low, the global median of the indices composing the ICM (IPS and TI) of all MS has to be used to calculate ICM.

Linear regression was established between the values of the national method and the common metric (ICM) so that the national boundaries could be translated to ICM using the equation.

f. Values of the global mean view of the HG and GM boundaries on the common metric scale for Member States who participated in the completed exercise.

Mean H/G (relevant for RM5): 0.914 (original); 0.914 (harmonized)
Mean G/M (relevant for RM5): 0.688 (original); 0.691 (harmonized)

2.5.2. Process

1. Calculate the common metric (ICM) on the national dataset.

The common metric used in the IC exercise (ICM) was calculated according to the formula:

$$ICM = (EQR_{IPS} + EQR_{TI})/2$$

EQR_{IPS} = Observed value / reference value*

QR_{TI} = (4-observed value) / (4-reference value*)

Reference values for IPS and TI represent the median value of each metric in the reference sites of the national database (in this case the reference sites complaining the reference selection criteria and corresponding to samples collected during the winter and spring campaigns, that were the ones subject of this IC exercise). ICM results are shown in Table 2.12.

Table 2.12. List of national winter and spring IC sites with its DIATMIB and ICM values, as well as the corresponding EQRs. The sample code indicated in the table is a combination of the site code + campaign (pri = spring; inv = winter) + year.

	National assessment		IC metrics				
Sample code	DIATMIB	EQR_DIATMIB	IPS	EQR_IPS	TI	EQR_TI	ICM
AB240pri06	2.920	0.990	19.1	1.055	2.96	1.014	1.034
AB240pri08	3.044	1.032	19.3	1.066	2.92	1.000	1.033
AB500pri08	0.602	0.204	4.6	0.254	0.70	0.240	0.247
ABB1000pri06	2.286	0.775	17.0	0.939	1.94	0.664	0.802
ABB1000pri08	2.354	0.798	16.6	0.917	2.10	0.719	0.818
AC19pri05	2.928	0.992	18.7	1.033	2.90	0.993	1.013
AC223pri05	0.609	0.206	5.6	0.309	0.82	0.281	0.295
AC223pri06	0.666	0.226	4.1	0.227	0.68	0.233	0.230
AC25pri05	2.954	1.001	17.6	0.972	2.96	1.014	0.993
AC25pri06	3.083	1.015	19.1	1.055	3.14	1.075	1.065
AC25pri08	3.023	1.025	17.7	0.978	3.12	1.068	1.023
AJ364pri06	1.033	0.350	10.0	0.552	1.10	0.377	0.465
AK28pri06	3.096	1.049	18.1	1.000	3.14	1.075	1.038
AN260pri08	3.078	1.043	18.7	1.033	2.00	0.685	0.859
AN271pri08	1.321	0.448	9.9	0.547	0.88	0.301	0.424
B1000pri08	2.217	0.751	12.8	0.707	1.60	0.548	0.628
B2000pri05	2.414	0.818	17.2	0.950	2.46	0.842	0.896
B2000pri06	3.019	1.023	19.7	1.088	3.02	1.034	1.061
B2000pri08	2.126	0.721	16.2	0.895	1.84	0.630	0.763
B2001pri05	2.830	0.959	14.7	0.812	2.02	0.692	0.752
B2001pri08	2.688	0.911	15.3	0.845	2.20	0.753	0.799
B216pri05	2.126	0.720	14.2	0.785	2.06	0.705	0.745
B216pri06	2.844	0.964	18.8	1.039	2.62	0.897	0.968
C454pri06	2.823	0.957	16.9	0.934	2.50	0.856	0.895
D5pri08	1.121	0.380	14.6	0.807	1.26	0.432	0.619
F459pri05	1.055	0.358	7.3	0.403	0.78	0.267	0.335
G3000pri05	3.040	1.030	18.5	1.022	3.08	1.055	1.038
G3000pri06	3.056	1.036	17.3	0.956	3.10	1.062	1.009

Table 2.12. Continued.

	National assessment		IC metrics				
Sample code	DIATMIB	EQR_DIATMIB	IPS	EQR_IPS	TI	EQR_TI	ICM
H12pri05	2.535	0.859	17.2	0.950	2.12	0.726	0.838
H12pri08	2.842	0.963	18.0	0.994	2.78	0.952	0.973
H220pri05	3.100	1.051	18.7	1.033	3.04	1.041	1.037
H220pri08	3.043	1.032	18.1	1.000	2.96	1.014	1.007
K2100pri05	2.629	0.891	17.0	0.939	2.74	0.938	0.939
K2101pri06	2.508	0.850	18.9	1.044	2.68	0.918	0.981
K2101pri08	2.581	0.875	17.2	0.950	2.24	0.767	0.859
K2600pri06	3.067	1.040	19.4	1.072	3.00	1.027	1.050
K2600pri08	3.033	1.028	17.2	0.950	2.98	1.021	0.985
K31pri08	2.480	0.841	17.6	0.972	2.40	0.822	0.897
L3000pri05	2.952	1.001	17.5	0.967	2.66	0.911	0.939
L3000pri06	2.716	0.921	18.1	1.000	2.78	0.952	0.976
L484pri05	1.860	0.630	13.7	0.757	1.42	0.486	0.622
N79pri05	1.737	0.589	14.9	0.823	1.36	0.466	0.644
N79pri06	1.247	0.423	13.1	0.724	1.36	0.466	0.595
N79pri08	1.910	0.647	14.6	0.807	1.38	0.473	0.640
Q520pri08	2.451	0.831	16.9	0.934	2.42	0.829	0.881
R380pri06	1.476	0.500	13.4	0.740	1.44	0.493	0.617
R508pri06	1.675	0.568	11.3	0.624	1.12	0.384	0.504
R516pri08	1.029	1.029	17.2	0.950	2.94	1.007	0.979
S468pri06	1.011	0.343	10.2	0.564	1.30	0.445	0.504
U470pri06	1.168	0.396	10.7	0.591	1.24	0.425	0.508
V3190pri05	1.833	0.621	15.7	0.867	1.76	0.603	0.735
V3190pri08	1.619	0.549	11.7	0.646	1.18	0.404	0.525
V319pri08	1.461	0.495	11.9	0.657	1.26	0.432	0.544
Y274pri05	1.980	0.671	16.6	0.917	2.14	0.733	0.825
Y288pri08	2.677	0.907	18.9	1.044	2.72	0.932	0.988
AB240inv06	2.556	0.866	16.8	0.928	2.22	0.760	0.844
AB240inv08	2.923	0.991	19.5	1.077	2.96	1.014	1.046
AC19inv06	2.355	0.798	14.8	0.818	2.38	0.815	0.816
AC19inv08	2.900	0.983	19.4	1.072	2.88	0.986	1.029
AC25inv06	2.841	0.963	19.3	1.066	3.04	1.041	1.054
AK28inv06	2.948	0.999	19.7	1.088	3.00	1.027	1.058
AN260inv06	2.892	0.980	19.0	1.050	3.06	1.048	1.049
AN260inv08	3.045	1.032	17.9	0.989	3.12	1.068	1.029
B1000inv06	2.432	0.824	15.7	0.867	2.44	0.836	0.852
B1000inv08	2.163	0.733	15.5	0.856	1.84	0.630	0.743
B2001inv06	2.998	1.016	19.2	1.061	2.84	0.973	1.017
B2001inv08	2.975	1.008	19.1	1.055	2.80	0.959	1.007
G3000inv06	2.882	0.977	15.9	0.878	2.96	1.014	0.946
G3000inv08	3.002	1.017	18.2	1.006	3.14	1.075	1.040
H12inv06	2.610	0.885	18.3	1.011	2.66	0.911	0.961
H12inv08	2.123	0.720	15.3	0.845	1.76	0.603	0.724
H220inv08	2.597	0.880	15.2	0.840	2.04	0.699	0.769
K2600inv06	2.398	0.813	19.2	1.061	2.94	1.007	1.034
K2600inv08	3.000	1.017	19.8	1.094	2.92	1.000	1.047
AB500inv08	0.672	0.228	5.5	0.304	0.86	0.295	0.299
ABB1000inv6	2.039	0.691	16.0	0.884	1.84	0.630	0.757

Table 2.12. Continued.

