JRC Scientific and Technical Reports

Water Framework Directive intercalibration technical report

Part 3: Coastal and Transitional waters

Edited by Alessandro Carletti and Anna-Stiina Heiskanen

EUR 23838 EN/3 - 2009

EUROPEAN COMMISSION

Institute for Environment and Sustainability

JRC Scientific and Technical Reports

Water Framework Directive intercalibration technical report

Part 3: Coastal and Transitional waters

Edited by Alessandro Carletti and Anna-Stiina Heiskanen

EUR 23838 EN/3 - 2009

EUROPEAN COMMISSION
Institute for Environment and Sustainability

The mission of the Institute for Environment and Sustainability is to provide scientific-technical support to the European Union's Policies for the protection and sustainable development of the European and global environment.

European Commission
Joint Research Centre
Institute for Environment and Sustainability

Contact information
Address: Via Enrico Fermi 2749
I-21020 Ispra (VA), Italy
E-mail: wouter.van-de-bund@jrc.ec.europa.eu
Tel.: +39 0332 789955
Fax: +39 032 789352

http://ies.jrc.ec.europa.eu/
http://www.jrc.ec.europa.eu/

EUR 23838 EN/3
ISBN 978-92-79-12568-3
ISSN 1018-5593
DOI 10.2788/19561

Luxembourg: Office for Official Publications of the European Communities

Printed in Italy

Contents

1 Introduction

This Technical Report gives an overview of the technical and scientific work that has been carried out in the Coastal and Transitional Waters intercalibration of ecological classification systems across the European Union as required by the Water Framework Directive (WFD).

The results of this exercise were published in the Official Journal of the European Union as "Commission Decision 2008/915/EC of 30 October 2008[1]

The Coastal and Transitional Waters intercalibration exercise was carried out within 4 Geographical Intercalibration Groups (GIGs) – Baltic, Black Sea, Mediterranean and North East Atlantic. Common intercalibration types shared by Member States within each GIG were defined for the intercalibration exercise. The results of the first intercalibration exercise are the status boundaries for the benthic invertebrate fauna quality element (all GIGs), metrics and boundaries representing the phytoplankton quality element (all GIGs), metrics representing the macroalgae and angiosperms quality elements (Baltic, Mediterranean and NE Atlantic GIGs) and provisional boundaries for the fish quality element (NE Atlantic GIG only). These boundaries are based on definitions of reference criteria and the application of the Boundary Setting Protocol (BSP) to set the high-good and good-moderate boundaries in line with the normative definitions for status class boundaries for each quality specified in the WFD.
This report includes descriptions common and national coastal and transitional water types, national methods, common and national boundary setting protocols, the results of harmonisation of these boundaries between Member States as well as discussion of problems and way forward.
This report is available electronically at the following internet address:

http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library?l=/intercalibration_2&vm=detailed&sb=Title

Annexes are not included in the printed version, but can be downloaded from the above address.

2 Background

The **Water Framework Directive** (WFD) establishes a framework for the protection of all waters (including inland surface waters, transitional waters, coastal waters and groundwater). The environmental objectives of the WFD set out that good ecological status[2] of natural water bodies and good ecological potential[3] of heavily modified and artificial water bodies should be reached by 2015.

One of the key actions identified by the WFD is to carry out a European benchmarking or intercalibration (IC) exercise to ensure that good ecological status represents the same level of

1 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:332:0020:0044:EN:PDF

2 'Ecological status' is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, classified in accordance with Annex V WFD; 'Good ecological status' is the status of a body of surface water so classified in accordance with Annex V.

3 'Good ecological potential' is the status of a heavily modified or artificial body of water, so classified in accordance with the relevant provision of Annex V.

ecological quality everywhere in Europe (Annex V WFD). It is designed to ensure that the values assigned by each Member State (MS) to the good ecological class boundaries are consistent with the Directive's generic description of these boundaries and comparable to the boundaries proposed by other MS. The intercalibration of surface water ecological quality status assessment systems is a legal obligation.

Intercalibration is carried out under the umbrella of Common Implementation Strategy (CIS) Working Group A - Ecological Status (ECOSTAT), which is responsible for evaluating the results of the IC exercise and making recommendations to the Strategic Co-ordination Group or WFD Committee. The IC exercise aims at consistency and comparability in the classification results of the monitoring systems operated by each MS for biological quality elements (CIS WFD Guidance Document No. 14; EC, 2005). In order to achieve this, each MS is required to establish Ecological Quality Ratios (EQRs) for the boundaries between high (H) and good (G) status and for the boundary between good (G) and moderate (M) status, which are consistent with the WFD normative definitions of those class boundaries given in Annex V of the WFD.

All 27 MS of the European Union are involved in this process, along with Norway, who has joined the process on a voluntary basis. Expert groups have been established for lakes, rivers and coastal/ transitional waters, subdivided into 14 Geographical Intercalibration Groups (GIGs -groups of MSs that share the same water body types in different sub-regions or ecoregions).

The IC exercise aims to ensure that the H/G and the G/M boundaries in all MS's assessment methods for biological quality elements correspond to comparable levels of ecosystem alteration (EC, 2005). Intercalibration guidance produced by CIS (WFD Guidance Document No. 14) warns that the process will only work if common EQR boundary values are agreed for very similar assessment methods or where the results for different assessment methods are normalised using appropriate transformation factors (EC, 2005). Different assessment methods (e.g. using different parameters indicative of a biological element) may show different response curves to pressures and therefore produce different EQRs when measuring the same degree of impact (EC, 2005).

In each GIG, the IC exercise will be completed for those MS that already have data and (WFD compliant) assessment methods to set boundary EQR values for some of the biological quality elements. Countries that do not have data or assessment methods already available, or do not actively participate in the current IC exercise, need to agree with the outcome of the IC exercise and harmonise their assessment methods, taking into account the results of the current exercise, when their data/methods becomes available.

The WFD refers to an 'intercalibration network', comprising sites selected from a range of surface water body types present within each ecoregion, as the basis for intercalibration (Annex V; 1.4.1). For each surface water body type selected, the WFD specifies that at least two sites corresponding to the boundary between high and good status, and between good and moderate status should be submitted by each Member State for intercalibration. However, as the IC exercise evolved, this network has become redundant, as these datasets were too small to permit robust intercalibration.

This Technical Report provides a detailed description of the work that was carried out in the framework of the EU Water Framework Directive intercalibration exercise. harmonising the classification scales of national methods for ecological classification scales for rivers across the European Union. The technical work was carried from 2004 to 2007 by groups of experts from all EU Member States, within the framework of the Common Implementation Strategy working group (2)A on Ecological Status, facilitated by a steering group lead by the European Commission Joint Research Centre (JRC) (Figure 1.1).

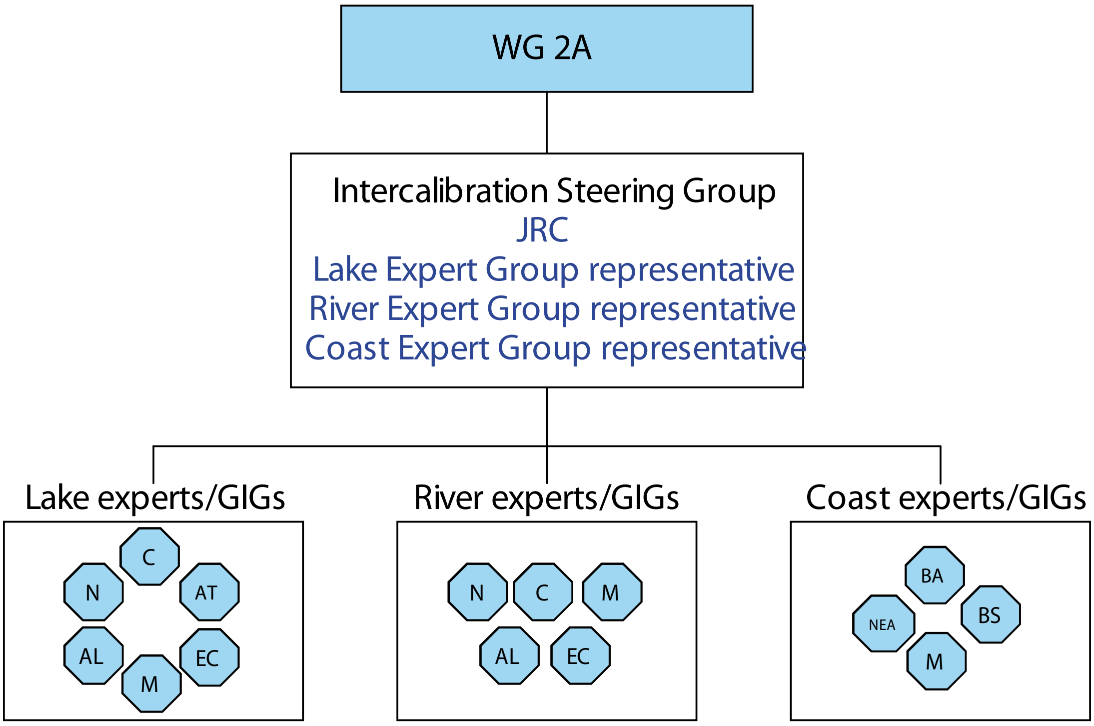


Figure 2.1: Overview of the organisational structure of the intercalibration process (from EC 2005).

Before the start of the intercalibration exercise a guidance document (EC 2005) was agreed describing the key principles and process options for the intercalibration exercise. The key principles of the intercalibration process as described in the guidance document are reproduced below.

Key principles of the intercalibration process (from Guidance on the Intercalibration Process, EC 2005)

1. The intercalibration process is aimed at consistency and comparability of the classification results of the monitoring systems[4] operated by each Member State for the biological quality elements[5]. The intercalibration exercise must establish values for the boundary between the classes of high and good status, and for the boundary between good and moderate status, which are consistent with the normative definitions of those class boundaries given in Annex V of the WFD[6].
2. The essence of intercalibration is to ensure that the high-good and the good-moderate boundaries in all Member State's assessment methods for biological quality elements correspond to comparable levels of ecosystem alteration. Intercalibration is not necessarily about agreeing common ecological quality ratio (EQR) values for the good status class boundaries as measured by different assessment methods. Common EQR values only make sense, and are only possible, where very similar assessment methods are being used or where the results for different assessment methods are normalised using appropriate transformation factors. This is because different assessment methods (e.g. using different parameters indicative of a biological element) may show different response curves to pressures and therefore produce different EQRs when measuring the same degree of impact.
3. The first phase of the process is the establishment of an intercalibration network for a limited number of water body types consisting of sites representing boundaries between the quality classes High-Good and Good-Moderate, based on the WFD normative definitions. The WFD requires that selection of these sites is carried out "using expert judgement based on joint inspections and all available information[7]".

[4] The term 'monitoring system' in the way it is commonly used includes the whole process from sampling, measurement and assessment including all quality elements (biological and other). In the context of WFD Annex V, 1.4.1, the term 'monitoring system' only refers to a biological assessment method, applied as a classification tool, the results of which can be expressed as ecological quality ratios. This guidance uses the term 'WFD assessment method' in place of the term 'monitoring system' that may be misleading in this context.

[5] The WFD intercalibration as described in Annex V, 1.4.1 does not concern the monitoring systems themselves, nor the biological methods, but the classification results

[6] WFD Annex V, 1.4.1 (ii), (iii), (iv), (vi)

[7] WFD Annex V, 1.4.1 (v)

4. The Intercalibration Guidance states that "some artificial or heavily modified water bodies could be considered to be included in the intercalibration network, if they fit in one of the natural water body types selected for the intercalibration network. Artificial and heavily modified water bodies that are not comparable with any natural water bodies should only be included in the intercalibration network, if they are dominant within a water category in one or more Member States; in that case they should be treated as one or several separate water body types". An artificial or heavily modified water body is considered to fit in a natural water type if the maximum ecological potential of the artificial or heavily modified water body is comparable to the reference conditions of the natural type for those quality elements considered in the intercalibration exercise[8].

5 In the second phase of the process, each Member State's assessment method must be applied to those sites on the register that are both in the ecoregion (or, as pointed out in section 2.8, in the Geographical Intercalibration Group (GIG)) and of a surface water body type to which the system will be applied. The results of the second phase must be used to set the EQR values for the relevant class boundaries for each Member States' biological assessment system. The results of the exercise will be published by the Commission by 22 December 2006 at the latest.

6. Intercalibration sites are selected by the Member States, and represent their interpretation of the WFD normative definitions of high, good and moderate status. There is no guarantee that different Member States will have the same views on how the normative definitions should be interpreted. Differences in interpretation are reflected in the intercalibration network[9]. A common interpretation of the normative definitions should be the main outcome of the intercalibration exercise. At the end of the intercalibration exercise the intercalibration network may need to be revised according to this common interpretation.

7. The Intercalibration Exercise is focused on specific type/biological quality element/pressure combinations[10]. The selection of these combinations is based on the availability of adequate data within the time constraints of the exercise. This means that the exercise will not identify good status boundary EQR values for all the type/biological quality element/pressure combinations relevant for the implementation of the WFD. However, the Intercalibration Exercise will identify, and test the use of, a procedure and criteria for setting boundaries in relation to any such combinations[11].

8. The intercalibration process described in this guidance is aimed at identifying and resolving:

   (a) Any major/significant inconsistencies between the values for the good ecological status class boundaries established by Member States and the values for those boundaries indicated by the normative definitions set out in Section 1.2 of Annex V of the WFD; and,

   (b) Any major/significant incomparability between the values established for the good status class boundaries by different Member States.

9. The process will identify appropriate values for the boundaries of the good ecological status class applicable to the ecological quality ratio EQR scales produced by the Member States' assessment methods.

10. The Intercalibration Exercise will be undertaken within GIGs rather than the ecoregions defined in Annex XI of the WFD. This is to enable intercalibration between a maximum number of Member States.

11. The Intercalibration Exercise assumes that all Member States will have developed their national WFD assessment methods to a sufficient extent to enable the consistency with the normative definitions, and the comparability between Member States, of the good status boundary EQR values for those methods to be assessed during 2005. It was recognized however that this assumption might be problematic. An inventory on the state-of-the-art in the developments of WFD compliant methods is carried out during the process of finalisation of the intercalibration network[12].

---

8 This is not the case for those quality elements that are significantly impacted by the hydromorphological alteration that has led to the water body to be designated as heavily modified.

9 Intercalibration Guidance, section 3.5

10 as described in the document' Overview of common Intercalibration types' (available at the intercalibration site submission web pages, http://wfd-reporting.jrc.cec.eu.int/Docs/typesmanual)

11 If the results of the method are significantly affected by biogeographical or other ecological differences within the intercalibration type, different boundary EQR values may be appropriate for different parts of the type

12 The metadata questionnaire is available at the intercalibration site submission web pages, http://wfd-reporting.jrc.cec.eu.int/Docs/ metadata

3 Geographical Intercalibration Groups (GIGs) and common intercalibration types

3.1 Geographical intercalibration groups

For coastal and transitional waters, four geographical intercalibration groups were agreed upon:

Baltic, (see chapter 1.1.3), including Denmark, Germany, Finland, Sweden, Latvia, Lithuania, Estonia and Poland.
Black Sea (see chapter 1.1.4) includes Bulgaria and Romania.
Mediterranean (see chapter 1.1.5), including Spain, France, Italy, Slovenia, Greece, Cyprus, Malta. Croatia as a potential accession country also participates.
North East Atlantic (see chapter 1.1.6), including Portugal, Spain, France, Ireland, UK, Belgium, Netherlands, Germany, Denmark, Sweden and Norway.

3.2 Common intercalibration types

The common coastal intercalibration types are characterised broadly by the descriptors of the WFD System B typology: geographical position (for latitude and longitude), tidal range and salinity as obligatory factors plus optional factors such as, exposure, depth, mixing characteristics, substratum composition, current velocity, residence time and ice cover where applicable.

Baltic

The Baltic GIG includes the whole or parts of the coastline of the following countries: DE= Germany, DK=Denmark, EE=Estonia, FI= Finland, LT=Latvia, LI=Lithuania, PL=Poland and SE=Sweden. The common intercalibration types were agreed using the basic factors of salinity and exposure with further delineation based on depth and number of ice cover days plus the identification of one lagoon type. The countries which have each type within their coastal waters are shown in the table below. All countries agreed to intercalibrate quality elements that respond to eutrophication pressures. The chosen elements in each type are also shown in the table below.

Table 2.2.1: Common intercalibration types.

Type	Pressure	Quality element	Countries involved (number of sites)
CW B0 Salinity 0.5-3, sheltered, shallow, >150 ice days	Eutrophication	**Phytoplankton:** Chlorophyll a **Benthic Fauna:** National indices	SE FI , SE
CW B2 Salinity 3-6, sheltered, shallow, 90-150 ice days	Eutrophication	**Phytoplankton:** Chlorophyll a **Benthic Fauna:** National indices	FI , SE FI, SE
CW B3 Salinity 3-6, sheltered, shallow, 90 ice days	Eutrophication	**Phytoplankton:** Chlorophyll a **Benthic Fauna:** National indices	FI FI, SE
CW B12 Salinity 6-22, sheltered, shallow	Eutrophication	**Phytoplankton:** Chlorophyll a **Angiosperms:** Eelgrass depth limit (DK + DE) **Benthic Fauna:** National indices	DE, DK, EE, SE
CW B13 Salinity 6-22, exposed, shallow	Eutrophication	**Phytoplankton:** Chlorophyll a **Benthic Fauna:** National indices	EE, LV, PL DK, LT, LT, PL
CW B14 Salinity 6-22, sheltered, shallow lagoons	Eutrophication	**Phytoplankton:** Chlorophyll a	PL DK, PL

Black Sea

Bulgaria and Romania are the only two countries participating in the Black Sea GIG and identified one common intercalibration type, BS1: based on salinity (mesohaline), depth (shallow waters) and substratum (mixed). In this phase of the intercalibration only the benthic invertebrates and phytoplankton quality elements have been considered, as these are the most developed indicators in the two countries.

Mediterranean

The Mediterranean GIG includes seven Member States, Spain, France, Italy, Slovenia, Greece, Cyprus and Malta plus Croatia as an accession country. The Mediterranean GIG has at this stage confined its work to coastal waters. Transitional waters were not included in this phase of the intercalibration exercise due to lack of sites and data in participating countries. Preliminary discussions on transitional water have taken place with a view to undertaking further assessments in phase two.

Mediterranean Coastal IC types were defined primarily on the substratum composition and the depth profile. Salinity was not seen as a primary discriminating factor as it is very similar across the whole Mediterranean basin. The Mediterranean GIG agreed four basic coastal water types as shown below:

Table 2.2.2: Mediterranean Coastal Waters Types.

Type	Name of Type	Substratum (1)	Depth (2)
CW - M1	Rocky shallow coast	rocky	shallow
CW - M2	Rocky deep coast	rocky	deep
CW - M3	Sedimentary shallow coast	sedimentary	shallow
CW - M4	Sedimentary deep coast	sedimentary	deep

(1) In many cases different seabed substrata will occur within one waterbody type. The dominant substratum should be selected.

(2) Depth division is based on 40 m depth at 1 mile distance from the coastline.

The following quality elements with respective countries' participation have been included in this phase of the intercalibration exercise: **Phytoplankton**: CY, ES (Balearic Islands, Catalonia, Valencia), F, GR, IT, SL and Croatia participating,
Benthic macroinvertebrates: CY, ES (Balearic Islands, Catalonia, F, GR, IT, SL participating,
Macroalgae: CY, ES (Catalonia, Valencia), F, GR and I, participating
Angiosperms (*P.oceanica*): ES (Catalonia, Valencia), F, GR, IT, Malta participating.

Typology
Not all types are included for each quality element: in some cases the type distinctions were not relevant for the IC exercise (e.g. Angiosperm) and new types have been defined for the specific analysis of phytoplankton, as shown below:

Table 2.2.3: details about typology.

All types included?	**All pressures included?**
Phytoplankton	
Revision of Typologies	Yes
Benthic invertebrate fauna	
Only M2 & M3	Yes
Macroalgae	
M1, M2 & M3	Yes
Angiosperms: *P.oceanica*	
No types distinction	Yes

Phytoplankton Typologies
Details on the process that was followed for this types' revision are found in the specific part Section 3. Phytoplankton.
For general information:
Three different water types, in an ecological perspective, have been described as follows:

Type 1 coastal sites highly influenced by freshwater inputs

Type 2 coastal sites not directly affected by freshwater inputs

Type 3 coastal sites not affected by freshwater inputs

Further discrimination within types based on geographical/ecological differences (e.g. eastern, western Mediterranean basins) was used, in some cases in order that the exercise produced biologically meaningful results.

North East Atlantic

The North East Atlantic GIG involves eleven countries, Portugal, Spain, France, Ireland, UK, Belgium, Netherlands, Germany, Denmark, Sweden and Norway. Common intercalibration types were agreed based on the obligatory factors salinity and tidal range, plus optional factors, depth, current velocity, exposure, mixing and residence time. After consideration of the relevance of the original types within the NE Atlantic complex, based solely on the above factors, it was decided that in some cases there was no biological differences between types in relation to the chosen quality element or metric(s) being intercalibrated and that some could be merged together. This resulted in the adoption of the following grouped types in this intercalibration exercise:

Table 2.2.4: NEA Coastal Waters Types.

New Type ID	Name	Salinity (PSU)	Tidal range (m)	Depth (m)	Current velocity	Exposure	Mixing	Residence time
CW –NEA1/26a	Open oceanic, exposed or sheltered, euhaline, shallow	Fully saline (> 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or sheltered	Fully mixed	Days
CW –NEA1/26b	Enclosed seas, exposed or sheltered, euhaline, shallow	Fully saline (> 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or sheltered	Fully mixed	Days
CW –NEA1/26c	Enclosed seas, exposed or sheltered, partly stratified	Fully saline (> 30)	Microtiadl/ Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or sheltered	Partly stratified	Days to weeks
CW –NEA1/26d	Scandinavian coast, exposed or sheltered, shallow	Fully saline (> 30)	Microtidal (<1)	Shallow (< 30)	Low (<1 knot)	Exposed or moderately exposed	Partly stratified	Days to weeks
CW –NEA1/26e	Areas of upwelling, exposed or sheltered, euhaline, shallow	Fully saline (> 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or sheltered	Fully mixed	Days
CW – NEA3/4	Polyhaline, exposed or moderately exposed (Wadden Sea type)	Polyhaline (18 - 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or moderately exposed	Fully mixed	Days
CW – NEA7	Deep, low current, sheltered	Fully saline (> 30)	Mesotidal (1 - 5)	Deep (> 30)	low (< 1 knot)	Sheltered	Fully mixed	Days
CW – NEA8	Polyhaline, microtidal, sheltered, shallow (Skagerrak inner arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Shallow (< 30)	low (< 1 knot)	Sheltered	Partially Stratified	Days-Weeks
CW – NEA9	Fjord with a shallow sill at the mouth with a very deep maximum depth in the central basin with poor deepwater exchange.	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	low (< 1 knot)	Sheltered	Permanently Stratified	Weeks
CW – NEA10	Polyhaline, microtidal exposed, deep (Skaggerak outer arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	low (< 1 knot)	Exposed	Permanently Stratified	Days
TW – NEA11	Transitional waters	Oligo-Euhaline (0 - 35)	Mesotidal (1 – 5)	Shallow (< 30)	Medium	Sheltered or moderately Exposed	Partially- or Permanently Stratified	Days-Weeks

The above types occur in Member State’s waters as detailed below:

Table. 2.2.5: Occurrence of coastal water types in NEA MSs.

Type	BE	DK	FR	DE	IE	NL	NO	PT	ES	SE	UK
CW – NEA1/26a			X		x		x		X		x
CW – NEA1/26b	X		X			x					x
CW – NEA1/26c		X		x							
CW – NEA1/26d		X									
CW – NEA1/26e								X	X		
CW – NEA3/4				x		x					
CW – NEA7							x				**X**
CW – NEA8		x					x			X	
CW – NEA9							x			x	
CW – NEA10							x			x	
TW – NEA11	**X**		X	X	X	X		X	X		X

In each type relevant biological quality elements have been included in this stage of the intercalibration process, linked to specified pressures. These are detailed in the table below:

Table 2.2.6: Quality elements and pressures included per types in the intercalibration.

Type	Biological Quality Elements	Pressures
CW – NEA1/26, NEA3/4, NEA7, NEA8, NEA9, NEA10	Benthic Invertebrates Phytoplankton Macroalgae/Angiosperms	Organic enrichment Hazardous substances Nutrient enrichment Morphological alteration
TW – NEA11	Benthic Invertebrates Macroalgae/Angiosperms Fish	Organic enrichment Hazardous substances Morphological alteration Organic enrichment (DO)

This does not mean that intercalibration for all quality elements (or metrics) has been completed in each type at this stage of the process.

4 Methodology and Results

Following the CIS guidance on intercalibration options the coastal GIGs selected the most appropriate for each quality element and metric selected. The following describes the options chosen within each GIG:

Baltic

The following intercalibration options were chosen for the relevant biological quality elements and metrics.

Phytoplankton:

a) Hybrid between option 2 and 3 (Denmark, Estonia, Finland, Germany, Latvia and Sweden) A common metric – summer mean of chlorophyll a from May/June to September - was agreed. In some parts of the Baltic Sea the summer period can be shorter. Intercalibration was performed by comparison of the results from the national assessment tools. Within each type member state essentially agreed on common reference and classifications values for the whole type or for sub areas in the type. (However, Type B3 in Sweden and Finland differ a lot from each other by their natural conditions, which complicates intercalibration).

b) Option 3
A combined data set from most member states participating in the Baltic GIG has been compiled and an overall relation between chl. a and TN established. Further and future work on establishing type or site specific chl. a-TN relationships and ranges of nutrient concentrations related to the different ecological classes may provide a useful tool for setting boundaries.

Benthic fauna: Option 3 (Estonia, Denmark, Finland, Germany, Latvia and Sweden)
Intercalibration is performed in three steps, comparison of species sensitivity classifications, comparison of indices and comparison of classification of water bodies based on national methods for assessment. Work is still ongoing.

Angiosperms: Hybrid between option 2 and 3. Bilaterally between Denmark and Germany Reference levels are based on historical data, expert judgement and modeling. Two approaches have been used for classification: 1) percent deviation (3 scenarios) and 2) modeling.

Denmark used the maximum depth of 5 % eelgrass cover to define the depth limit. Germany used historical records of depth limit to define reference and light modeling to define depth limits.

Black Sea

Taking into account that both countries are aiming to use at national level the same methods for phytoplankton and macrozoobenthos, Option 1 was initially chosen however the final intercalibration used option 3 in the first instance as only common metrics for each quality element could be agreed on rather than common whole classification systems covering the whole element.

Mediterranean

For each biological quality element considered, different options were used, mainly hybrids.

Phytoplankton

A hybrid between options 1 and 2 is used. No national methods were intercalibrated. Only common statistical analysis on chl-α, nutrients and physico-chemical data and some multivariate techniques have been performed as a starting point (exploratory data analysis) for the intercalibration process.

Benthic invertebrates

A hybrid between options 2 and 3 is used.

Macroalgae

Option 3 has been used; i.e. Member States have set national boundaries using their own nationally developed assessment methods. These systems are then compared between Member States by application of these methods on each country's data.

Angiosperms (*P.oceanica*)

Option 3 has been used; i.e. Member States have set national boundaries using their own nationally developed assessment methods. These systems are then compared between Member States by application of these methods on each country's data.

North East Atlantic

The following intercalibration options have been used for the biological quality elements.

Benthic Invertebrates

Option 3 has been used in all types i.e. Member States have set national boundaries using their own nationally developed assessment methods. These systems are then compared between Member States by application of these methods on each country's data. Initial appraisal is made of the level of agreement on status assessments between countries methods when data is exchanged, and national boundaries adjusted post this comparison until a satisfactory degree of agreement is reached (e.g. > 80 % comparability).

Phytoplankton

Option 2 has been used in all types. Metrics have been selected that form part of the overall quality element assessment that are used in all Member States own classification systems where a common approach can be adopted to setting boundaries for these metrics at the GIG level. These boundaries may differ according to the type selected and in some cases region specific boundaries within types have been adopted. At this stage the selected metrics are, chlorophyll-a (90^{th} percentile over a six year period), frequency of *Phaeocystis* cell counts above 10^6 cells/l over a six year period and frequency of microphytoplanton cell counts above 10^5 cells/l.

Macroalgae and Angiosperms

A hybrid approach between option 2 and 3 has been used for the macroalgae and angiosperms metrics. Metrics have been selected that form part of the overall quality element assessment that are used in all Member States own classification systems where a common approach can be adopted to setting boundaries for these metrics at the GIG level. These boundaries may differ according to the type selected and in some cases region specific boundaries within types have been adopted. At this stage the selected metrics are, opportunistic macroalgae (areal extent and/or biomass), perennial intertidal and subtidal algae (extent and species composition), intertidal and subtidal seagrass (areal extent, density and species composition) and saltmarsh (areal extent and species composition). For seagrass a combination of options 2 and 3 was used because UK and NL use broadly the same metrics but derived boundary conditions separately which were then compared and harmonized.