	National assessment		IC metrics				
Sample code	DIATMIB	EQR_DIATMIB	IPS	EQR_IPS	TI	EQR_TI	ICM
AC223inv06	0.182	0.062	1.9	0.105	0.70	0.240	0.172
AC223inv08	0.881	0.299	8.7	0.481	0.80	0.274	0.377
AF700inv08	2.729	0.925	17.4	0.961	2.84	0.973	0.967
AG254inv06	0.025	0.008	5.1	0.282	0.80	0.274	0.278
AL3200inv06	3.083	1.045	19.8	1.094	3.02	1.034	1.064
AN271inv08	1.377	0.467	11.7	0.646	1.06	0.363	0.505
AO89inv06	1.507	0.511	10.7	0.591	1.22	0.418	0.504
B213inv06	0.915	0.310	11.1	0.613	1.32	0.452	0.533
B216inv08	2.660	0.902	16.8	0.928	2.54	0.870	0.899
C454inv06	2.804	0.950	18.1	1.000	2.70	0.925	0.962
E221inv06	0.476	0.161	8.5	0.470	0.84	0.288	0.379
F459inv06	0.323	0.110	9.5	0.525	0.80	0.274	0.399
H1000inv08	2.914	0.988	19.1	1.055	2.92	1.000	1.028
K2100inv06	2.829	0.959	18.5	1.022	2.60	0.890	0.956
K23inv08	2.120	0.719	15.8	0.873	1.88	0.644	0.758
K26inv06	2.923	0.991	19.2	1.061	2.94	1.007	1.034
O502inv06	0.651	0.221	6.1	0.337	0.82	0.281	0.309
R380inv06	0.582	0.197	10.4	0.575	1.18	0.404	0.489
R508inv06	0.671	0.227	10.1	0.558	0.92	0.315	0.437
U470inv06	0.952	0.323	8.8	0.486	0.94	0.322	0.404
V3190inv08	1.757	0.595	16.1	0.890	1.84	0.630	0.760
V319inv08	1.480	0.502	13.4	0.740	1.40	0.479	0.610
Y274inv08	2.232	0.757	15.3	0.845	2.36	0.808	0.827
Y286inv06	0.728	0.247	4.0	0.221	0.82	0.281	0.251
Y286inv08	0.503	0.170	2.0	0.110	0.88	0.301	0.206
Y288inv06	2.818	0.955	17.8	0.983	2.96	1.014	0.999

2. Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.

The MED GIG provides numbers of reference samples instead of reference sites and we followed the same approach. After checking the MED GIG requirements for identifying benchmarking, a total of 37 reference samples (from the total of 49 available in the whole dataset for the winter and spring campaigns) were accepted as references and used in this IC exercise (Table 2.13).

Table 2.13. Balearic Islands winter and spring samples fulfilling criteria for benchmarking.

Sample code	Site name	Sampling year	IPS	TI	ICM	DIATMIB	EQR DIATMIB
AB240inv06	D'Almadra torrent	2006	16.8	2.22	0.844	2.556	0.866
AB240inv08	D'Almadra torrent	2008	19.5	2.96	1.046	2.923	0.991
AB240pri06	D'Almadra torrent	2006	19.1	2.96	1.034	2.920	0.990
AB240pri08	D'Almadra torrent	2008	19.3	2.92	1.033	3.044	1.032
AC19inv06	Comafreda-Guix torrent	2006	14.8	2.38	0.816	2.355	0.798
AC19inv08	Comafreda-Guix torrent	2008	19.4	2.88	1.029	2.900	0.983
AC19pri05	Comafreda-Guix torrent	2005	18.7	2.90	1.013	2.928	0.992
AC25inv06	Mancor de la Vall headwaters	2006	19.3	3.04	1.054	2.841	0.963
AC25pri05	Mancor de la Vall headwaters	2005	17.6	2.96	0.993	2.954	1.001
AC25pri08	Mancor de la Vall headwaters	2008	17.7	3.12	1.023	3.023	1.025
AK28inv06	Matzoc torrent	2006	19.7	3.00	1.058	2.948	0.999
AK28pri06	Matzoc torrent	2006	18.1	3.14	1.038	3.096	1.049
AN260inv06	Coccons	2006	19.0	3.06	1.049	2.892	0.980
AN260inv08	Coccons	2008	17.9	3.12	1.029	3.045	1.032
AN260pri08	Coccons	2008	18.7	2.00	0.859	3.078	1.043
B1000inv06	Gorg Blau	2006	15.7	2.44	0.852	2.432	0.824
B1000inv08	Gorg Blau	2008	15.5	1.84	0.743	2.163	0.733
B1000pri08	Gorg Blau	2008	12.8	1.60	0.628	2.217	0.751
B2000pri05	Ternelles 3	2005	17.2	2.46	0.896	2.414	0.818
B2000pri08	Ternelles 3	2008	16.2	1.84	0.763	2.126	0.721
B2001inv06	Ternelles 5	2006	19.2	2.84	1.017	2.998	1.016
B2001inv08	Ternelles 5	2008	19.1	2.80	1.007	2.975	1.008
B2001pri05	Ternelles 5	2005	14.7	2.02	0.752	2.830	0.959
B2001pri08	Ternelles 5	2008	15.3	2.20	0.799	2.688	0.911
G3000inv06	Ses Comer torrent	2006	15.9	2.96	0.946	2.882	0.977
G3000inv08	Ses Comer torrent	2008	18.2	3.14	1.040	3.002	1.017
G3000pri05	Ses Comer torrent	2005	18.5	3.08	1.038	3.040	1.030
G3000pri06	Ses Comer torrent	2006	17.3	3.10	1.009	3.056	1.036
H12inv06	Gorg Blau	2006	18.3	2.66	0.961	2.610	0.885
H12inv08	Gorg Blau	2008	15.3	1.76	0.724	2.123	0.720
H12pri08	Gorg Blau	2008	18.0	2.78	0.973	2.842	0.963
H220inv08	Lluc	2008	15.2	2.04	0.769	2.597	0.880
H220pri05	Lluc	2005	18.7	3.04	1.037	3.100	1.051
H220pri08	Lluc	2008	18.1	2.96	1.007	3.043	1.032
K2600inv06	Biniaratx-Camidel L´Ofre	2006	19.2	2.94	1.034	2.398	0.813
K2600inv08	Biniaratx-Camidel L´Ofre	2008	19.8	2.92	1.047	3.000	1.017
K2600pri08	Biniaratx-Camidel L'Ofre	2008	17.2	2.98	0.985	3.033	1.028
		Median	18.1	2.92			

3. Standardise the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise. If benchmark standardisation was concluded not to be required in the completed exercise the mean CM value of the joining method's benchmark sites must lie inside the range of mean values of the benchmark sites of the methods already intercalibrated for this conclusion to remain applicable. If the joining method's benchmark sites lie outside of this range the joining method must benchmark standardise its sites relative to the global mean CM value of the benchmark sites included in the completed exercise. These scenarios are illustrated in Table 1 and 2.

The MED GIG report didn't provide the range of mean CM values of benchmark sites of the methods already intercalibrated for all the MS. With the purpose of determining the need of standardisation in this IC exercise, the mentioned range is inferred from a diagram for the common metric values from all benchmark sites provided in the MED GIG report for the RM5 common type (Fig. 2.3).

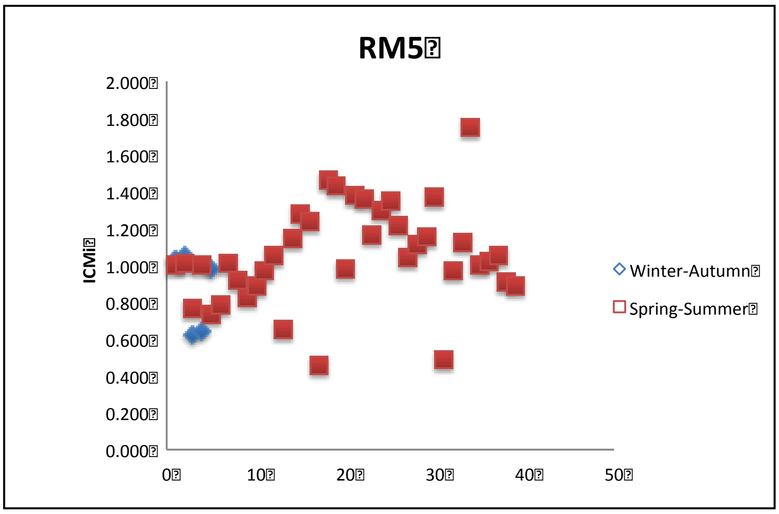

Figure 2.3. Comparison between winter-autumn and spring-summer ICM values for RM5 type, considering all MS and only benchmarks (from MED GIG report).

The mean of the CM values from the joining method benchmark sites is 0.944 (range 0.628–1.058), while the range from the other MS of the finalized IC exercise is approximately 0.5–1.8. It could be concluded that both the mean value and the whole range of the common metric values from de Balearic Island data object of this IC exercise, fall within the approximated range of the CM values from the MED GIG. Thus, as stated in the CIS Guidance Document nº30, no standardisation is required.

4. Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x). A specialist case is that when a joining method relies exclusively on the common metric developed in the completed exercise for its classification rather than devising an original method (then being more like Option 1). In such cases a regression would be meaningless as y is directly dependent on x. The goal for an MS choosing to use the CM as the basis for their method is simple – after any benchmarking their boundaries must simply lie within one quarter of class of the global mean view.

The regression between the EQR values from the national assessment method (EQR_DIATMIB) and those obtained from the intercalibration common metric (ICM) is highly significant, with $R^2 = 0.9234$ (Figure 2.4).

EQR_DIATMIB and ICM were also strongly correlated (Pearson test: R = 0.961, $P<0.001$) (The requirement during the IC exercise was R>0.5).