Fish

Option 3 has been used i.e. Member States have set national boundaries using their own nationally developed assessment methods. These systems are then compared between Member States by application of these methods on each country's data. Initial appraisal is made of the level of agreement on status assessments between countries methods when data is exchanged, and national boundaries adjusted post this comparison until a satisfactory degree of agreement is reached (e.g. > 80 % comparability). The original intention was to pursue an **option 1** solution. This is still the preferred option and may be possible in Phase II.

5 Discussion

5.1 Comparability between quality elements

There has not been an exercise in phase one of the intercalibration process to directly compare the results for common quality elements between the GIGs. A range of intercalibration options have been used for each quality element in the different GIGs. Therefore it is not possible at this time to demonstrate that where similar metrics or classification systems have been used that they show the same level of comparability between the GIGs as within individual GIGs. However in many cases the biology of the geographical regions is very different e.g. it is very difficult to compare the Baltic with the Mediterranean. However further evaluation is needed where the same basic metric is being used e.g. depth limitation, to investigate if there is more scope for further harmonization of approaches.

5.2 Open issues and need for further work

The main area where further work is needed in the second phase of the process is in transitional waters. Almost none of the results presented in phase one of the process are applicable in transitional waters, except for a few metrics developed for coastal waters that can be used in the higher salinity areas. One key quality element in transitional waters is fish. No agreed results were produced in phase one, making this a key priority in phase two. However a lot of work has been done on fish in the North East Atlantic GIG which may be applicable in other GIGs. It is anticipated that results for fish can be produced quite early in the next phase. Another issue is whether the basic typology needs to be redefined in some GIGs. There have been some difficulties in phase one with the broad nature of the typology and further subdivisions have been necessary in order to produce results. Transitional waters will definitely need a new typology for all quality elements. Further work is also needed to cover the key pressures that have not been assessed in phase one. Some methods and metrics for which results have been agreed so far respond to specific pressures. It is also important to look in future at how biological indicators respond to different combinations of pressures. Another issue is how assessments of the status of whole waterbodies are done using the agreed methods and metrics. Further work is needed to ensure a common approach is adopted to classification of waterbodies across all GIGs. This work should be linked to the development of classification rules, data aggregation and assessment and common approaches to sampling and analysis strategies.

6 Summary and Conclusions

In summary results have been produced in all GIGs for at least two quality elements. In many cases these results for which intercalibrated results have been agreed are only part of the overall national classification systems. Therefore a main conclusion is that while common values have been agreed principally at the metric level much more work is required in the next phase to compare Member States' whole quality element methods and apply these to the status assessment of whole waterbodies. Only after this has been done can it be truly demonstrated that there is an equal level of ambition across GIGs and within GIGs.

Section 2 – Benthic Invertebrates

1 Introduction

Three of the four coastal water GIGs have been able to produce results for the benthic invertebrate quality element. Each GIG has several typologies that are found in these waters. Not all countries within each GIG have all the types within its borders. Information about the types and countries with each type in each GIG is described in the sections below.

All the GIGs have chosen Option 3 in this phase of the intercalibration process. Therefore Member States have developed their own classification schemes, these have been assessed against each other through the exchange and evaluation of data and the boundaries in each scheme harmonized to give an acceptable level of agreement.

It is important to note that the methods intercalibrated in this phase are habitat specific. All methods are for use in soft sediment habitats. Methods for other habitats are largely still under development and cannot be assessed at this stage.

2 Methodology and results

2.1 Baltic GIG

2.1.1 Intercalibration approach

The Baltic Sea Geographical Intercalibration Group (GIG) carried out intercalibration of the macroinvertebrate assessment systems for coastal waters developed in four Member States: Denmark, Finland, Germany and Sweden. The results from the intercalibration between Sweden and Finland are presented in this technical report. Comparability between the Danish, German, and Swedish macroinvertebrate methods could not be demonstrated and thus those results are not included in the technical report. In addition, macroinvertebrate assessment methods have been developed in Estonia, but the intercalibration exercise could not be completed as the other countries sharing the same coastal types with Estonia had not yet their national methods ready[13].

The Option 3, as described in the Guidance of the Intercalibration Process[14], was used for the Benthic Invertebrate fauna quality element. The Member States have developed their own classification schemes, these have been assessed against each other through the exchange and evaluation of data and the boundaries in each scheme harmonized to give an acceptable level of agreement. The setting of the reference conditions and high-good and good-moderate boundaries for each Member States' assessment systems was first carried out separately and then the outcomes of these systems were compared on water body (or sample) level against each others by applying

13 Report of the Estonian assessment method and the preliminary evaluation of the comparability with methods from other Baltic Sea countries is presented in the Baltic Sea Milestone Report 6. Quality element: Benthic Fauna. Version 16 June 2006. Rev. 3, 30 March 2007. Available at: http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library, in folder: GIG Milestone Reports

14 Guidance on Intercalibration Process, No 14. Available at: http://circa.europa.eu/Public/irc/env/wfd/library

different national assessment methods to local data sets from countries sharing the same common types. At this stage the assessment systems of four countries, Denmark, Finland, Germany and Sweden could be compared bilaterally against each others using data from the coastal types that these countries share. Finally, only the results between Sweden and Finland were concluded to be sufficiently comparable and could be included in the final decision.

Intercalibration was performed in three steps, 1) comparison of species sensitivity classifications, 2) comparison of indices, and 3) comparison of water body classifications using different national assessment methods applied to local datasets.

Baltic Sea Common intercalibration types

The Baltic Sea Geographical Intercalibration Group (GIG) includes the whole or parts of the coastline of the following countries: Germany, Denmark, Estonia, Finland, Latvia, Lithuania, Poland and Sweden (Table 2.1.1).

The common coastal water types are characterised by the descriptors of the System B typology. The typology factors are based on the common typology framework presented in the guidance on the typology for the coastal and transitional waters[15]. In the Baltic Sea GIG, the common intercalibration types were characterized using basic salinity and exposure with further delineation based on depth and number of ice cover days (Table 2.1.1). One transitional water type (TW B 13) was identified. All countries agreed to focus the intercalibration on the quality elements that are sensitive to eutrophication pressures.

Table 2.1.1: Description of Baltic Sea Common intercalibration types that have included in the intercalibration exercise.

Type	Salinity psu	Exposure	Depth	Ice days	Other Characteristics
CW B0	0.5- 3	Sheltered	Shallow	> 150	Sites in Botnian Bay (Northern Quark)
CW B2	3-6	Sheltered	Shallow	90-150	Sites in Bothnian Sea
CW B3 a	3-6	Sheltered	Shallow	~90	Sites in the area extending from the southern Bothinian Sea to the Archipelago Sea and the western Gulf of Finland
CW B3 b	3-6	Exposed	Shallow	~90	
CW B12 b Western Baltic Sea	8 - 22	Sheltered	Shallow	-	Sites at the Southern Swedish coast and the South western Baltic Sea open coast along Denmark and Germany
CW B13	6-22	Exposed	Shallow	-	Sites along the coast of the Estonia, Latvia and Lithuania, the Polish coast and the Danish island “Bornholm”
CW B 14	6-22	Sheltered	Shallow	-	Lagoons
TW B 13	6-22	Exposed	Shallow		Transitional water. Sites along the coast of Lithuania and Poland

15 Guidance document No. 5 *‘Transitional and Coastal Waters - Typology, Reference conditions, and Classification systems’*. Common Implementation Strategy of the Water Framework Directive, Available at: http://forum.europa.eu.int/Public/irc/env/wfd/library

The common intercalibration types were characterised by the following descriptors:

– Salinity (using practical salinity scale): low (0,5-3) and high (3-6) oligohaline, mesohaline (6-22)
– Depth: all shallow (<30 m)
– Exposure (using agreed Pan-European scale[16]): exposed, sheltered and very sheltered
– Duration of ice cover: >150 days/ year, 90-150 days/ year, no or very short ice cover

At this stage four countries, Denmark, Finland, Germany and Sweden had developed benthic macroinvertebrate assessment systems that could be intercalibrated for five Baltic Sea common types:

Types CWB0, CWB2, CWB3a, CWB3b: Finland, Sweden.

Type CWB12b: Germany, Denmark, Sweden.

At this stage, intercalibration was completed for the four types shared between Finland and Sweden in the Bothnian Sea and Bothnian Bay. The list of the two national methods intercalibrated is presented below (Table 2.1.2.).

Table 21.2: The national assessment methods for the classification of ecological quality based on benthic macroinvertebrates that were compared during the intercalibration exercise.

Country	Assessment Method
Finland	BBI- Finnish Brackish Water Benthic Index
Sweden	BQI–Swedish multimetric biological quality index (soft sediment infauna)

2.1.2 National methods that were intercalibrated

Macroinvertebrates have for decades been an integrated part of monitoring programmes setup to detect marine pollution or eutrophication. Soft bottom macrofauna is a well-suited parameter to use since it is stationary, relatively long-lived, easy to collect quantitatively, restricted to very limited vertical distribution in or just below the sediment surface, and they can be identified to species also after unlimited preservation. The impact of pollution in the water column is not necessarily the same as in the bottom and at the sediment-water interface, in which the long-term effects of discharged pollutants, may be better monitored by using sessile or sedentary organisms as indicators.

Changes in abundance, biomass and species composition of the benthic communities are signs of eutrophication. Some of these species will respond to changes in food supply and/or sedimentation rates and/or lowered oxygen concentrations (Diaz & Rosenberg 1995; Gray et al. 2002). The complex benthic communities respond to anthropogenic loading and stress by establishing a new community structure more tolerant to the increasingly unfavorable physio-chemical conditions (Leppäkoski 1975). Karlson et al. (2002) gives a good review on eutrophication and oxygen deficiency and their effects on the benthic community in Baltic coastal waters. Many indices and approaches have been developed for the assessment of the status of zoobenthos communities in marine waters. However, many of them are not applicable in the brackish Baltic Sea ecoregion due to the low biodiversity. The gradually decreasing salinity and diminishing species richness towards

[16] According to the definitions of the common European exposure categories; Guidance document No. 5

north- and east in the Baltic Sea make only region-specific comparisons of data and assessment systems meaningful.

Finland:

The Finnish Brackish Water Benthic Index, BBI, follows the theory that biodiversity increases with increasing distance from a pollution source along a gradient of disturbance (Pearson & Rosenberg 1978). The index is very similar to other multimetric indices developed for marine conditions (Danish DKI-index and British BMI-index) (Borja et al. 2007), but it has additional features which make it apt to brackish water conditions, typical for the Baltic Sea (Perus et al. 2007). The index is a construction of the Baltic Sea-adapted BQI-index (Rosenberg et al. 2004, Blomqvist et al. 2007) with additional biodiversity and abundance factors incorporated into the matrix. Therefore the multimetric BBI index meets the specification of the Annex V in the WFD. The BBI Index values are continuous and therefore useful for further analysis and interpretations in classification systems (Perus et al. 2007).

The index compare observed BQI- and Shannon-Weaver (H´) values against highest recorded values within individual types (serving as reference values) and further deducts the value for stations showing low biodiversity or abundance. A value of zero (0) indicates conditions without any benthic macroinvertebrates (azoic) and value one (1) indicate unpolluted bottom conditions.

The multimetric BBI-index includes relative abundance (%) of sensitive or tolerant species. The list of the species sensitivity or tolerance is same as used for the Swedish BQI-index (Rosenberg et al. 2004; Blomqvist et al. 2007). The evaluation of sensitivity and tolerance of each species is based on literature information (Anger 1975, 1977; Borja et al. 2000; Helawell 1986; Järvekülg 1970; Landner et al. 1977; Leppäkoski 1975; Mandaville 2002; Wiederholm 1973) and expert judgment. The levels of sensitivity/tolerance are:

1 – Very tolerant to pollution
5 – Tolerant
10 – Pollution sensitive
15 – Very pollution sensitive

The BBI is calculated as following:

$$BBI = \frac{\left[\left(\frac{BQI}{BQI\max}\right)+\left(\frac{H'}{H'\max}\right)\right]}{2} * \frac{\left[\left(1-\frac{1}{ABtot}\right)+\left(1-\frac{1}{S}\right)\right]}{2}$$

where, *BQI* is the Swedish Biological Quality Index (Rosenberg et al. 2004, Blomqvist et al. 2007), *H´* is the (log2-base) Shannon-Weaver diversity, *AB* is species abundance, and *S* the species richness. A detailed presentation of the BBI index and its application for the national Finnish classification and boundary setting is described in Perus et al. (2007).

Sweden:

The Benthic Quality Index (BQI) is based on the distribution of sensitive and tolerant species, the number of species and the number of individuals. The complete classification method is described by Blomqvist et al. (2007), and the original background paper presenting the characteristics and composition of the BQI is published by Rosenberg et al. (2004).

The distribution between sensitive and tolerant species comprises the base of the BQI index. This value generally varies between 1 and 15. Low values indicate high proportion of tolerant species and high values indicate high proportion of sensitive species. The factor enumerating the number of species will increase the index value when there are more than 9 species per sample and will decrease the index value when there are less than 9 species per sample. The factor enumerating the number of individuals has been derived in order to handle situations when there are only a few individuals per sample. In a few instances, these individuals may represent sensitive species, and thus produce an unjustifiably high index value. When fewer than about 20 individuals are present in a sample (0.1 m^2), the index is significantly depressed by this factor.

$$BQI = \left[\sum_{i=1}^{S_{classified}} \left(\frac{N_i}{N_{totalclassified}} * Sensitivityvalue_i \right) \right] *^{10}\log(S+1) * \left(\frac{N_{total}}{N_{total} + 5} \right)$$

where, S is the number of taxa, $S_{classified}$ is the number of taxa having a sensitivity value, N_i is the number of individuals of taxon I, $N_{totalclassified}$ is the total number of individuals of taxa having a sensitivity value, N_{total} is the total number of individuals per 0.1 m^2 and the $Sensitivityvalue_i$ is the sensitivity value for taxa i.

The Baltic Sea coastal fauna is already under physiological stress due to low salinity. The fauna was classified based on national expert knowledge and literature information. Each macroinvertebrate species was given a value of 1, 5, 10 or 15 depending on its’ sensitivity for anthropogenic disturbance. A high value indicates high sensitivity for disturbance and a low value high tolerance against disturbance. The major pressure in the Swedish coastal waters is excess nutrient loading leading to eutrophication and resulting in increased organic load to the seafloor and decreased oxygen content in the sediments and deep water. This classification of different species into sensitive and non-sensitive species is shared between Finland and Sweden. The Sensitivity values for different taxa are presented in an Excel-file at:

http://www.naturvardsverket.se/sv/Arbete-med-naturvard/Vattenforvaltning/Handbok-20074/

Since benthic invertebrates display large natural spatial variation, we chose to base the estimate for determining status using the entire water body, instead of basing it on individual sample results. For this estimate, we chose to follow the precautionary principle and used the 20th percentile instead of the median of the BQI values from a water body, when comparing with class boundaries per water body type. The 20th-percentile was calculated using a special method based on 9,999 randomly selected mean values from the existing index values in a water body. Naturally, the status estimate becomes more robust the greater the number of sampling stations in a water body. As a rule of thumb we recommend five or more sampling stations per water body.