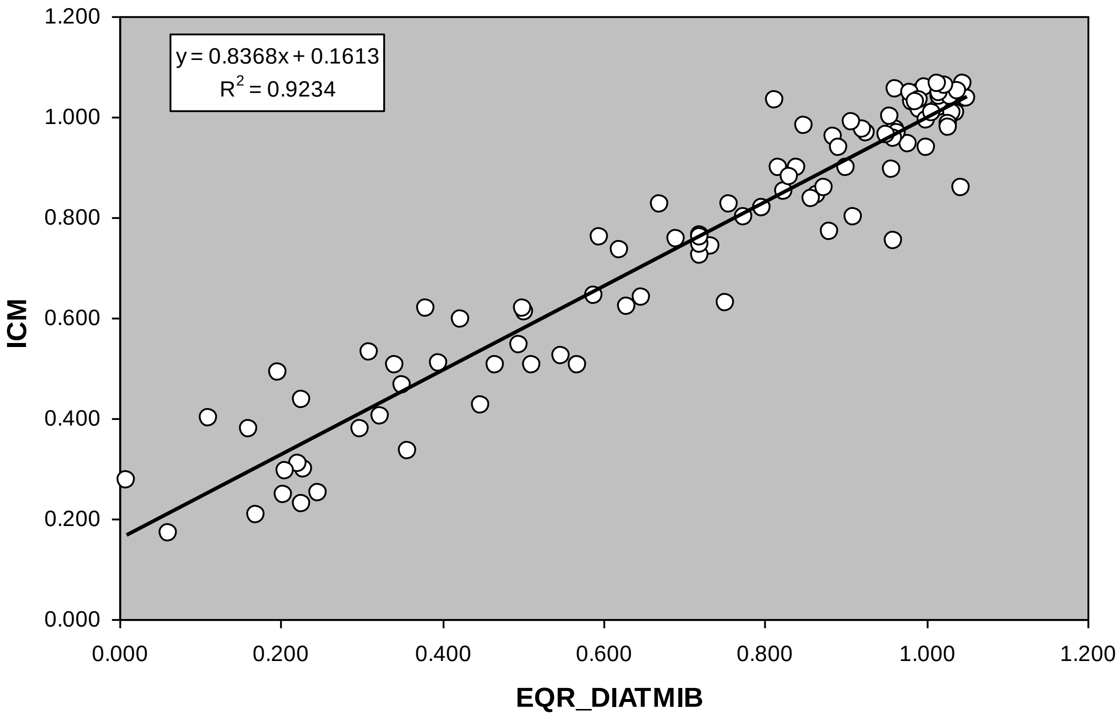

Figure 2.4. OLS regression to establish the relationship between the Balearic Islands joining method and the intercalibration common metric (ICM), for the IC river type RM5.

5. Predict the position of the national class boundaries (MP, GM, HG and reference) on the CM_bm scale.

National class boundaries predicted on the ICM scale using the relationship established from the OLS regression, are provided in Table 2.14.

Table 2.14. Positions of the class boundaries according to the DIATMIB method and predicted positions after translation to the ICM scale.

Boundary	EQR_DIATMIB	Boundaries on ICM scale
High/Good	0.93	0.940
Good/Moderate	0.73	0.772
Moderate/Poor	0.50	0.580
Poor/Bad	0.25	0.371

6. Apply the comparability criteria as summarised in Chapter 6 of the manual.

The global mean views of the H/G and G/M boundaries for IC river type RM5 in the MED GIG were extracted from the final table of harmonized class boundaries:

H/G boundary global mean view of ICM = 0.914

G/M boundary global mean view of ICM = 0.691

As the national **G/M boundary** on ICM scale lies above the global mean view, the amount of this deviation was calculated and expressed as a proportion of the class width (CW) of the Moderate (national) status on the same scale. The obtained deviation was >0.25. According to the manual, in that case the G/M boundary can be lowered until the deviation between the national boundary on the ICM scale and the global view on the same scale is ≤0.25 class widths (but there is no obligation to make this adjustment). However if the deviation is >0.5, then an adjustment is strongly recommended. In the Balearic Islands method case the deviation for the G/M boundary is <0.5, it is still quite high (0.422) (Table 2.15). Thus the G/M boundary was finally lowered. Although to achieve a final deviation <0.25 it would be necessary to lower the boundary from 0.73 to 0.67, as the adjustment is not obligated the final decision was to lowering it only to 0.68. The reason was that this is the G/M boundary adjustment for the other biological quality element (macroinvertebrates) subject of IC for rivers of the Balearic Islands, and it is more practical to have a single boundary for both elements. Besides, the final deviation with an adjustment of 0.68 is not much greater than 0.25 (Table 2.15).

Table 2.15. Summary of boundary values, class widths and boundary bias obtained for the G/M boundary before and after adjustment.

	Original G/M Boundary	Adjusted G/M Boundary
G/M Boundary of the EQR_DIATMIB	0.73	0.68
G/M Boundary on ICM scale	0.772	0.730
Global mean view of the G/M boundary	0.691	0.691
Deviation	0.081	0.039
Class width good status	0.167	0.209
Class width moderate status	0.192	0.151
Deviation as proportion of moderate status CW	0.422 (**>0.25**)	0.261(**>0.25**)

The national **H/G boundary** on ICM scale also lies above the global mean view. Thus, the amount of this deviation was calculated and expressed as a proportion of the class width (CW) of the Good (national) status on the same scale. The obtained deviation was <0.25 (Table 2.16). According to the manual, in that case the H/G boundary meets the comparability criteria and no adjustment is needed.

Table 2.16. Summary of boundary values, class widths and boundary bias obtained for the H/G boundary.

	Original H/G Boundary
H/G Boundary of the EQR_DIATMIB	0.93
H/G Boundary on ICM scale	0.940
Global mean view of the H/G boundary	0.914
Deviation	0.026
Class width high status	0.118
Class width good status	0.167
Deviation as proportion of good status CW	0.153 (**<0.25**)

However the adjustment of the G/M boundary to 0.68 entailed a change of the good status class width, and hence a change in the final deviation of the H/G boundary expressed as proportion of the mentioned class width. Thus the final deviation for the H/G boundary was 0.122 (<0.25, no adjustment is still needed).

2.6. CONCLUSIONS ON THE BALEARIC ISLANDS RM5 TYPE IC

The DIATMIB assessment method for the temporary streams (common IC type RM5) of the Balearic Islands (Spain) was compared with the finalized IC exercise of the Mediterranean Rivers GIG following the procedure described in the IC guidance nº30. The DIATMIB is a fully compliant WFD assessment method as demonstrate in this report. According Wilby et al. (2014), the DIATMIB is considered a comparable assessment method with the existing intercalibrated methods. The relationship of the DIATMIB-method with the common intercalibration common metric was very good R = 0.961. The H/G boundary on the common metric scale was within the accepted class boundary. The G/M boundary was above the boundary on the common metric scale, we partially adjusted it by lowering the class boundary for the DIATMIB and consequently its EQR. After the adjustment, the deviations of the national view on the class boundaries expressed as proportions of the corresponding class width were 0.261 (G/M) and 0.122 (H/G). It is recommended to submit the method to the ECOSTAT group for official approval.

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CHAPTER 3

THE MIBIIN A NEW CLASSIFICATION METHOD TO ASSESS WITH BENTHIC INVERTEBRATES THE ECOLOGICAL STATUS OF TRANSITIONAL WB IN THE BALEARIC ISLANDS (MED GIG TW)

CONTENTS

3.1. INTRODUCTION

- **SPAIN**
- **Benthic invertebrates**
- **Transitional water bodies (Oligohaline, Mesohaline chocked and restricted, and Poly-euhaline chocked)**

The TW Mediterranean GIG finalised the intercalibration (IC) exercise for Benthic invertebrates with the participation of 3 Member States (Italy, France and Greece), that compared and harmonized their national assessment systems for the common types Mediterranean *Coastal lagoon Poly-euhaline restricted* and *Mesohaline Chocked*. The national methods addressed the eutrophication pressure, and used an IC Option 3 (when a common biological metric was not used in the completed exercise). There were not enough data for the IC of the other GIG common types *Coastal lagoon Oligohaline*, *Coastal lagoons Poly-euhaline chocked* and *Estuaries*.

With this report we intend to fill in the existing gap (Gap N 3) for the Illes Balears (Spain), showing that the Regional authorities of the Balearic Islands have developed a fully Water Framework Directive (WFD) compliant benthic invertebrates classification method for the common types ***Oligohaline, Mesohaline chocked, Mesohaline restricted*** and ***Poly-euhaline chocked*** that exist in the Balearic Islands.

The way forward for filling intercalibration gaps by MS is described in the document "Intercalibration of biological assessment methods – remaining open issues and proposed way forward". The gaps have to be filled by 2016, and in order to achieve this, the IC reports (from the GIGs or Member States) have to be:

- Submitted to the Intercalibration steering
- Reviewed by the IC review panel (in cases if no problems, reviews can be omitted);
- Presented at ECOSTAT meetings and agreed by ECOSTAT working group.

In particular, Gap N 3 addresses the situation where the GIG work has been finalised and it is not possible to IC the method according to the final results. In the case of the Balearic Islands the reasons are: 1) that the finalised GIG results (MED TW GIG) did not include the IC most common types covered by the new classification method (e.g. Oligohaline, Mesohaline restricted and Poly-euhaline chocked) developed for the Balearic Islands, and 2) that for the type Mesohaline chocked it was not possible to IC using the metric already intercalibrated in the MED GIG TW exercise (M-AMBI) as common metric for the Balearic Islands case.

The way forward, is that Member States have to show that their methods are compliant with the

WFD normative definitions, with a detailed description of the assessment methods and evaluation of their WFD compliance, and a description of the reasons why an IC was not feasible (or refer to the GIG report, if this is explained in the GIG report).

3.2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The **MIBIIN** was developed using Benthic invertebrate samples collected by the Illes Balears Government monitoring program between 2005 and 2008 (Campaings: May–June 2005, November–December 2005, February–March 2006, May–June 2006, February–March 2008 and May–June 2008). Here we describe the MIBIIN compliance with WFD in relation with its sampling method, taxonomic resolution, calculation, national reference criteria, class boundaries and presure-impact relationships.

3.2.1. Methods and required BQE parameters

Table 3.1. Overview of the metrics included in the national method according to common types.

MS	Type	Taxonomic composition	Abundance	Sensitive taxa	Tolerant taxa	Diversity
ES	Oligohaline	%CY-PO	%SENGEN %CY-PO	%SENGEN	%CY-PO	GEN-S
ES	Mesohaline (chocked and restricted)	BC to order level	%AM-GA-IS	SENGEN-S	NO	BC SENGEN-S
ES	Poly-euhaline (chocked)	%ARSA	%ARSA	SENGEN-S	%ARSA	SENGEN-S

%SENGEN (frequency of sensitive genera); GEN-S (genera richness); %CY-PO (frequency of *Cyprides torosa* + Polychaeta; SENGEN-S (sensitive genera richness); BC (similarity of Bray-Curtis (to level order); %AM-GA-IS (frequency of Amphipoda + Gastropoda + Isopoda); %ARSA (frequency of *Artemia salina*)

The classification system developed in this study consists of a multimetric index (MIBIIN), type-specific for the different Balearic Islands coastal lagoons salinity types: oligohaline, mesohaline and euhaline, corresponding to the IC common types Oligohaline, Mesohaline chocked, Mesohaline restricted and Poly-euhaline chocked for TW. The criteria followed to select the metrics were: (1) to comply with the normative definitions of the WFD (European Union 2000), (2) to respond to the gradient of pressure, (3) to be able to discern reference values from disturbed sites, and (4) the metrics selected should be non-redundant.

The MIBIIN is compliant with the WFD, as it is based on the use of individual metrics selected following the requirements of the normative definitions (i.e. abundance, diversity and ratio of sensitive-tolerance taxa) (see Lucena-Moya et al. 2012 for further details). Based on current literature (e.g. Barbour et al. 1999; Kashian and Burton 2000) a great number metrics commonly used in assessment and covering all the required BQE parameters were selected as candidates (e.g., richness at the genus level, relative richness (e.g. number of genera with respect to total richness), relative abundance of several taxa (e.g. %*Ablabesmyia*/*Tanytarsus*, %Orthocladiinae/Chironomidae), several diversity measures (e.g., Shannon–Wiener, Simpson's index, percentage of the three dominant taxa)). Finally only 8 of the individual metrics (3 for oligohaline, 3 for mesohaline and 2 for euhaline) made up the MIBIN. The final selection of these eight metrics was due to their best performance fulfilling the aforementioned criteria and for its potential as indicator, as their response were analysed along to pressure gradients, thus explaining possible absences in some parameter for some coastal lagoon type. The finally selected metrics per type were averaged in the Multimetric Index of Balearic Islands based on Invertebrates (MIBIIN). The three MIBIIN indices selected for each intercalibrated salinity type (Table 3.2) are:

oligo-MIBIIN = Σfrequency of *sensitive genera* + genera richness + frequency of (*Cyprideis torosa* +Polychaeta),

meso-MIBIIN = Σ*sensitive genera* richness + Bray–Curtis similarity + frequency of (Amphipoda + Gastropoda + Isopoda)

euh-MIBIIN = Σ*sensitive genera* richness + frequency of *Artemia salina*.

To combine individual metrics to make up the MIBIIN they have to be in the same scale, thus is previously necessary to standardise them dividing by the median of the *reference* sites. After that individual metrics can be added to produce the MIBIN.

Table 3.2. Metrics composing the MIBIIN multimetric for the oligohaline, meshohaline and euhaline transitional waters types for the Balearic Islands. The expected responses to pressure, the transformations and the normalizations necessary to obtain it are also indicated (from Lucena-Moya et al. 2012).

Type	MIBIIN	Description MIBIIN	Expected response to pressure	Reverse
Oligohaline	%SENGEN	Frequency of sensitive genera	-	No
	GEN-S	Genera richness	-	No
	%CY-PO	Frequency of *Cyprides torosa* + Polychaeta	+	Yes
Mesohaline	SENGEN-S	Sensitive genera richness	-	No
	BC	Similarity of Bray-Curtis (to level order)	-	No
	%AM-GA-IS	Frequency of Amphipoda + Gastropoda + Isopoda	-	No
Euhaline	SENGEN-S	Sensitive genera richness	-	No
	%ARSA	Frequency of *Artemia salina*	+	Yes

The ecological status is defined on the basis of the Ecological Quality Ratio (EQR), calculated by dividing each resulting value of the MIBIIN by the median value of the reference data:

EQR_MIBIIN = measured MIBIIN value / median MIBIIN values of references

Then the obtained values ranged from 0 (worst quality) to >1 (best quality), being the ecological status classified by one of five classes (high, good, moderate, poor and bad) shown in Table 3.3.

Table 3.3. Ecological status levels and corresponding national boundaries.

Boundaries	Level of disturbance	Quality class	Colour
		Reference	
> 0.93	Minimal	High	
0.93-0.73	Slight	Good	
0.73-0.50	Moderate	Moderate	
0.50-0.25	Major	Poor	
< 0.25	Severe	Bad	

3.2.2. Sampling and data processing

Invertebrate samples (macro- and micro- invertebrates) were collected using a hand-net (25x25 cm frame net, 250-mm mesh size) and following a protocol based on a multi-habitat survey (e.g. Barbour et al. 1999). Different habitats (vegetated shores, shores without vegetation, bare soft sediment, and submerged macrophytes) are taken into account in the sampling. Depending on each coastal lagoons habitat heterogeneity and extension, several sampling sites were differentiated (22 lagoons with 1 site *vs*. 12 lagoons with ≥2-6 sites).

Abundance (individuals by sample) is related to number of "sweeps" (capture per unit effort, CPUE). 10 sweeps per sampling site were took in 2005–2006 and 20 sweeps in 2008 (this change was produced to harmonize the sampling protocols with the remaining Spanish research groups, with a view to the European IC exercises).

The area sampled in each sweep was constant, 0.125m^2 (25-cm width by 50-cm length). The sampling was carried out in a littoral band (ca. 50 x 3 m) of the coastal lagoon. The technique used to sample different habitats afore mentioned was similar to one used by Trigal et al. (2007) (i.e., plants were disturbed by sweep netting up and down in the vegetation stands to a depth of about 5–10 cm off the bottom sediment; then, dislodge material and animals were collected. In sediment and shores without vegetation, first the bottom sediment was disturbed with the net edge to resuspend the animals living in it; and next, the net was swept around to collect the resuspended material and animals). This methodology allows capturing the maximum biodiversity, which in coastal lagoons is generally found at shoreline rather than in deeper water. Besides, due to the shallowness of the coastal lagoons studied, especially in the littoral area, planktonic taxa (living in the benthos many of them) are present in the water column and are inevitable to collect.

The samples were preserved in 70% ethanol and once in the laboratory, they were sorted using sieves of different pore size (5 mm, 1 mm and 0.1 mm). Individuals were identified to the lowest taxonomic level possible (i.e., species or genus); although some taxa whose identifications were more complicated, were identified to superior taxominc levels (e.g., Diptera –family–, Nematoda –order–, Oligochaeta –class– or Acari –class–). Moreover, some individuals were identified to family level because their small size that did not allow better taxonomic resolution (e.g., some individuals of the orders Odonata and Heteroptera).

3.2.3. National reference conditions

Evaluation of the level of pressures at the catchment level; collection of information on land use within determined bands around the wetlands and field ascertainment (Lucena-Moya et al., 2009). A modified methodology based on U.S. EPA 2000, was applied with this purpose. An a priori screening of pressures was initially performed based on the existing regional wetlands inventories and cartography, as well as in satellite and aerial photographs. This a priori analysis consisted on: a) evaluation of the level of pressures at the catchment level, or in the influential surrounding areas whereas the stream inputs are not relevant; b) collection of information on land use within determined bands around the wetlands; and c) field ascertainment on the absence of pressures. Two buffer zones were distinguished around the selected sampling sites to evaluate the pressures. The first one corresponds to the area immediate to the edge (< 50 m band) and the second band goes from the 50 m that limit the first band to the following 300 m. In the first buffer zone, the selected pressures have to be absent: agriculture (extensive or intensive), roads-trails, artificial/urban uses, non site pollution, channels/sluice gates or recreational activities (Table 3.4). In this first buffer any exotic species can be present among the vegetation, thus only autochthonous vegetation is possible (Table 3.4). The second buffer is a more permissive zone for pressures, allowing the presence of low levels of extensive agriculture (but not intensive) and recreational activities, as well as wooded ways or trails (not asphalted roads), channels or sluice gates that not influence water connexion; and the presence of some exotic species (Table 3.4). The a posteriori evaluation of the a priori selected sites consisted on checking for consistency using information on water physico-chemistry and biological communities. A total of 6 sites were identified and used as references for the development of the MIBIIN index (2 per salinity type) (see Lucena et al. 2012 for further details). In table 3.5 there is a summary of the main environmental variables defining the reference conditions for each coastal lagoon type.