A complete classification requires the following calculations in order to derive a value for a water body in order to compare with the class boundaries for the type of that water body:

1. Calculate the BQI based on species and abundance information from each individual sample
2. Calculate the mean BQI for each station
3. Calculate the 20th-percentile using randomization based on the mean BQI values from all stations
4. Compare the value for the 20th percentile with the class boundaries

2.1.3 Reference criteria and class boundary setting

Finland:

Reference conditions and boundary setting

Areas not influenced by human activities are regarded not to be present in the Baltic Sea today. Historic reference data is almost completely lacking from Finnish coastal areas and use of old data is therefore not an option in determining reference conditions. Number of stations for the Baltic common intercalibration types B0, B2, B3a, and B3b in Finland are presented in Table 2.1.3. Bulk of the benthic macroinvertebrate data in the national database are sampled between 1990 and present. In order to determine reference conditions, a method that is used in lakes and running waters (Vuori et al. 2006) was modified to be applicable for the coastal waters.

Bäck et al. (2006) concludes on the Finnish coastal zoobenthos monitoring that “Long-term zoobenthos monitoring has been carried out only in one area with two depth zones since 1964, with few observations from the 1920s (Kangas et al. 2001). The zoobenthos monitoring is a part of the HELCOM monitoring for the assessment of the state of the Baltic Sea. In addition to the national monitoring, there is some regional zoobenthos monitoring carried out along the Finnish coast. However, all available benthic invertebrate (or zoobenthos) data for setting the WFD reference conditions and good-moderate boundary is gathered from the pollution control monitoring system, which covers all the essential Finnish coastal areas impacted by nutrient loading with several hundreds sampling stations near the sites that are recipients for the loading.”

Baltic Sea common intercalibration types B0, B2, B3a, and B3b are spatially larger than the Finnish national coastal type areas. The environmental conditions (e.g. hydromorphological and topographic) characteristics of these large intercalibration types vary considerably within respective types and in order to produce more reliable analysis types were subdivided into smaller subtypes. In the macroinvertebrate analyses a depth separation of the water column (0 to 10 m and deeper than 10 m) was used in relating the ecological meaningfulness on macroinvertebrate communities to the typology. This was done to improve the interpretation of environmental status in individual types (Perus et al. 2004).

The Finnish Brackish water Benthic Index (BBI) incorporates all parameters required in Annex V of the WFD. The index takes into account relative abundance of sensitive/tolerant species, abundance and biodiversity (Shannon-Wiener and Species Richness) and can thus identify data of different ecological status.

The determination of the reference conditions for the BBI was carried out as following:

1) The median of the 10 % highest BBI-values were chosen to represent reference conditions for each type and depth interval.
2) The EQR was calculated by dividing Observed values with the Reference values
3) The value for the high-good ecological status boundary is set at the 10 %-percentile of reference EQR-values. Values below this boundary are divided into 5 equal classes Good 2/5, Moderate 1/5, Poor 1/5 and Bad 1/5) to represent classes below high ecological status.

Boundary values were further validated by checking species richness, abundance, diversity values and community composition of tolerant/sensitive species. The species tolerance or sensitivity was determined according to species classification list made for the Swedish BQI-index in the Baltic Sea, which was also used for the Finnish BBI (Perus et al. 2007). Definition criteria for high, good, and moderate status in the coastal waters were following description in Annex V of the Directive.

Table 2.1.3: The classification boundary values for the Finnish Brackish Water Benthic Index (BBI). Lower boundary values for High (H), Good (G), Moderate (M) and Poor (P) status as BBI- and EQR values. Number of stations (Stations) and reference stations (Ref.) visited. These are the final boundary values adjusted after intercalibration with Sweden.

		#	#	**BBI-EQR**					**BBI-values**				
Type	Depth	Stations	Ref.	H	G	M	P	B	H	G	M	P	B
B 3 Middle	0-10	52	5	0,93	0,56	0,37	0,19	<0,19	>,70	0,42	0,28	0,14	<0,14
	10+	290	29	0,89	0,53	0,36	0,18	<0,18	>,53	0,32	0,21	0,11	<0,11
B 3 Outer	0-10	45	5	0,92	0,55	0,37	0,18	<0,18	>,74	0,44	0,29	0,15	<0,15
	10+	210	21	0,90	0,54	0,36	0,18	<0,18	>,62	0,37	0,25	0,12	<0,12
B 2 Inner	0-10	280	28	0,94	0,56	0,38	0,19	<0,19	>,52	0,31	0,21	0,10	<0,10
	10+	96	10	0,95	0,57	0,38	0,19	<0,19	>,71	0,42	0,28	0,14	<0,14
B 2 Outer	0-10	55	6	0,88	0,53	0,35	0,18	<0,18	>,67	0,40	0,27	0,13	<0,13
	10+	58	6	0,92	0,55	0,37	0,18	<0,18	>,60	0,36	0,24	0,12	<0,12
B 0 Outer	0-10	41	4	0,94	0,56	0,38	0,19	<0,19	>,71	0,43	0,28	0,14	<0,14
	10+	46	5	0,98	0,59	0,39	0,20	<0,20	>,64	0,38	0,25	0,13	<0,13

For each individual national type comprehensive species lists have been compiled and reference criteria can be set as a percentage value of species (alternatively number of sensitive species) that need to be present for reference criteria to be met. At present such a percentage value have not been set.

Boundary values are further validated by checking species richness, abundance, diversity values and community composition of tolerant/sensitive species. The same species list and species classification is used in both Sweden and Finland (Blomqvist et al. 2007; Perus et al., in 2007).
Regional expertise has been contacted to determine whether or not the selected station visits are appropriate (e.g. located in area where periodic hypoxia/anoxia may have occurred in periods when no sampling has taken place.)

The example in Figure 2.1.1, illustrates how the boundary values of the BBI are linked with sensitivity of the macroinvertebrate species groups for one common intercalibration type B0. The species sensitivity groups 15 and 10 should be dominant or sub-dominant above the Good-Moderate

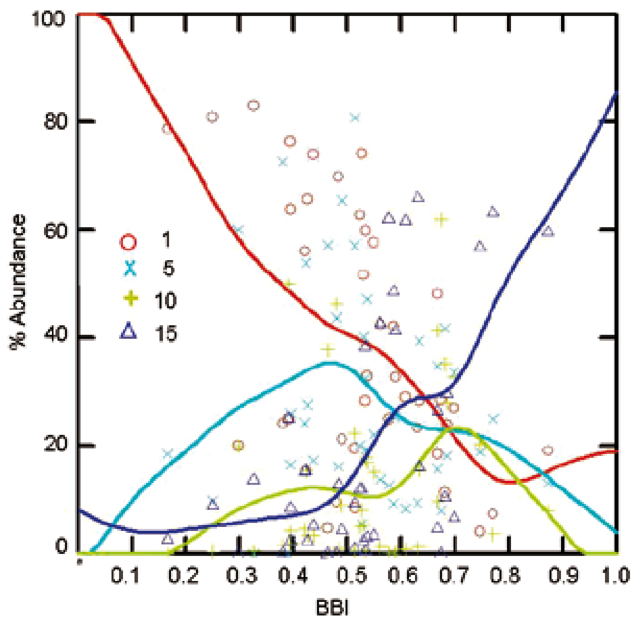


Figure 2.1.1: Distribution of the relative abundance of the four macroinvertebrate sensitivity groups as a function of the BBI values for the common the intercalibration type B0 (Data from the corresponding national Finnish type). The distributions are illustrated with the least square smoothing lines (DWLS, stiffness=0.5). Group 15 is the most sensitive and group 1 the most tolerant group of species.

boundary (BBI value 0.43 from Table 3). The species sensitivity groups 5 and 1 should be dominant or sub-dominant below the Good-Moderate boundary (Fig. 1). Additionally, diversity was higher above Good-Moderate boundary than below.

Sweden:

Class boundary setting procedure and reference criteria

It was not possible to describe reference conditions using spatial approach or historical data, since there are no unaffected areas in the Baltic nowadays. Also historical data was not collected using comparative methodology as today. Instead the best available data from areas without local discharges were used as a proxy for reference conditions in the boundary setting. Data from these regions was assumed represent at least good ecological status.

In deriving the type-specific class boundaries, the greatest emphasis was placed on the good–moderate boundary. This boundary was primarily determined using data from high–good areas. Comparative data was chosen for each national type from regions lacking local discharges; in practice from areas with the highest mean BQI values existing for that type. Sequential tests identifying the level of BQI (20^{th} percentile) where a water body significantly differs from the comparison material were made to assist in the setting of good-moderate boundary. In those types where the amount of data was insufficient, expert assessments were conducted based on existing data and data from nearby types with similar properties.

Once the good–moderate boundary was determined, it was deemed acceptable to consider the area from the good–moderate boundary up to the highest observed index value in the existing type as mainly constituting a status of good. Two-thirds (2/3) of the span exceeding the good–moderate boundary was assigned a status of good, while the upper third was reserved for the status of high (Fig. 2.1.2). The area below the good–moderate boundary was divided into three equal intervals for the remaining boundaries.

For calculation of EQR we have used the 20^{th} percentile of BQI in a water body divided by the highest observed BQI-value in a type.

The low salinity in the Baltic put a big natural stress on the fauna resulting in low number of taxa. For this reason one can not expect a perfect fit to the normative definition.
More details on this approach are available in Blomqvist et al. (2007).

2.1.4 Results of the comparison

Three steps were carried out in the intercalibration exercise: 1) comparison of species sensitivity classifications between the methods, 2) comparison of index values when applied for different coastal types, and 3) comparison of ecological status classifications on water body level.

Comparisons between Swedish and Finnish methods

Due to differences in monitoring and assessment methods[17], two separate comparisons were made. Firstly, the comparison of the Finnish BBI and Swedish BQI macroinvertebrate classification systems was made by applying both assessment tools using Swedish monitoring data. Secondly, both methods were applied to Finnish monitoring data.

[17] In Sweden macroinvertebrate monitoring is carried out using 0.1 m^2 van Veen grab and 1 mm sieve, while in Finland 0.025 m^2 Ekman grab and 0.5 mm sieve are used. The Swedish BQI index values are calculated separately for each grab while the Finnish BBI values are calculated for five (5) pooled grab samples.

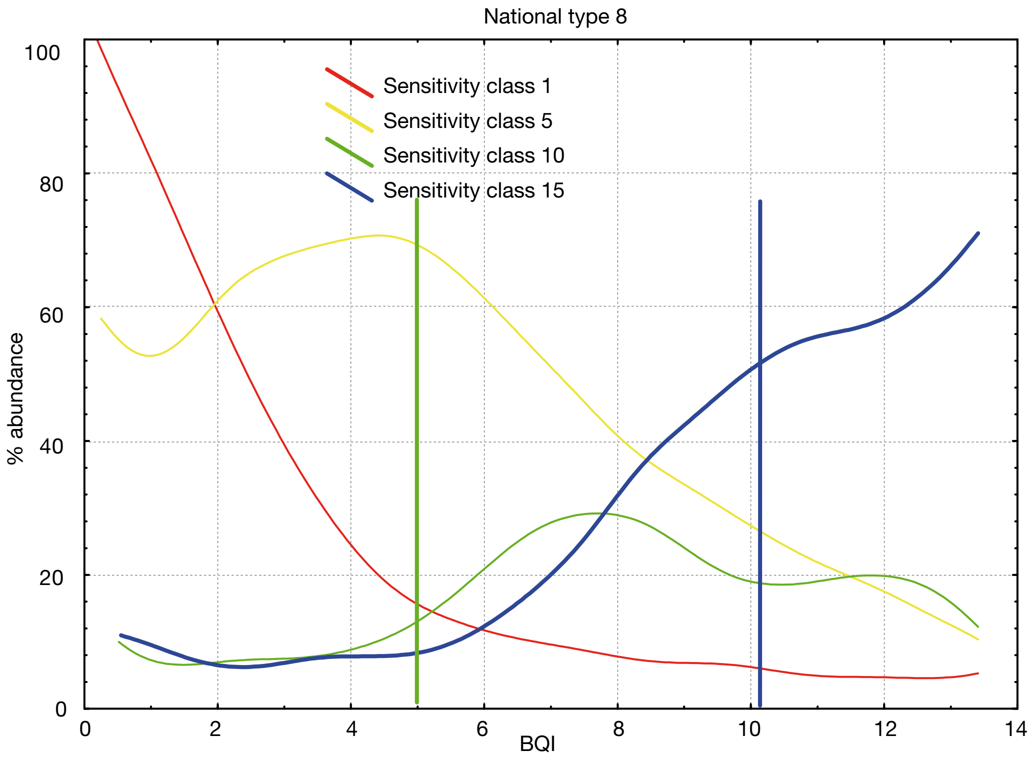


Figure 2.1.2: Distribution of the relative abundance of the four macroinvertebrate sensitivity groups as a function of the BQI values. Group 15 is the most sensitive and group 1 the most tolerant group of species. Good-moderate boundary is indicated with a green and the High-good boundary with a blue vertical line.

Comparison based on Swedish data

The first comparison of the two national methods was carried out by Sweden using Swedish national and regional monitoring data from the Gulf of Bothnia. The data included in this exercise comes from the national types No. 16 (Coastal waters of South Bothnian Sea, inner parts), 18 (Coastal waters of North Bothnian Sea, Höga kusten, inner parts), 20 (Coastal waters of the Quark, inner parts) and 22 (Coastal waters of North Bothnian Bay, inner parts). Only data deeper than 5 m were used from these types. Due to methodological differences between Sweden and Finland, it was not possible to apply the Finnish national boundaries on Swedish data. The intercalibration is based on applying the Finnish method for setting of boundaries on Swedish data and comparing this with the Swedish boundaries derived on the same Swedish data.

The intercalibration exercise was carried out in two steps: 1) Comparison of index values in different environments, and 2) Comparison of classification status on water body level using both BBI and BQI methods.

1. Comparison of index values

In the first step the Swedish BQI and The Finnish BBI indices were calculated for each grab sample (~0.1 m^2) separately and the results where plotted against each other (Fig. 2.1.3). The Swedish BQI and the Finnish BBI indices were relatively well correlated, but the scatter increased in the two northern types (types 20 and 22).