Tabla 3.4. List of pressures evaluated within the two buffer bands used to define reference conditions and thus to select reference sites in the small coastal lagoons of the Balearic Islands (Lucena-Moya et al. 2009).

Pressure	Buffer 1 (0-50 m)	Buffer 2 (50-300 m)
Autochthonous vegetation	X	X
Absence of agriculture	X	Absence of intensive (irrigation) agriculture
Absence roads, no trails	X	Wooder or trails
Absence artificial/urban use/non point pollution	X	X
Absence of channels, sluice gates, concrete structures, quarries	X	No influence on water connexion
Absence of exotic species	X	Presence of exotics, nor invasive cover
Absence of recreational activities	X	Low use for recreational activities

Table 3.5. Major environmental features (mean ± SD) that characterize the reference samples collected in the reference sites for the Balearic Island transitional waters types. The number of samples is indicated in brackets (adapted from Lucena-Moya et al. 2012).

Characteristic	Oligohaline n = 12	Mesohaline n = 8	Euhaline n =7
P_PO_4 (mg/L)	0.06 ± 0.04	0.02 ± 0.01	0.05 ± 0.03
TP (mg/L)	0.11 ± 0.04	0.04 ± 0.01	0.22 ± 0.10
DIN (mg/L)	0.09 ± 0.03	0.03 ± 0.02	0.05 ± 0.02
TN (mg/L)	0.82 ± 0.21	0.71 ± 0.13	2.64 ± 0.66
O_2 (mg/L)	7.76 ± 0.84	8.34 ± 1.15	5.61 ± 1.14
Salinity (%)	2.31 ± 0.38	9.03 ± 0.63	56.38 ± 13.88
Chl a (μg/L)	2.57 ± 0.65	2.36 ± 0.38	15.46 ± 5.72
AFDM (g/L)	0.003 ± 0.001	0.003 ± 0.001	0.087 ± 0.045

3.2.4. National Boundary setting

- Methodology used to set H/G boundary: The H/G was set as the 25th percentile (P25) of the EQR_MIBIIN reference values.
- Methodology used to set the remaining boundaries: Below the 25th percentile, the remaining quality class boundaries were defined into equal bands.

The G/M boundary was set as H/G boundary- (P_{25} /4)

The M/P boundary was set as G/M boundary- (P_{25} /4)

The P/B boundary was set as M/P boundary- (P_{25} /4)

We **averaged** the three values per boundary to obtain one single value for each class boundary across the three salinity types. A posterior adjustment and confirmation of the boundaries was produced looking at the crossing between the fitted regression lines of paired individual metrics, for its ecological interpretation following the "Guidance on the intercalibration process 2008-2011" (Schmedtje et al. 2009). The obtained class boundaries are illustrated in Table 3.6.

Table 3.6. Officially used class boundaries for the MIBIIN method.

Class Boundary	High/Good	Good/Moderate	Moderate/Poor	Poor/Bad
MIBIIN	0.93	0.73	0.5	0.25

3.2.5. Pressures addressed

A Multimetric index and the individual metrics have to respond to pressure gradients. To obtain the pressure gradients Factor Analysis (FA), with the Principal Component Analysis (PCA) was performed. For the MIBIIN and for each individual metric, PCA scores were evaluated as explanatory variables of their response to the gradient of pressure. We fitted a general lineal model (multiple regressions); we searched for the best subset of explanatory variables (PCA axes) using a forward stepwise procedure selection. For these analyses the MIBIIN values were introduced under the form of EQR. The obtained results on regression and correlation analysis confirmed the function of the MIBIIN multimetric index as indicator (Fig. 3.1). In Table 3.7 the relation among the environmental variables and the axes of the PCAs defining the pressure gradients are given.

Table 3.7. Variable loading in axes (PC1 to PC4) obtained from factor analysis for each type of coastal lagoon. Numbers in bold indicate values above (0.7).

	Oligohaline				Mesohaline				Euhaline			
Characteristics	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
P_PO_4 (mg/L)	**0.926**	-0.028	0.079	-0.036	**0.845**	0.271	-0.148	0.038	0.277	0.053	0.16	0.165
TP (mg/L)	**0.921**	0.005	-0.022	0.277	**0.919**	0.000	0.118	0.105	0.286	-0.306	-0.015	0.45
DIN (mg/L)	0.046	**0.986**	0.013	0.017	0.04	**0.968**	0.053	-0.50	-0.189	0.244	**0.933**	0.03
TN (mg/L)	0.004	**0.977**	0.135	0.026	0.23	**0.892**	-0.211	0.092	0.075	**0.900**	0.349	-0.095
O_2 (mg/L)	0.059	-0.177	0.129	0.023	0.219	-0.026	-0.047	0.144	**-0.842**	0.170	0.064	-0.384
Salinity (%)	0.054	0.211	**0.874**	-0.197	0.06	-0.079	**0.945**	0.024	**0.825**	-0.045	-0.231	0.122
Chl a (μg/L)	0.651	0.216	-0.562	-0.130	0.212	0.042	-0.602	0.624	-0.246	**0.920**	0.024	0.089
AFDM (g/L)	0.106	0.042	-0.129	**0.969**	0.042	0.005	0.041	**0.931**	0.354	0.068	0.039	**0.848**
% Variation	26.9	25.7	14.2	13.4	21.4	22.7	16.8	16.3	22.2	23.1	13.5	14.1

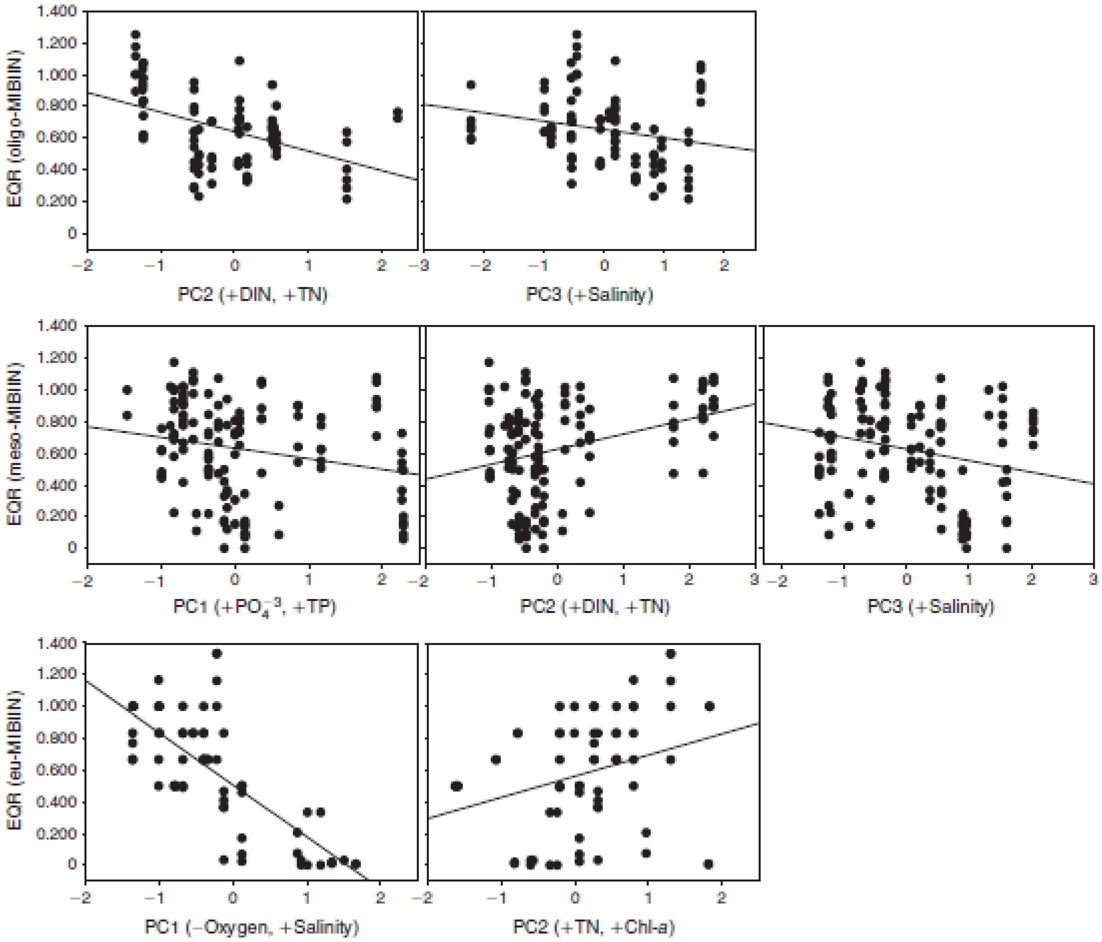

Figure 3.1. Dispersion plots showing the significant trends of the multimetric index of the Balearic Island based on invertebrates (MIBIIN) as ecological quality ratio values (EQR) against the gradients of pressure for each type.

3.3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria (Table 3.8).

Table 3.8. List of the WFD compliance criteria and the WFD compliance checking process and results.