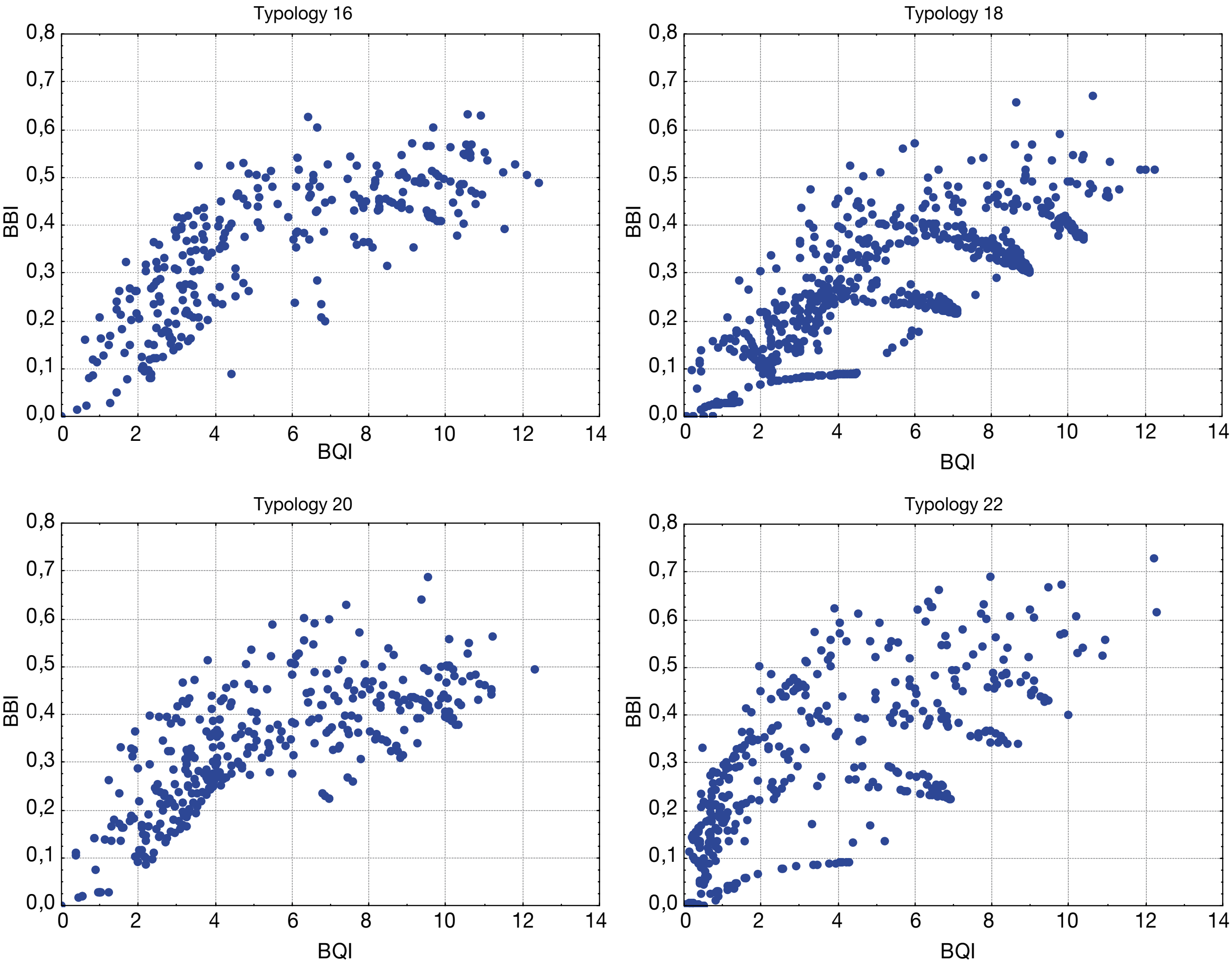


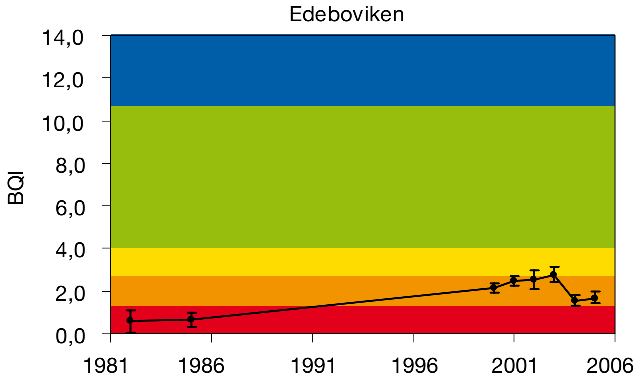
Figure 2.1.3: Correlation between the BQI and BBI using Swedish national and regional monitoring data from the types 16, 18, 20 and 22 (inner coastal waters, south to north, corresponding to common intercalibration types B3, B2 and B0, respectively).

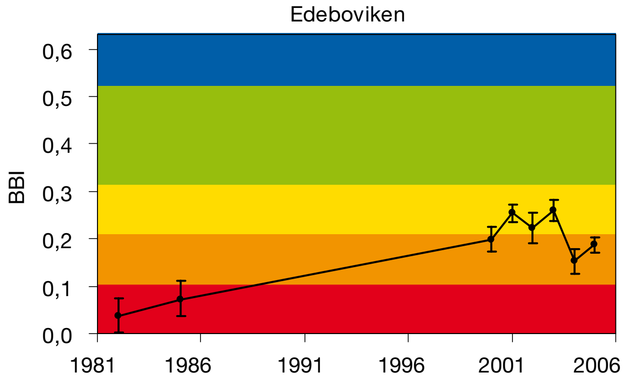
2. Comparison of classification status on water body level

The classification status of water bodies was calculated applying both BBI and BQI on Swedish monitoring data. The boundary setting was done following both Finnish and the Swedish approaches for identifying reference status and the boundary setting principles for each station and year separately.

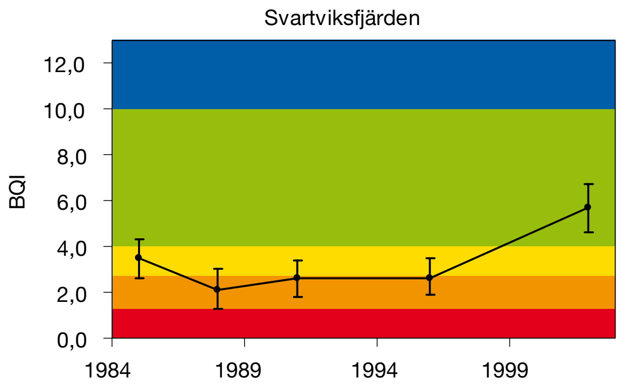
The comparison was made using time series data from seven water bodies from the Swedish national types 16, 18, 20 and 22. The Swedish method states that at least five stations per year should be present in a water body for an assessment to be made (Blomqvist et al 2007). This rule has been applied to both assessment methods. Both the Swedish and Finnish methods (Jens Perus, pers. comm. March 2007) use the 20th percentile for water body assessment. The results of comparison of the two methods are shown in (Fig. 2.1.4).

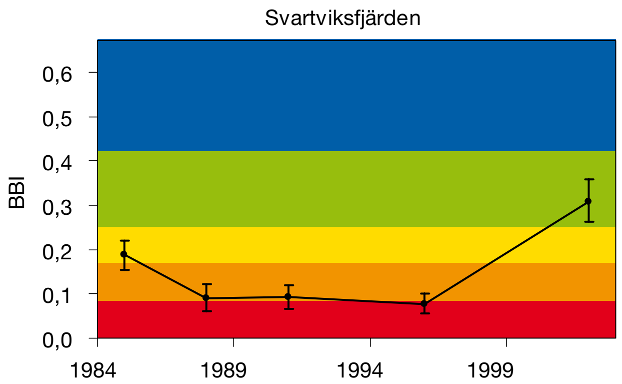
Swedish National type 16

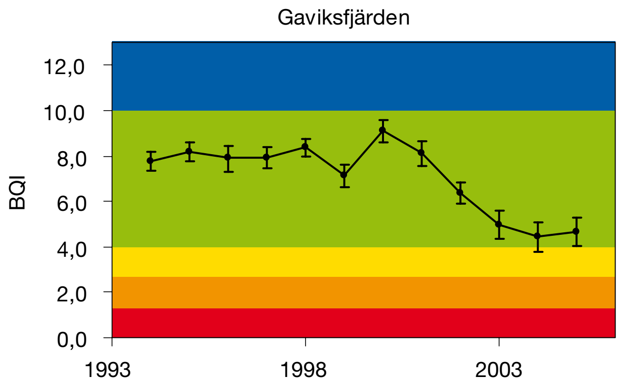


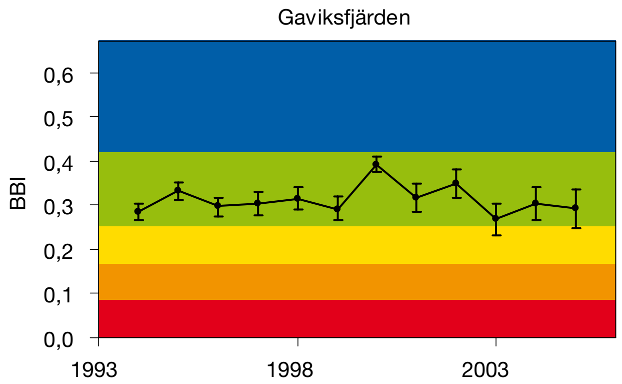


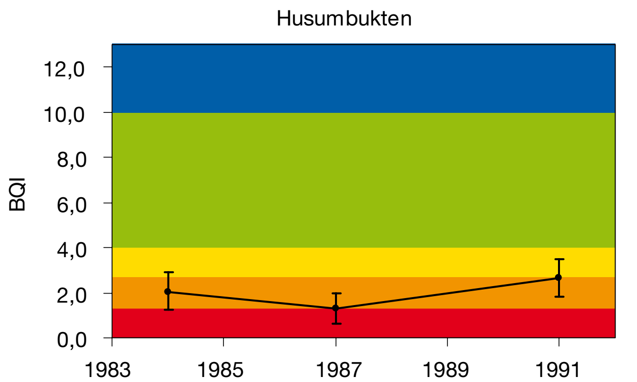
Swedish National type 18

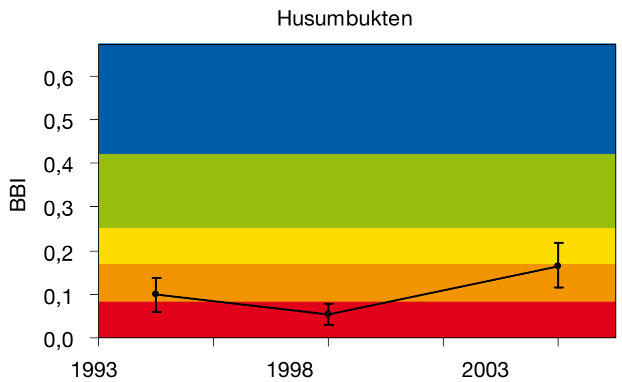




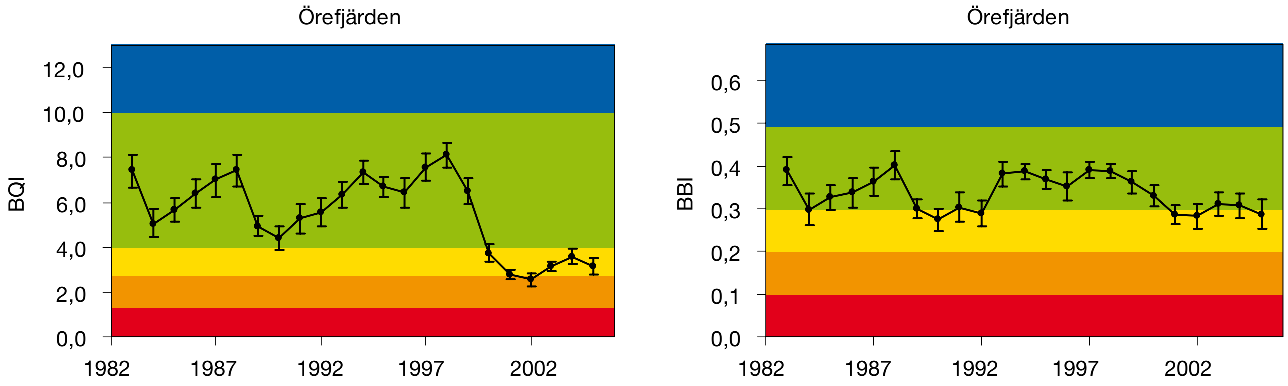








Swedish National type 20



Swedish National type 22

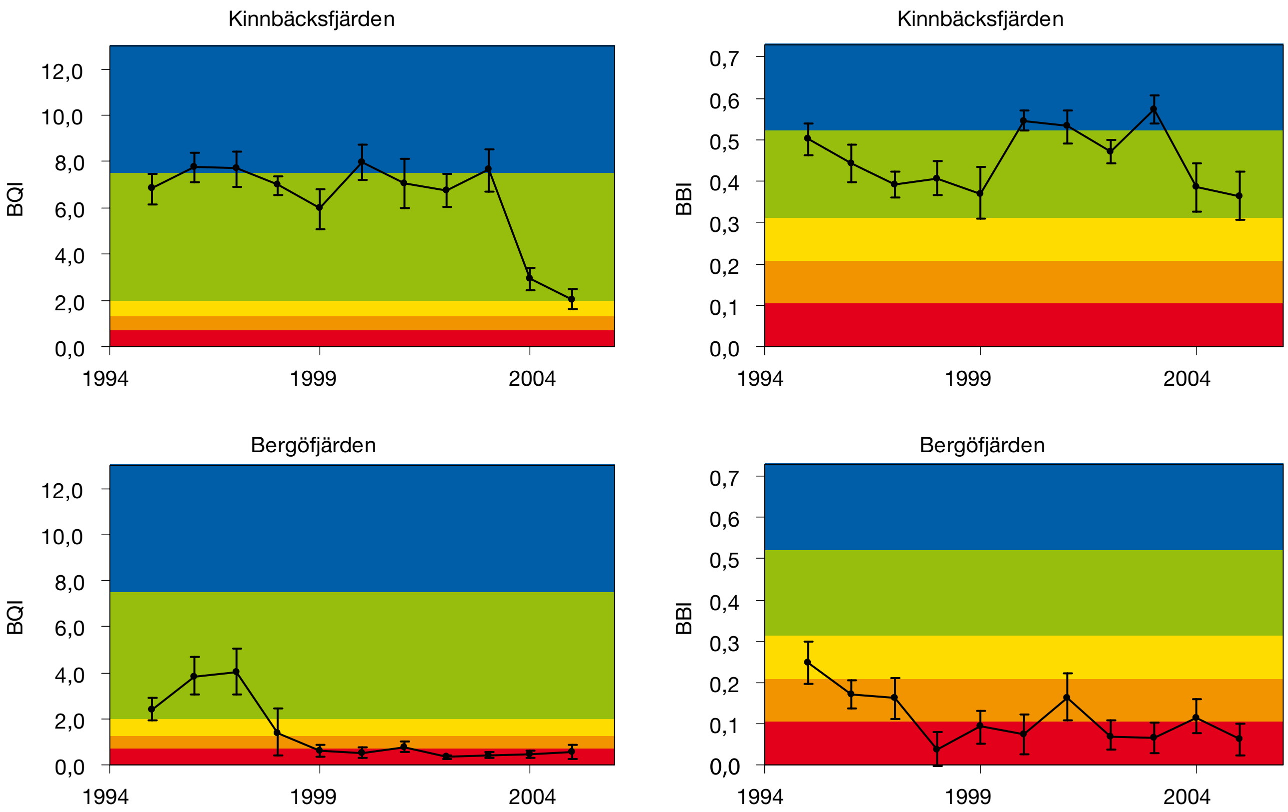


Figure 2.1.4: Comparisons of the water body assessments using Swedish (BQI; left column) and Finnish (BBI; right column) macroinvertebrate methods applied to Swedish monitoring data from the national Swedish types No. 16 (Coastal waters of South Bothnian Sea, inner parts), 18 (Coastal waters of North Bothnian Sea, Höga kusten, inner parts), 20 (Coastal waters of the Quark, inner parts) and 22 (Coastal waters of North Bothnian Bay, inner parts). Error bars denotes 20th and 80th percentile. Note that the 20th percentile (lower error bar) is used to determine the calssification status. Boundaries hose are from March 2007 (Jens Perus, pers. comm. and Blomqvist et al. 2007).