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by one of five classes (high, good, moderate, poor and bad).	**Yes**
2. High, good and moderate ecological status are set in line with the WFD's normative definitions (Boundary setting procedure)	**Yes**, see subsection "National boundary setting".
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.	**Yes**, the MIBIIN index is an average of different normalized metrics depending on the stream national type and covering all relevant parameters.
4. Assessment is adapted to intercalibration common types that are defined in line with the typological requirements of the WFD Annex II and approved by WG ECOSTAT	**Yes**, the assessed sites were all assigned to the common types .
5. The water body is assessed against type-specific near-natural reference conditions	**Yes**, coastal lagoons are assessed against existing type-specific near-natural reference conditions.
6. Assessment results are expressed as EQRs	**Yes**
7. Sampling procedure allows for representative information about water body quality/ ecological status in space and time	**Yes**
8. All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure	**Yes**, the sampling procedure consider abundance, diversity an ratio of sensitive-tolerance taxa.
9. Selected taxonomic level achieves adequate confidence and precision in classification	**Yes**, species or genus (with exceptions). See subsection 3.2.2

After the compliance check we concluded that the MIBIIN method fulfils all the requirements of the WFD (Table 3.8).

However a comment should be done regarding the inclusion of the zooplankton as part of MIBIN. The exclusion of the plankton as biological quality element in the WFD has already been criticized by other authors (Boix et al. 2005; Moss et al. 2003). Microcrustaceans have properties (e.g., they are ubiquitous, respond to disturbance gradients) which make them suitable as quality indicator (see Boix et al. 2005 and references therein) and have been used as such by several authors (e.g., Bianchi et al. 2003; Boix et al. 2005). The collection of the plankton fraction together with the benthic one is inevitable in shallow systems as coastal lagoons are; they should be indispensable for the determination of the ecological quality of shallow lagoons (Scheffer 1998; Moss et al. 2003).

3.4. IC FEASIBILITY CHECKING

3.4.1. Typology

The CL-Mesohaline chocked type was intercalibrated between France, Italy and Greece. The other three types existing in the Islands, Oligohaline, Mesohaline restricted and Poly-euhaline Chocked were not intercalibrated in the IC exercise. The correspondence with the coastal lagoons common IC types and the types main characteristics are shown in table 3.9.

Table 3.9. Common IC types existing in the Balearic Islands.

Common IC type in the Balearic Islands	Type characteristics
CL-Oligohaline	Coastal lagoons (Salinity <5 psu)
CL-Mesohaline (Chocked and restricted)	Coastal lagoons (Salinity 5-18 psu)
CL-Poly-euhaline (Chocked)	Coastal lagoons (Salinity 18-40 psu)

3.4.2. Pressures addressed

Yes, the MIBIIN address the pressures of Eutrophication intercalibrated in the TW MED GIG. See above *subsection 3.2.5.* Pressures addressed, to see the significant response of the EQR_MIBIIN to nutrients and organic pollution variables.

3.4.3. Assessment concept

The already IC method in the TW MED GIG exercise (M-AMBI) is focused on soft bottom macroinvertebrates, based on the abundance of sensitive/tolerant species faced with the increased or decreased disturbance. The 3 MS that IC their methods (M-AMBI) in the GIG exercise, collected samples by grab sampler; while in the MIBIIN case a litoral net sweeping for 2.5 m^2 in different habitats approach is used.

3.4.4. Conclusion on the intercalibration feasibility

There are two main reasons why de IC is not attainable for the MIBIIN case according to the final results of the MED GIG TW intercalibration exercise.

1) Three of the 4 common types covered by the MIBIIN have not been intercalibrated.

2) The Balearic Islands multimetric (MIBIIN) is not conceptually comparable with the method already intercalibrated (M-AMBI) for the common type Mesohaline chocked.

Regarding methodological aspects, the M-AMBI samples of the intercalibrated methods for the three MS are collected by grab sampler; while in the MIBIIN case a littoral net sweeping for 2.5 m^2 area with a multiple habitat sampling approach is used.

Considering the application of the metric, initially the AMBI was designed to establish the ecological quality of European coastal waters. This index has been validated for use with coastal and estuarine benthic fauna. However, the sampling locations initially selected for this IC exercise are mostly located within endorreic lagoons or are the final section of temporary rivers in the coast, with highly variable salinity fluctuations (Basset et al. 2012). The observed fauna is characterised by a low proportion of marine taxa and high proportions of epicontinental taxa, adapted to the ecological characteristics of these lagoons. This fact has resulted in the virtual impossibility of applying the M-AMBI for the three common types existing in the Balearic Islands (Poly-euhaline, mesohaline and oligohaline), according to the recommendations and truncation rules given in Borja et al. 2005.

Attending to the exclusion criteria exposed in Borja et al. 2005, excepting for the mesohaline type stations with salinity <10, the initial matrices for all the other types were reduced in more than 50% prior to the index calculation. Thus the use of a biotic ecological quality index, omitting such amount of fauna seems not to be the best approach. The three main criteria (truncation rules) that have been necessary to apply were as follows:

- Remove all freshwater taxa
- In salinity >10, remove insecta
- Remove epifaunal taxa

Being the insects (most of which resulted to be freshwater taxa), the ones that contributed the most to the matrices reduction. By the way, this significant reduction has also entailed that some samples resulted to be with no taxa and therefore the calculation of AMBI would not be possible for them, as well as some other samples remained only with 1 to 3 taxa, fact that according to Borja et al. 2005 may reduce the robustness of the calculation.

Finally, in addition to the already high reduction of the matrices, often the percentage of taxa not assigned to any group in the reduced matrices was above 20 and even 50% for many samples of the eu- and oligohaline types. According to Borja et al. 2005 when the percentage of non assigned taxa is >20%, the results should be evaluated with care, and when it is >50%, the AMBI should not be used. Attending each typology individually we found that:

- For the euhaline type the initial matrix was reduced in a 73% according to the truncation rules exposed above. This reduction also implies that of the 21 available samples 9 had no taxa, 8 ended with 1 to 3 taxa, 4 with a percentage of non assigned taxa >20% and 2 of them >50%.

- For the mesohaline type with salinity >10, the initial matrix was reduced in a 62% according to the truncation rules exposed above. This reduction also implies that of the 17 available samples 9 ended with only 1 to 3 taxa.
- For the meshohaline type with salinity <10, the initial matrix was reduced in a 48% according to the truncation rules exposed above.
- For the oligohaline type the initial matrix was reduced in a 68% according to the truncation rules exposed above. This reduction also implies that of the 30 available samples 5 ended with only 1 to 3 taxa, 9 with a percentage of non assigned taxa >20% and 4 of them >50%.

For all that reasons, the use of M-AMBI as common metric for IC the MIBIIN for the Mesohaline chocked common type, was not possible for this intercalibration exercise.

3.5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

Although general data on the benthic invertebrates communities are publicated for the Balearic Islands coastal lagoons (see Lucen-Moya et al. 2010 for further details), the publication and description of the biological communities along degradation gradients is still under study. Once the Illes Balears Government obtains more samples from its monitoring program, the biological communities at each status class will be defined.

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CHAPTER 4

THE PHYTOMIBI A NEW CLASSIFICATION METHOD TO ASSESS WITH PHYTOPLANKTON THE ECOLOGICAL STATUS OF TRANSITIONAL WB IN THE BALEARIC ISLANDS (MED GIG TW)

CONTENTS

4.1. INTRODUCTION

- **SPAIN (Balearic Islands)**
- **Phytoplankton**
- **Transitional water bodies (Oligohaline and mesohaline Coastal Lagoons)**

The TW Mediterranean GIG finalised the intercalibration (IC) exercise for Phytoplankton with the participation of 3 Member States (Italy, France and Greece), that compared and harmonized their national assessment systems for the common type "Mediterranean Coastal lagoon Poly-euhaline". The national methods addressed the eutrophication pressure, and used an IC Option 2 (indirect comparison of assessment methods using a common metric). The intercalibration was performed only for summer Chla data. There were not enough data for the IC of the other GIG common types *Coastal lagoon Oligohaline*, *Coastal lagoons Mesohaline* and *Estuaries*.

With this report we intend to fill in the existing gap (Gap N 3) for the Illes Balears (Spain), showing that the Regional authorities of the Balearic Islands have developed a fully Water Framework Directive (WFD) compliant phytoplankton classification methods for the common types ***Oligohaline*** and ***Mesohaline Coastal Lagoons*** that exist in the Balearic Islands.

The way forward for filling intercalibration gaps by MS is described in the document "Intercalibration of biological assessment methods – remaining open issues and proposed way forward". The gaps have to be filled by 2016, and in order to achieve this, the IC reports (from the GIGs or Member States) have to be:

- Submitted to the Intercalibration steering
- Reviewed by the IC review panel (in cases if no problems, reviews can be omitted)
- Presented at ECOSTAT meetings and agreed by ECOSTAT working group.

In particular, Gap N 3 addresses the situation where the GIG work has been finalised and it is not possible to IC the method according to the final results. This is the case of the Balearic Islands. The finalised GIG results (MED TW GIG) did not include the IC of the common types covered by the new classification method (e.g. Oligohaline and Mesohaline) developed for the Balearic Islands.

The way forward, is that Member States have to show that their methods are compliant with the WFD normative definitions, with a detailed description of the assessment methods and evaluation of their WFD compliance, and a description of the reasons why an IC was not feasible (or refer to the GIG report, if this is explained in the GIG report).

4.2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The **PHYTOMIBI** was developed using Phytoplankton samples collected by the Illes Balears Government monitoring program in winter and spring of 2008. Here we describe the PHYTO-MIBI compliance with WFD in relation with its sampling method, taxonomic resolution, calculation, national reference criteria, class boundaries and presure-impact relationships.