In most cases the two methods show the same general pattern for the water body assessments. In some years there are differences in the two assessment methods, e.g. the collapse of the dominating *Monoporeia affinis* in Kinnbäcksfjärden year 2004 and Örefjärden year 2000 is more clearly detected by the Swedish assessment method.

Comparison based on Finnish data

The other comparison of the Finnish BBI and Swedish BQI macrozoobenthos assessment methods was done by using Finnish national and regional monitoring data from the Baltic Sea. Data used in the comparison was representative for the four national Finnish water body types (Table 2.1.4). Only data from below 10 m depth was included in the exercise. The national Finnish typology separates coastal types into two depth zones: above 10 m and below 10 m depth intervals. The national boundaries are set separately for different depth intervals within the same type.

Table 2.1.4: Correspondence of the common intercalibration types for the Northern Baltic Sea with the Finnish national types (all deeper than 10 m; Perus et al. 2004) and Swedish national types (NFS 2006:11) included in the intercalibration exercise. All the common types were characterized to be deeper than 10 m.

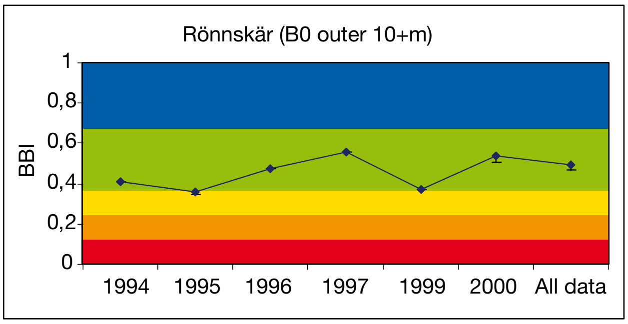
Intercalibration type	Finnish type	Swedish type
B0	Mu	20
B2	SeS	16
B3a	Lv	12
B3b	Lu	14

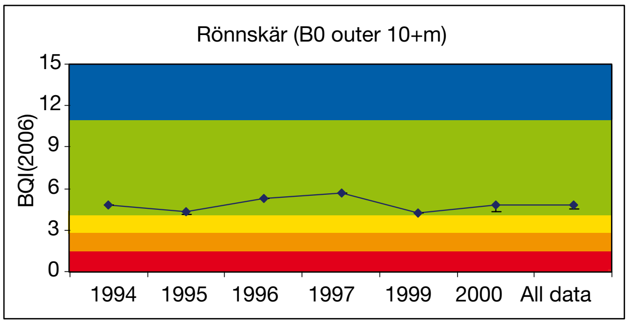
The Finnish BBI and Swedish BQI assessment systems were compared by setting the boundaries for the Finnish water bodies belonging to the intercalibration types B0, B2 and B3 and using the Swedish assessment method. The Swedish calculation method cannot be applied directly on Finnish data due to methodological differences[18]. However, this problem was overcome using the boundary setting principle of the Swedish method (pers. comm. Mats Blomqvist, Hafok AB). According to this approach 80 % (20th percentile) of the natural variation within a data set, originating from an area without local discharges, can be regarded as high or good status i.e. thus being above the good-moderate boundary. This good-moderate boundary value was set for each of the four intercalibration types (Fig. 2.1.5).

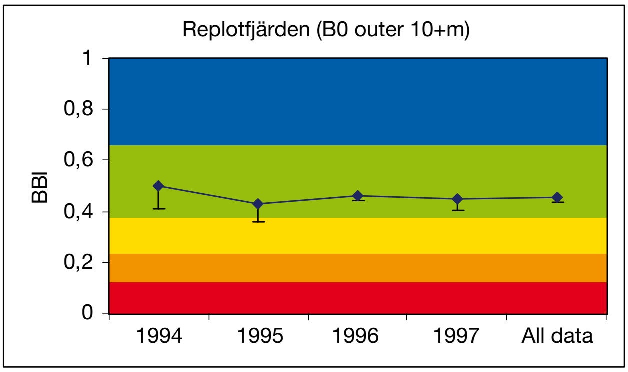
A common species sensitivity classification list is used for both indices and no comparison of differences in species sensitivity classification is thus needed.

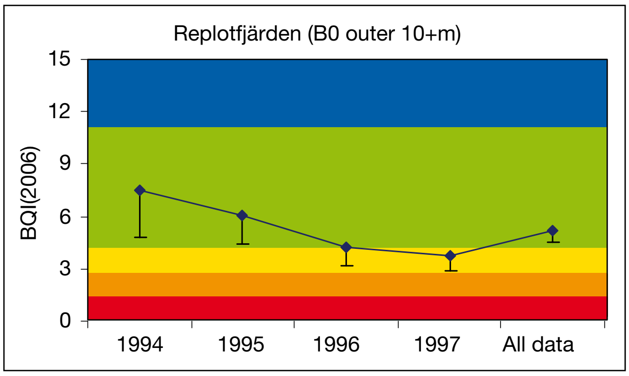
After comparison of the Finnish-Swedish classification results, a change in the Finnish boundaries was proposed. The original Finnish classification boundaries for the classes Good-Moderate-Poor-Bad were set as four equally sized classes beneath the High-Good boundary. The proposal is that the boundary for High-Good remains the same, but that the remaining BBI-index spectra should be divided in to five equally sized classes out of which the Good status class should occupy two fifths (2/5) and classes Moderate, Poor and Bad one fifth each (1/5). A new specification on the Finnish assessment of status is that the use of the 20th percentile value as assessment value would be taken into practice (identical to Swedish practice).

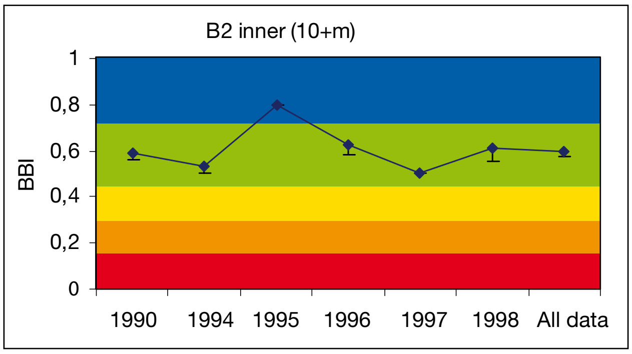
18 In Finland 5 pooled (mean of abundances (ind/m^{-2}) samples taken by Ekman-Birge grab samples (0.5mm mesh) are required and total species richness needs to be determined while in Sweden individual (0,1 m^2) Van-Veen grab samples (1mm mesh) are required to be taken. Also the Swedish method requires at least five stations per year for assessment

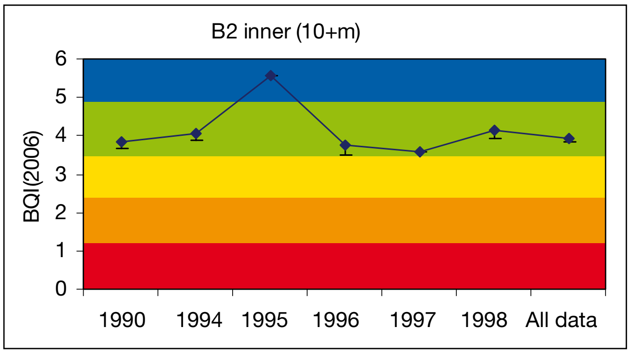

Rönnskär (B0 outer 10+m)
BBI
1
0,8
0,6
0,4
0,2
0
1994
1995
1996
1997
1999
2000
All data

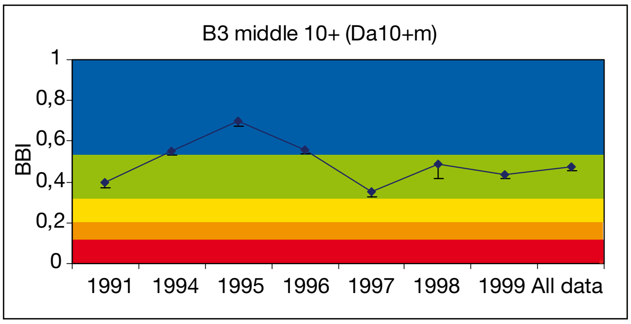

Rönnskär (B0 outer 10+m)
BQI(2006)
15
12
9
6
3
0
1994
1995
1996
1997
1999
2000
All data

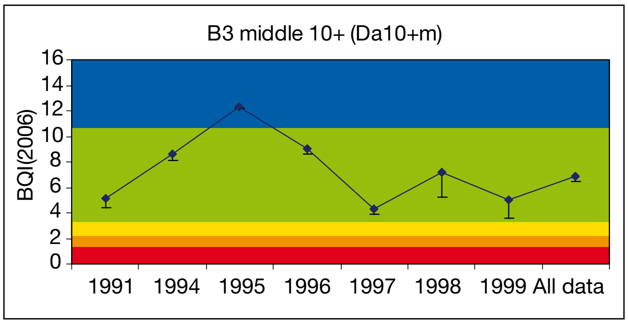

Replotfjärden (B0 outer 10+m)
BBI
1
0,8
0,6
0,4
0,2
0
1994
1995
1996
1997
All data

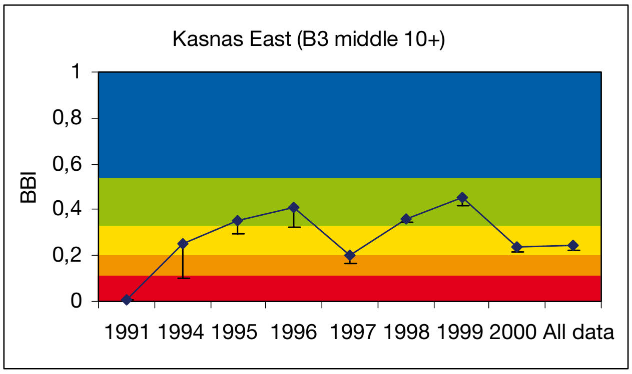

Replotfjärden (B0 outer 10+m)
BQI(2006)
15
12
9
6
3
0
1994
1995
1996
1997
All data

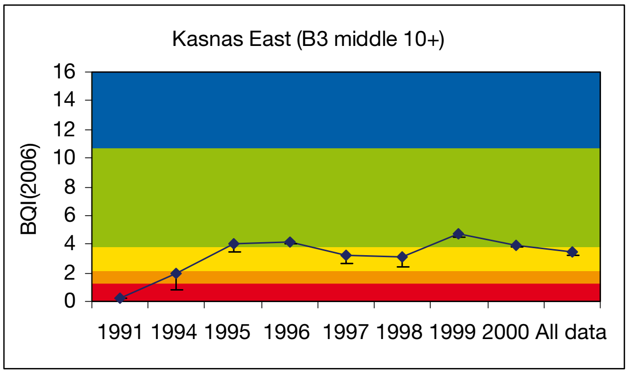

B2 inner (10+m)
BBI
1
0,8
0,6
0,4
0,2
0
1990
1994
1995
1996
1997
1998
All data


B2 inner (10+m)
BQI(2006)
6
5
4
3
2
1
0
1990
1994
1995
1996
1997
1998
All data


B3 middle 10+ (Da10+m)
BBI
1
0,8
0,6
0,4
0,2
0
1991
1994
1995
1996
1997
1998
1999
All data


B3 middle 10+ (Da10+m)
BQI(2006)
16
14
12
10
8
6
4
2
0
1991
1994
1995
1996
1997
1998
1999
All data


Kasnas East (B3 middle 10+)
BBI
1
0,8
0,6
0,4
0,2
0
1991
1994
1995
1996
1997
1998
1999
2000
All data


Kasnas East (B3 middle 10+)
BQI(2006)
16
14
12
10
8
6
4
2
0
1991
1994
1995
1996
1997
1998
1999
2000
All data

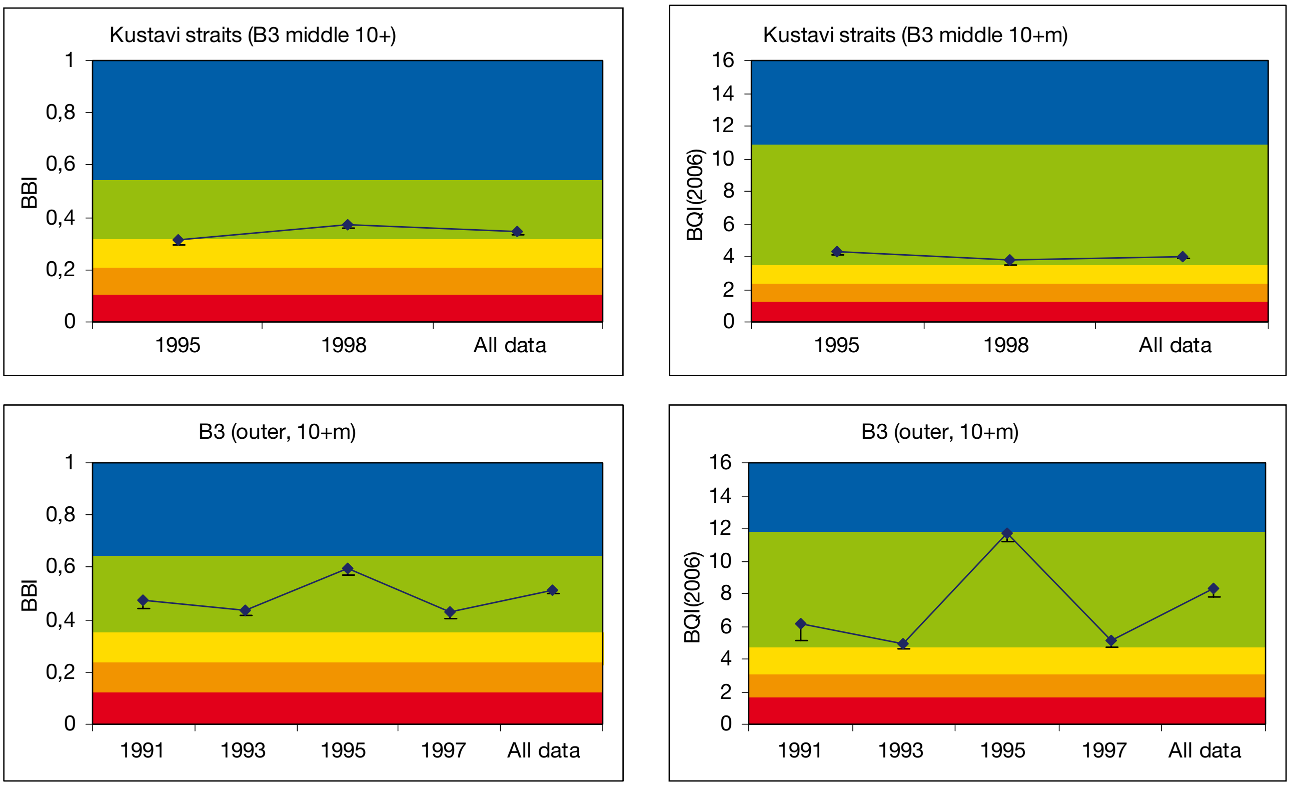


Figure 2.1.5: Comparisons of the water body assessments using Swedish (BQI; left column) and Finnish (BBI; right column) macroinvertebrate methods applied to national Finnish monitoring data from water bodies belonging to common intercalibration types B0, B2, B3a (B3 middle, Kasnäs East, Kustavi Straits) and B3b (B3 outer, WB Jussarö at the entrance of the Gulf of Finland (GOF), WB Rosala-Hitis). Error bars denotes 20th and 80th percentile. Note that the 20th percentile (lower error bar) is used to determine the classification status. Detailed results are presented in Annex 2.1.

Table 2.1.5: The intercalibrated Finnish and Swedish EQR-values for the high-good (HG) and good-moderate (GM) boundaries calculated for the national coastal types representative for the common intercalibration types B0, B2 B3a, and 3b.

Type	Country	National type	EQR HG	EQR GM
B0	Finland	Mu 10+m	0,99	0,59
	Sweden	20	0.77	0.31
B2	Finland	Ses 10+m	0,95	0,57
	Sweden	16	0.76	0.29
B3a	Finland	Lv 10+m	0,89	0,53
	Sweden	12	0.76	0.29
B3b	Finland	Lu 10+m	0,90	0,54
	Sweden	14	0.76	0.29

2.1.5 Results of the harmonization – Boundary EQR values

Biological Quality Element	Benthic invertebrate fauna
Results: Ecological quality ratios of the national classification systems intercalibrated	

Type and country	**National classification system intercalibrated**	**Ecological Quality Ratios**	
		High-Good boundary	***Good-Moderate boundary***
CW B0			
Finland	BBI- Finnish Brackish water Benthic Index	0.99	0.59
Sweden	BQI–Swedish multimetric biological quality index (soft sediment infauna)	0.77	0.31
CW B2			
Finland	BBI- Finnish Brackish water Benthic Index	0.95	0.57
Sweden	BQI–Swedish multimetric biological quality index (soft sediment infauna)	0.76	0.29
CW B3 a			
Finland	BBI- Finnish Brackish water Benthic Index	0.89	0.53
Sweden	BQI–Swedish multimetric biological quality index (soft sediment infauna)	0.76	0.29
CW B3 b			
Finland	BBI- Finnish Brackish water Benthic Index	0.90	0.54
Sweden	BQI–Swedish multimetric biological quality index (soft sediment infauna)	0.76	0.29

2.1.6 Open issues and need for further work

Intercalibration need to be extended to cover all Baltic Sea countries and all common types. Currently results are completed at quality element level only for two countries (FI, SE) covering three (3) common types in the Northern Baltic Sea (Bothnian Sea and Bothnian Bay).

Denmark, Sweden, and Germany carried out intercalibration of their national methods for the common type B12. However, at this stage comparability was not sufficiently demonstrated, and the results are not included in the final intercalibration decision. New comparisons have to be carried out when more monitoring data will be available for common intercalibration types in the Southern Baltic Sea.

Other countries still need to develop and intercalibrate WFD compliant assessment methods for benthic invertebrates for the Baltic coastal waters.

Currently most national methods have been developed for the soft bottom sediment habitats only, with the exception the German method that is a multi-habitat method. Assessment methods for other coastal habitats (sandy and rocky bottoms. littoral zone) have to be developed. methods for the transitional water types in the Baltic have to be developed.

2.2 Black Sea GIG

2.2.1 Intercalibration approach

Typology

The countries participating at this intercalibration exercise into the Black Sea region are Bulgaria and Romania. One common water body type CW-BL 1 was defined, as is shown below:

Salinity: Mesohaline
Tidal Range: Microtidal
Depth: Shallow
Subsratum: Mixed
Wave Exposure: Moderately Exposed

2.2.2 National methods that were intercalibrated

Boundaries were agreed in this intercalibration exercise for the Shannon diversity index H', AMBI, M-AMBI, applicable to both Bulgaria and Romania.

2.2.3 Reference conditions and class boundary setting

As Option 3 has been selected, both countries have used the same methods for sampling, laboratorial analyses, reference conditions and EQRs. Those were derived using literature, historical data and expert judgment.

The criteria for establishment of biological reference conditions and the boundary setting procedure are described below separately for each country.

2.2.4 Results of the comparison

The method of sampling and analytical methods are as provided by the Manual "Quantitative sampling and sample treatment of marine soft bottom – macrozoobentos" (Tsenka Konsulova, Valentina Todorova – 2005), agreed by the Black Sea Commission. The species names used in the process of AMBI calculation should be in accordance with the valid names from the same manual.

The macroinvertebrates experts, from both countries, agreed to use three indices to assess the ecological status of the selected common type: Shannon Diversity Index H', AMBI and M-AMBI.

Both countries agreed to use "one out all out principle" to establish the ecological status.

BULGARIA

Reference conditions and EQR values were developed for Benthic invertebrate fauna in Bulgarian waters by the following scientists from the Marine Biology and Ecology Department; INSTITUTE OF OCEANOLOGY, BAS, Varna:

Assoc. Researcher Antoaneta Trayanova;

Dr. Assoc. Researcher Valentina Todorova;

Dr. Assoc. Professor Tsenka Konsulova;

For the Bulgarian Black Sea coastal waters, no reliable data of the "pristine" period (before the 1970s) collected with the same methodology as we use today, exist. Use of historical data is therefore not an option in determining reference conditions. Another approach is screening for un-impacted areas, i.e. communities not affected by human activities. Unaffected areas are regarded as absent in the Bulgarian Black Sea coastal waters nowadays. The remaining option to establish condition comparable to a reference is to derive it from areas as unaffected by human activities as possible using the data of slightly disturbed benthic communities (having at least good status). An expert judgment and knowledge of the conditions under evaluation were applied in obtaining of "virtual" reference conditions.

The study area covers the one nautical mile zone along the entire Bulgarian Black Sea coast. Nineteen sites have been sampled for macrozoobenthos in the period 2002-2006 (Figure 2.2.1).

Figure 2.2.1: Monitoring sampling network of the Bulgarian Black Sea coastal waters (used for intercalibration exercise)

Table 2.2.1: Number of samples per station by sampling years, months and totally.

Station (1nm) / Sampling year and month	2002		2003		2004		2005				2006	Number of samples per station
	8	11	3	6	6	9	3	6	9	11	7	
10 (100) Krapetz	1	1		1	1	1	1	1	1	1	1	10
20 Shabla											1	1
30 Tjulenovo											1	1
40 (111) Rusalka Resort					1	1	1	1	1	1	1	7
200 Kaliakra (in front of the cape)	1	1	1	1								4
50 (211) Kaliakra (under the cape)					1	1	1	1	1	1	1	7
60 Albena Resort											1	1
70 (B3) Varna Bay							1	1	1	1	1	5
80 (301) cape Galata	2	2	1	1	1	1	1	1	1	1	1	13
90 Kamchia River											1	1
100 Dvoinitsa River											1	1
411 Irakli							1	1	1	1		4
110 (500) Slanchev Briag	3	1	1	2	2	1	1	1	1	1	1	15
120 Burgas Bay											1	1
130 (501) Burgas	1	1	1	1	1	1	1	1	1	1	1	11
140 - Sozopol											1	1
150 (600) Achtopol	1	1		1		1		1	1	1	1	8
160 - Varvara											1	1
170 (611) Veleka River								1	1		1	3
Total number of samples	9	7	4	7	7	7	8	10	10	9	17	95

a. Shannon diversity index H’

Although the identification of reference and bad status values rely on expert assessment the limited number of samples and/or replicates results in high degree of uncertainty.
When deriving the type-specific class boundaries, the greatest emphasis was placed on the good–moderate boundary. This boundary was primarily determined with the aid of data from high–good areas.
The approach in identifying reference conditions is based on the hypothesis that the high ecological status represents certain percentage of the good status.
The reference value for water bodies with sandy and mixed sediments is derived with the assumption that the average community diversity index of stations reaching good ecological status constitutes 75 % from the high status. The reference value for water bodies with muddy sediments have been determined from the average community diversity index of stations reaching good status and having more than 25 species per sample, than the reference value is extrapolated from current status deemed as 70 % from reference. The percentage is lower due to the specificity of the muddy sediments characterized by higher sensitivity of the developing communities to environmental disturbances.

The boundaries between the ecological classes are set as percentage from average reference values. The interval of 0.2 between the ecological status boundaries is equally scaled (80 % for good, 60 % for moderate, 40 % for poor and 20 % for bad status). For determination of EQRs each class boundary value has been divided by the average reference value – 3.6 for water bodies with muddy sediments and 4.5 for water bodies with sandy and mixed sediments (Table 2.2.2). The data available do not allow developing of separate classification schemes for water bodies with sandy and for water bodies with mixed sediments. It is highly recommended the boundary values to be readjusted after new data are available as well classification scale for water bodies with mixed sediments to be developed.

Table 2.2.2. Classification scheme for Shannon community diversity index (H').

Water bodies with muddy sediments					
Ecological status	**High**	**Good**	**Moderate**	**Poor**	**Bad**
H' average	3.6	2.9	2.2	1.5	0.7
Range	H' ≥ 3.3	3.3 > H' ≥ 2.5	2.5 > H' ≥ 1.8	1.8 > H' ≥ 1.1	H' < 1.1
EQR	≥ 0.92	0.69	0.50	0.31	< 0.31
Water bodies with sandy and mixed sediments					
Ecological status	**High**	**Good**	**Moderate**	**Poor**	**Bad**
H' average	4.5	3.6	2.7	1.8	0.9
Range	H' ≥ 4	4 > H' ≥ 3.1	3.1 > H' ≥ 2.2	2.2 > H' ≥ 1.3	H' < 1.3
EQR	≥ 0.89	0.69	0.49	0.29	< 0.29

b. A Marine Biotic Index (AMBI)

The method is based on the assignment of species to five ecological groups, according to the sensitivity to an increasing stress gradient (Gray and Glemarec (1997):

- Group I – species very sensitive to organic enrichment and present under unpolluted conditions (initial state). They include the specialist carnivores and some deposit feeding tubicolous polychaetes;
- Group II – species indifferent to enrichment, always present in low densities with non-significant variations with time. These includes suspension feeders, less sensitive carnivores and scavengers;
- Groups III – species tolerant to excess organic matter enrichment. These species may occur under normal conditions, but their populations are stimulated by organic enrichment. They are surface deposit – feeding species, as tubiculous spionids;
- Group IV – Second order opportunistic species. Mainly small sized polychaetes; subsurface deposit feeders, such as cirratulids;
- Group V – First order opportunistic species. These are deposit feeders, which proliferate in reduced sediments;

The reference value (AMBI ≤ 1.2) is derived as dominance of sensitive and indifferent taxa in the abundance. The bad status value (AMBI > 5.5) is achieved when second order and first order opportunists dominate. The boundaries between the ecological classes are those identified by Borja *et al.,* (2000, 2003) and Muxica *et al.*, (2005). EQR is determined by subtraction of the boundary value divided by the maximal value 7 from 1 (Table 2.2.3). AMBI is not sediment dependant which allows to be applied for all WBT regardless of their typology

Table 2.2.3: Classification scheme for A Marine Biotic Index (AMBI).

Ecological status	High	Good	Moderate	Poor	Bad
Range	0.2 < AMBI ≤ 1.2	1.2 < AMBI ≤ 3.3	3.3 < AMBI ≤ 4.3	4.3 < AMBI ≤ 5.5	5.5 < AMBI ≤ 7.0
EQR	≥ 0.83	0.53	0.39	0.21	< 0.21

c. Multivariate AMBI (M-AMBI)

The default EQR boundaries of M-AMBI are set through intercalibration exercise for North Atlantic ecoregion (Borja *et al.*, 2006). Because no proper intercalibration is carried out for the Black Sea ecoregion we adopted the default boundaries between the ecological classes. Further changes of these values for each WBT are advisable after intercalibration procedure and harmonisation of these boundaries between Member States took place. The default EQR boundaries of high and bad status have been accepted for all WBT

Table 2.2.4. Classification scheme for Multivariate AMBI (M-AMBI).