4.2.1 Methods and required BQE parameters

Table 4.1. Overview of the metrics included in the national method.

MS	Type	Taxonomic composition	Abundance	Frequency and intensity of algal blooms
ES	Oligohaline Coastal Lagoons	%Cyanobacteria	Chlorophill a	no
ES	Mesohaline Coastal Lagoons	% Diatoms + Cryptophyceae + Prasinophytes	Chlorophill a	no

The classification system developed in this study consists of a multimetric index (PHYTO-MIBI) for each of the two types of coastal lagoons, oligohaline and mesohaline. The criteria followed to select the metrics were: (1) to comply with the normative definitions of the WFD (i.e., abundance, diversity and ratio of tolerant-sensitive taxa), (2) to respond to the gradient of pressure able to discern references from disturbed sites.

The multimetrics are composed by metrics describing composition and abundance of the phytoplanktonic taxa, and phytoplankton biomass. The metrics composing the PHYTO-MIBI include phytoplankton biomass (Chlorophyll-a in µg/L), percentages of Cyanobacteria, Diatoms, Cryptophyceae and Prasinophytes (Table 4.2). Each individual metric was selected for its potential as indicator, analyzing their response to pressure gradients for each lagoon type (Oligohaline and mesohaline). The final metrics selected per type were averaged in a multimetric index (Pardo et al., 2010).

The PHYTOMIBI does not include the frequency and intensity of algal blooms. The Balearic lsland’ s transitional coastal lagoons are small and numerous. A major effort was performed to sample all them initially in the development of the method. Meanwhile, bloom frequency was very difficult to assess with precision, because the water bodies are numerous (n=30) and remotely located, and the impossibility of adjusting sampling timing with possible bloom existence was very low. For that, and taking into account that the other metrics of abundance and composition composing the PHYTOMIBI responded in a significant way to existing pressures in the Balearic Islands, this parameter of the phytoplankton was not considered in the assessment method development.

Table 4.2. Summary of metrics composing the PHYTOMIBI multimetrics for the Oligohaline and Mesohaline lagoons, its response to pressure, transformation and normalization to calculate EQR.

Type		Metric	Expected response to pressure	Reverse	Transformation	Normalisation
Oligohaline	Biomass	*Chl a* (µg/L)	+	yes	/Max. Serie	Median ref.
	Composition	% Cyanobacteria	+	yes	no	Median ref.
Mesohaline	Biomass	*Chl a* (µg/L)	+	yes	/Max. Serie	Median ref.
	Composition	%Pras+Diato.+Cript	+	yes	no	Median ref.

4.2.2. Sampling and data processing

Phytoplankton samples were collected in winter and spring 2008. Chlorophyll a and ash-free dry mass (AFDM) were estimated from three separate replicates of a known volume of water filtered *in situ* with a hand-pump through a glass fibre filter (Whatman GF/F with a pore size 0.45 µm; in the case of the AFDM the filters were pre-weighted) (see Lucena-Moya et al., 2009 for further details).

Phytoplankton composition was assessed with water samples taken in 125ml glass bottles at around 20 cm depth and preserved in situ with 2.5mL of glutaraldehide 2%. Samples were hold at 4ºC in darkness conditions until its process in the laboratory. Phytoplankton analyses were carried out filtering 10 ml samples through a 0.2µm membrane filter (Millipore GTTP de Ø 25 mm), drying later the filtered material. Because of the sample salinity, the salt retained on the filters was removed adding 5ml of distilled water and the samples were again filtered and dried. After this, the material on the filter was dehydrated by washing successively with 50%, 80%, 90% and 99% aqueous ethanol. Each dried filter was placed onto a drop of immersion oil in the centre of a slide and 2 more drops were added on the top side of the filter. Finally, a coverglass was placed on the top of the filter. Algal counts were made by epifluorescence microscopy with a Nikon Optiphot microscope, using a 100× oil-immersion objective. This technique is based on the observation of morphological characters and the pigments colour to classifly phytoplankton. A minimum of 300 cells was counted and at least 100 cells of the species or genera more abundant were counted with an error lower than 20% (Lund et al., 1958).

The assessment concept is based on the different phytoplankton functional groups depending on different TW typologies. Water samples are taken in the superficial layer, and recounts are at Class level and higher taxonomic level than Genus.

4.2.3. National reference conditions

The evaluation of the level of pressures was performed at the catchment level, combined with the collection of information on land use within determined bands around the wetlands and field confirmation (Lucena-Moya et al., 2009). A modified methodology based on U.S. EPA 2000, was applied with this purpose. An a priori screening of pressures was initially performed based on the existing regional wetlands inventories and cartography, as well as in satellite and aerial photographs. This a priori analysis consisted on: a) evaluation of the level of pressures at the catchment level, or in the influential surrounding areas whereas the stream inputs are not relevant; b) collection of information on land use within determined bands around the wetlands; and c) field ascertainment on the absence of pressures. Two buffer zones were distinguished around the selected sampling sites to evaluate the pressures. The first one corresponds to the area immediate to the edge (< 50 m band) and the second band goes from the 50 m that limit the first band to the following 300 m. In the first buffer zone, the selected pressures have to be absent: agriculture (extensive or intensive), roads-trails, artificial/urban uses, non site pollution, channels/sluice gates or recreational activities (Table 4.3). In this first buffer any exotic species can be present among the vegetation, thus only autochthonous vegetation is possible (Table 4.3). The second buffer is a more permissive zone for pressures, allowing the presence of low levels of extensive agriculture (but not intensive) and recreational activities, as well as wooded ways or trails (not asphalted roads), channels or sluice gates that not influence water connexion; and the presence of some exotic species (Table 4.3). The a posteriori evaluation of the a priori selected sites consisted on checking for consistency using information on water physico-chemistry and biological communities.

Tabla 4.3. List of pressures evaluated within the two buffer bands used to define reference conditions and thus to select reference sites in the small coastal lagoons of the Balearic Islands (Lucena-Moya et al. 2009).

Pressure	**Buffer 1 (0-50 m)**	**Buffer 2 (50-300 m)**
Autochthonous vegetation	X	X
Absence of agriculture	X	Absence of intensive (irrigation) agriculture
Absence roads, no trails	X	Wooder or trails
Absence artificial/urban use/non point pollution	X	X
Absence of channels, sluice gates, concrete structures, quarries	X	No influence on water connexion
Absence of exotic species	X	Presence of exotics, nor invasive cover
Absence of recreational activities	X	Low use for recreational activities

4.2.4. National Boundary setting

The methodology used to set up the Boundaries was the same for both TW types of the PHYTOMIBI (Pardo et al. 2010). The 25th percentile of the Reference Conditions was used as the H/G boundary. A posterior adjustment and confirmation of the boundary was produced looking at the crossing between the two metrics, for its ecological interpretation following the boundary setting protocol.

For the G/M boundary. Below the 25th percentile, the remaining quality class boundaries were defined into equal bands. A posterior adjustment and confirmation of the boundary was produced looking at the crossing between the two metrics, for its ecological interpretation following the boundary setting protocol (Pardo et al. 2010). The final boundaries are indicated in table 4.4.

Two tables with the mean values and median values of each metric included in the MIBIIN for the two coastal lagoon types are given (Tables 4.5 and 4.6).

Table 4.4. Officially used class boundaries for the PHYTOMIBI method.

Class Boundary	High/Good	Good/Moderate	Moderate/Poor	Poor/Bad
PHYTOMIBI	0.93	0.73	0.5	0.25

Table 4.5. Mean, median and SE of Chla a values for the references and status classes of the EQR PHYTOMIBI in Oligohaline Coastal Lagoons.

		Class EQR PHYTOMIBI Phytoplanktoncton		
		High	**Good**	**Moderate**
Chl-*a* µg/L	Mean	3.06	5.27	7.78
	Median	1.25	5.17	4.21
	SE	0.60	0.93	2.10
% Cyanobacteria	Mean	0.10	0.31	0.75
	Median	0.09	0.31	0.74
	SE	0.03	0.07	0.05

Table 4.6. Mean, median and SE of metric values for the references and status classes of the EQR PHYTOMIBI in Mesohaline Coastal Lagoons.

		Class EQR PHYTOMIBI Phytoplanktoncton		
		High	**Good**	**Moderate**
Chla µg/L	Mean	3.14	7.2	9.63
	Median	3.17	2.91	3.26
	SE	0.66	1.84	3.98
% Diatoms +	Mean	2.10	2.41	2.59
Cryptophyceae +	Median	2.11	2.45	2.70
Prasinophytes	SE	0.12	0.11	0.11

4.2.5. Pressures addressed

A Multimetric index and per ende the individual metrics have to respond to pressure gradients. Summary of the Pearson correlation coefficients (Table 4.7) and regressions values between the PHYTOMIBI and independent pressure variables (Figs. 4.1 and 4.2), for each type of coastal lagoons. Dependent variables were the PHYTOMIBI (as EQR), composition groups and Chl *a*, and the independent variables were the variables related with pressures (TP, P_PO_4, DIN, $N\text{-}NO_3$, N_NO_2, DIN/P_PO_4, TRIX (calculated for both types without Chl *a*, and for the mesohaline without O_2)) (Table 4.8).

Table 4.7. Summary of the Pearson correlation coefficients between the PHYTOMIBI and independent pressure variables. Marked in bold Pearson correlations p <0 .05.

	OLIGOHALINE (IB)			MESOHALINE (IB+VA)		
	Chl *a*	%Cyanobacteria	EQR PHYTOMIBI	Chl *a*	Cryptophyta + Diatom + Prasinophyce	EQR PHYTOMIBI
P-PO4	0.4391 p=.102	0.33 p=.230	-0.3597 p=.188	**0.3748 p=.022**	-0.0761 p=.654	-0.0948 p=.577
PT	**0.5392 p=.038**	**0.5731 p=.026**	**-0.5776 p=.024**	**0.6854 p=.000**	0.1034 p=.543	**-0.4454 p=.006**
N-NO2	0.3499 p=.201	0.2899 p=.295	-0.2745 p=.322	0.3137 p=.059	**0.5466 p=.000**	**-0.5609 p=.000**
N-NO3	0.2537 p=.362	0.1693 p=.546	-0.2664 p=.337	0.0358 p=.833	**0.5234 p=.001**	**-0.3642 p=.027**
N-NH4	0.3413 p=.213	0.146 p=.604	-0.2453 p=.378	-0.1071 p=.528	0.071 p=.676	-0.02 p=.907
DIN	0.19 p=.498	0.2993 p=.279	-0.297 p=.282	-0.111 p=.513	**0.4656 p=.004**	-0.2315 p=.168
DIN/PPO4	-0.1335 p=.635	-0.2641 p=.341	0.2013 p=.472	-0.1845 p=.274	**0.4145 p=.011**	-0.1937 p=.251
TRIX (with O2)	**0.5665 p=.028**	**0.5689 p=.027**	**-0.6305 p=.012**			
TRIX without O2)	0.269 p=.332	0.3177 p=.248	-0.2876 p=.299	0.2269 p=.177	**0.4653 p=.004**	**-0.4256 p=.009**

Table 4.8. Summary of the pressures addressed and the strength of the relationships with PHYTOMIBI.

Method	Metrics tested	Pressure	Pressure indicators	Strength of relationship(R^2, p)
PHYTO-MIBI	EQR_PHYTOMIBI Chl *a* % Cyanobacteria Chl a %Criptophycea + %Diatoms + %Prasinophyceae	Eutrophication and organic	**Mesohaline:** TP, $P\text{-}PO_4$, $N\text{-}NO_3$, $N\text{-}NO_2$, Nutrient index (TRIX without O2 and Chl *a*), DIN, DIN/P-PO4. **Oligohaline:** TP, (TRIX without Chl *a*).	**Mesohaline:** R^2=0.20 p<0.0057 **Oligohaline:** R^2=0.33 p=0.024

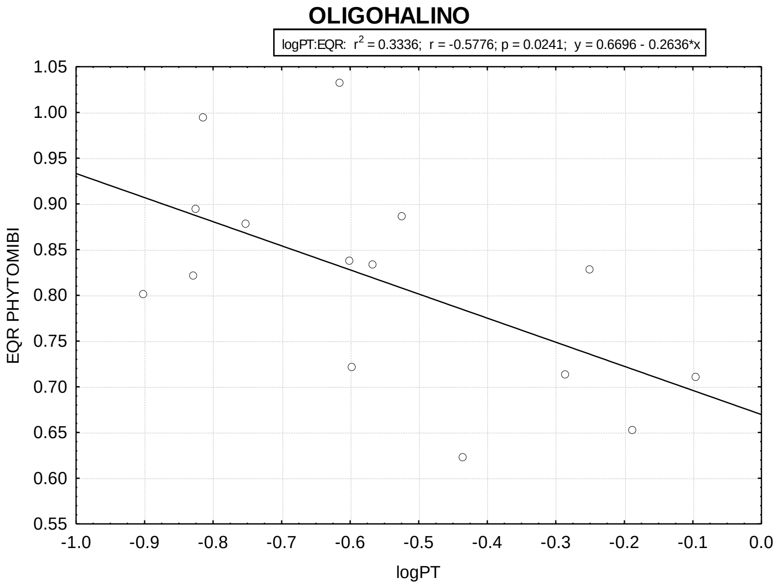

Figure 4.1. Regression between mean values of EQR_PHYTOMIBI and TP for the Oligohaline type.

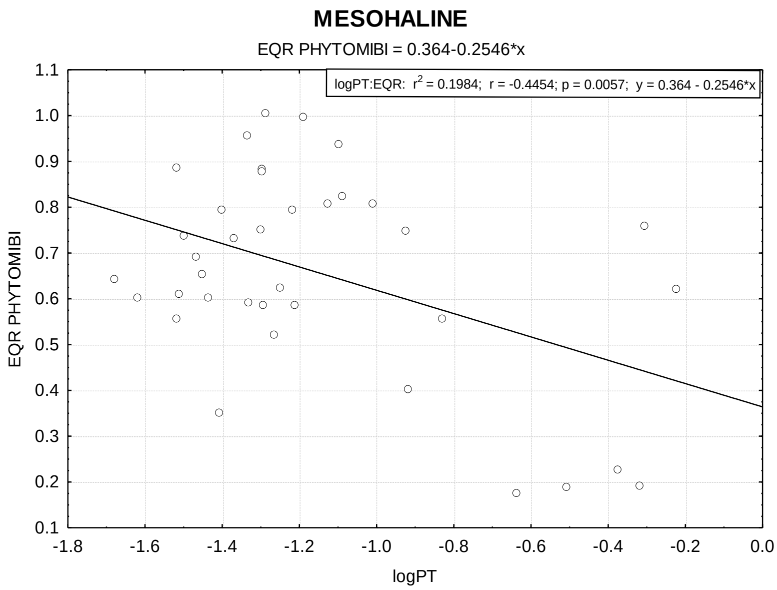

Figure 4.2. Regression between mean values of EQR_PHYTOMIBI and TP for the Mesohaline type.

4.3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria (Table 4.9).

Table 4.9. List of the WFD compliance criteria and the WFD compliance checking process and results.

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	**Yes**
High, good and moderate ecological status are set in line with the WFD's **normative definitions (Boundary setting procedure)**	**Yes**
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	**Yes.** Except for Bloom frequency that have not been included due to impossibility to assess accurately in space and time their occurrence
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and	**Yes**
The water body is assessed against **type-specific near-natural reference conditions**	**Yes**
Assessment results are expressed as **EQRs**	**Yes**
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	**Yes**
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	**Yes**
Selected taxonomic level achieves adequate **confidence and precision** in classification	**Yes**

4.4. IC FEASIBILITY CHECKING

4.4.1. Typology

The CL-Poly-euhaline type was intercalibrated between France, Italy and Greece. All types in the Balearic Islands are included within the IC common types. The Balearic Islands do not have an assessment method for the Poly-euhaline intercalibrated type for reasons explained below. The other two types existing in the Islands, Oligohaline and Mesohaline were not intercalibrated in the IC exercise. The correspondence with the coastal lagoons common IC types and the types main characteristics are shown in table 4.10.

Table 4.10. Common IC types existing in the Balearic Islands.

Common TW IC type in the Balearic Islands	Type characteristics
CL-Oligohaline	Coastal lagoons (Salinity <5 psu)
CL-Mesohaline	Coastal lagoons (Salinity 5-18 psu)
CL-Poly-euhaline	Coastal lagoons (Salinity 18-40 psu)

4.4.2. Pressures addressed

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

Yes, the FHYTOMIBI address the pressures of Eutrophication and organic pollution intercalibrated in the TW MED GIG. See above *subsection 4.2.5.* Pressures addressed, to see the significant response of the EQR_PHYTOMIBI and its individual metrics to nutrients and organic pollution variables.

4.4.3. Assessment concept

With respect to the Feasibility checking, the only divergence is the species level identification for Phytoplankton methods within the TW MED GIG. The taxa in our data was instead was identified by another valid method (Epifluorescence microscope) and the taxonomic level was to the different phytoplankton functional groups, with counts at Class level and higher taxonomic level than Genus.

4.4.4. Conclusion on the intercalibration feasibility

Justification of the absence of a classification system for the single intercalibrated common type in the TW MED GIG, the euhaline type (poly-euhaline IC common type) in the Balearic Islands

The Balearic Islands Poly-euhaline Coastal Lagoons are saltworks (salines), most of them in use for salt extraction, which adjust to the definition of heavily modified water bodies. During the development of the PHYTOMIBI multimetrics for each type of the TW existing in the Balearic Islands, many statistical analyses were performed taking into account both biological quality elements, invertebrates and phytoplankton. It resulted that invertebrates were better indicators for the Poly-euhaline type, as phytoplankton failed to respond to physical changes occurring in the Saltworks. It proved impractical to define a multimetric for the phytoplankton in Saltworks, since higher concentrations of chlorophyll a and biomass were found in those water bodies under minor impairment. However, these are the conclusions we arrived with the methodology used to assess phytoplankton (Chl a), it is possible that other methods (i.e. remote sensing) are more effective, at least for euhaline, in detecting phytoplankton changes.

In short, in the case of the euhaline type in the Balearic Islands (Salines) it will be more correct to use a method that will respond to hydromorphological pressures, rather to eutrophication.

4.5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

The publication and description of the biological communities along degradation gradients is under study. We have supplied the reference values for the different metrics composing the PHYTOMIBI above for the Oligohaline and Mesohaline coastal Lagoons.

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