Ecological status	High	Good	Moderate	Poor	Bad
Range	M-AMBI ≥ 0.85	0.85 > M-AMBI ≥ 0.55	0.55 > M-AMBI ≥ 0.39	0.39 > M-AMBI ≥ 0.20	0.20 > M-AMBI
EQR	≥ 0.85	0.55	0.39	0.20	< 0.20

The hierarchical clustering of stations based on the Bray-Curtis similarities of log (x + 1) transformed abundance was employed to reveal the similarity pattern of macrozoobenthic community. Five main groups have been differentiated on the dendrogram corresponding to the type of the sediment (Figure 2.2.2). In some cases the WB typology differentiates from the sediment type defined by the clustering technique, which is indication of misclassification of sediments resulting in inaccurate water bodies' typology.

Because the sediment type determine the development of specific macrozoobenthic communities, it is reasonable the assignment of high and bad status values for the selected metrics to be done according to the cluster groupings.

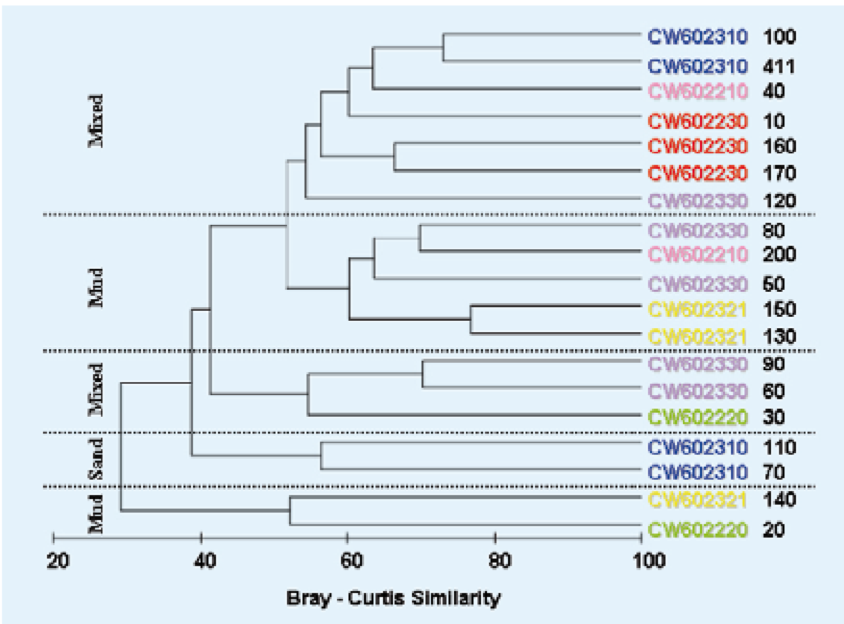


Figure 2.2.2: Dendrogram for hierarchical clustering (group-average linking) of WBT and stations.

ROMANIA

The study area covers the southern part of the Romanian Black Sea coast, from Singol Cape to Vama Veche (Fig.1). To assess the ecological status it was use the data from four sampling stations, during the period 2002-2006 (Table 2.2.4).

Table 2.2.4 Sampling stations / Number of samples per station.

Station/ Sampling year and month	**2002**		**2003**	**2004**		**2005**		**2006**	**Number of samples / station**
	May	Aug	May	May	Aug	May	Oct	Apr	
Eforie	-	-	-	1	1	1	1	1	**5**
Costinesti	1	1	1		1	1	-	1	**6**
Mangalia	-	-	-		1	1	1	-	**3**
Vama Veche	1	1	1		1	1	1	1	**7**
Total number of samples	**2**	**2**	**2**	**1**	**4**	**4**	**3**	**3**	**21**

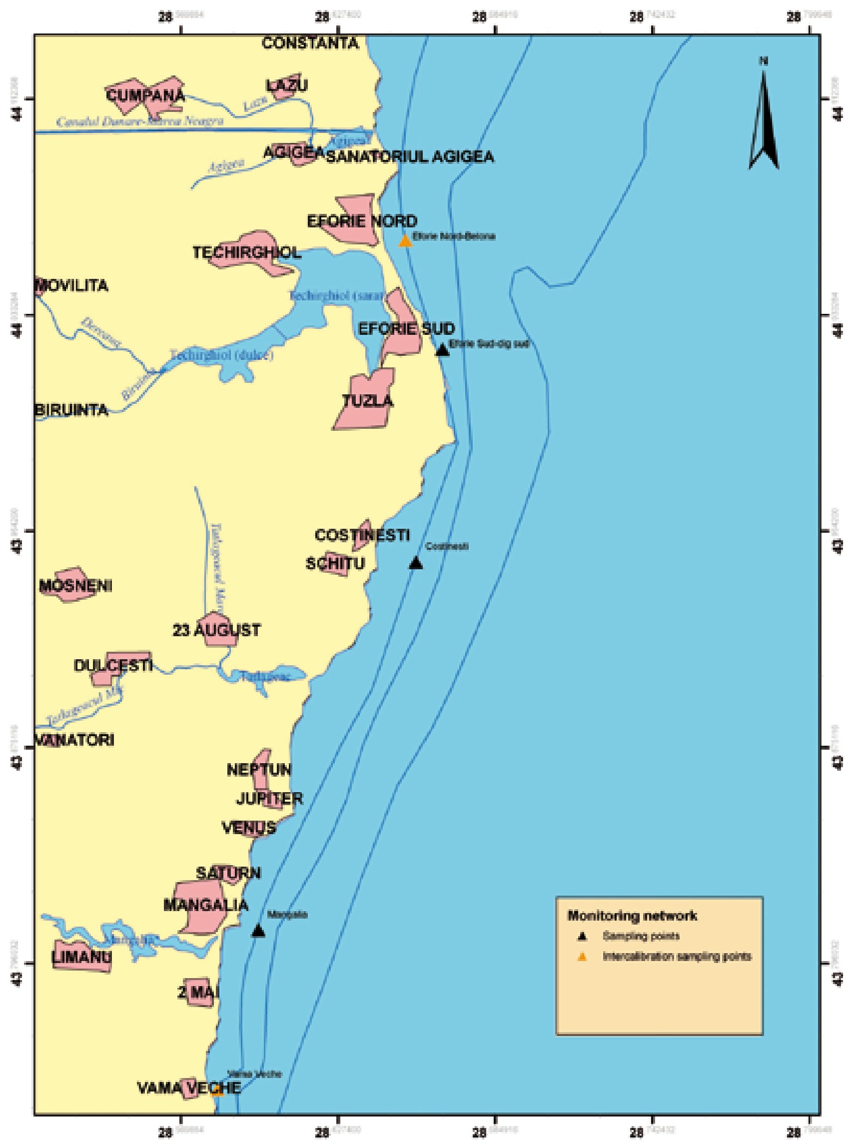


Figure.2.2.3: Monitoring sampling network of the Romanian Black Sea coastal waters (used for intercalibration exercise)

The sampling and analytical methods used were those provided in the manual Quantitative sampling and sample treatment of marine soft bottom – macrozoobentos" (Tsenka Konsulova, Valentina Todorova – 2005), approved by the Black Sea Commission.

a. A Marine Biotic Index (AMBI)

The AMBI was calculated using the data from the period 2002 – 2006. Results are shown in Table 2.2.5.

Table 2.2.5: AMBI values for the Romanian sampling network.

Sites	Eforie South	Costinesti	Mangalia	Vama Veche
2002	-	4.12		3.06
2003	-	3.85		3.25
2004	3.04	4.17	2.96	2.89
2005	3.12	3.13	3.01	2.14
2006	3.31	2.86		1.58
Average	3.2	3.6	3	2.6

It was estimated that the general status was moderate ($3.3<AMBI\leq4.3$).

The boundaries between ecological classes are those identified by Borja et al. (Table 2.2.6).

b. Multivariate AMBI (M-AMBI)

The M-AMBI was calculated using the data exported by the AMBI software for each of the stations. Based on the results and on expert judgment it was decided that the boundaries identified by Borja will be considered in assessment of the ecological status.

The boundaries values were considered as were identified by Borja et al. (Table 2.2.7).

c. Shannon diversity index H'

For the Romanian coastal waters, no reference sites were available for macro invertebrates. Although, the experts were used data from scientific literature and based on expert judgment, they set the values for reference conditions.

Using the AMBI software, the Shannon diversity index was calculated and based on acknowledge of the experts regarding the actual ecological status, the boundaries were considered as shown in Table 2.2.8.

Table 2.2.6: H/G and G/M boundaries for AMBI

Ecological status	H/G	G/M
Value	1.2	3.3
EQR	0.83	0.53

Table 2.2.7: H/G and G/M boundaries for M-AMBI

Ecological status	H/G	G/M
Value	0.85	0.55
EQR	0.85	0.55

Table 2.2.8: H/G and G/M boundaries for Shannon diversity index H.

Ecological status	H/G	G/M
Value	4	3
EQR	0.8	0.6

The evaluation was made processing the data from the period 2002-2006.

2.2.5 Results of the harmonisation – Boundary EQR values

AMBI

In was established that the AMBI should be calculated according to the "Guidelines for the use of AMBI (AZTI's Marine Biotic Index) in the assessment of the benthic ecological quality".
The species names used with AMBI should be according to the valid names from the manual "Quantitative sampling and sample treatment of marine soft bottom – macrozoobentos" (Tsenka Konsulova, Valentina Todorova – 2005).
In the assessment of the ecological status, both countries decided to use the boundaries defined by Borja (Table 2.2.9).

Table 2.2.9: AMBI boundary values.

Ecological status	High	Good	H/G	G/M
Range	AMBI ≤1.2	1.2<AMBI≤3.3	1.2	3.3
EQR	≥ 0.83	≥0.53	0.83	0.53

Shannon H'

Both countries agreed that the Shannon diversity index should be calculated using $\log_{2}$, as Bulgarian experts did in their assessment:

Table 2.2.10: Shannon Diversity boundary values

Ecological status	High	Good	H/G	G/M
Range	H' ≥ 4	4> H' ≥ 3.1	4	3.1
EQR	≥ 0.89	≥ 0.69	0.89	0.69

M-AMBI

The process of evaluation of M-AMBI was made taking into consideration some values for high status and bad status for richness and diversity (required by the program), established by common agreement as being characteristic to the Black Sea common type.

Table 2.2.11: M-AMBI boundary values.

Ecological status	High	Good	H/G	G/M
Range	M-AMBI≥0.85	0.85>M-AMBI≥0.55	0.85	0.55
EQR	≥ 0.85	≥0.55	0.85	0.55

In the process of calculation, the reference and bad status values for Species richness (S), diversity (H’) and AMBI were:

Table 2.2.12: Reference and bad status values for species richness, Shannon diversity, and AMBI.

	high	**bad**
Richness	50	15
Shannon	4	1.3
AMBI	1.2	5.5

For macroinvertebrates, both countries agreed to use the “one out, all out” principle to establish the ecological status.

2.2.6 Open issues and need for further work

Refining of the typology of water bodies is needed after the sediment types are accurately classified.

The determination of the reference values for the community diversity index (H’) was derived from actual data for good ecological status and based on expert judgement and knowledge. The limited number of stations and/or replicates in one nautical mile zone allowed partial application of statistical approaches and did not permit the development of classification scale for water bodies with mixed sediments. Revision of the boundary values is recommended after accumulation of further data.

The default boundary values of M-AMBI have to be readjusted for each WBT after intercalibration procedure for the whole Black Sea ecoregion takes place.

2.3 Mediterranean GIG

2.3.1 Intercalibration approach

Participation of countries in the Benthic Invertebrates subgroup:

- Cyprus
- France
- Greece
- Italy
- Slovenia
- Spain (Catalonia, Balearic Islands)

TYPOLOGY

In the Mediterranean 4 basic intercalibration types have been agreed. These are shown in table 2.3.1 below:

Table 2.3.1: Mediterranean coastal common intercalibration types.

Type	Name of Type	Substratum (1)	Depth (2)
CW - M1	Rocky shallow coast	rocky	shallow
CW - M2	Rocky deep coast	rocky	deep
CW - M3	Sedimentary shallow coast	sedimentary	shallow
CW - M4	Sedimentary deep coast	sedimentary	deep

(1) In many cases different seabed substrata will occur within one water body type. The dominant substratum should be defined.
(2) Depth division is based on 40 m depth at 1 mile distance from the coastline.

Benthic invertebrates experts defined that the four Mediterranean coastal IC types (see section 1 General part), primarily defined on substratum composition and depth profile, are not relevant to the MED-GIG benthic invertebrates intercalibration.

It is important to note that the intercalibrated methods in this phase are habitat specific (e.g soft bottom, hard bottom). *All methods are for use in soft sediment habitats*. Methods for other habitats are still under development and cannot be assessed at this stage.

OPTION 3 (direct comparison of national methods) has been used.

All pressures are considered but the methods are particularly sensitive to organic enrichment.

2.3.2 National methods that were intercalibrated

Member State	Method	Status	Boundary agreement
Cyprus	Bentix	Finalized	yes
France	Multimetric approach (AMBI, Shannon Diversity, BQI Trophic Index)	Under development	no
Greece	Bentix	Finalized	yes
Italy	AMBI, M-AMBI, Bentix	Under development	no
Slovenia	M-AMBI	Finalized	yes
Spain - Catalonia	MEDOCC	Officially accepted	yes
Spain- Balearic is.	MEDOCC	Officially accepted	yes

2.3.3 Reference conditions and class boundary setting

As option 3 has been selected for the intercalibration of this quality element each Member State has derived biological reference conditions and the boundaries according to their classification systems. The criteria for establishment of biological reference conditions and the boundary setting procedure are described below separately for each country.

GREECE and CYPRUS

Reference conditions

Species sensitivity

General reference conditions are based on the normative definition, which states, "All the disturbance-sensitive taxa associated with undisturbed conditions should be present".
Reference sites from Greek data were defined, presenting reference conditions for biological element macroinvertebrates. The fauna is composed of mostly sensitive species (GI) and corresponding mean Bentix values are amongst the highest: Bentix>5. In these cases the composition of the fauna corresponds to sensitive species over 75 %. In special cases where muddy bottoms are encountered within a reference site Bentix values are expected to reach values over 4 and sensitive species percentage over 50 %.

Species ecology

Another aspect biological reference conditions setting is based on the autoecology of species. Each species is designated with an ecological identity as extracted from scientific literature, so it is possible to identify the species belonging to, or characterizing each type of community > habitat > water body, and thus to establish reference conditions on an ecological basis. Species reference lists are established for each kind of habitats-communities (Simboura & Zenetos, 2002) and the link among community-habitat-water body type is given (Simboura et al., 2005, Sete presentation, Simboura) following the EUNIS habitat classification scheme for European coasts.

Diversity indices

Other indices as the Shannon Diversity index and species Richness are expected to be among the highest according to the given type of habitat, in the sites under reference conditions. For example over a large set of data from Greek coastal areas and for a sample size of 0.1m2, Hmax=6.3 and Smax=110 for mixed sediments, while for muds the respective values were Hmax=4,6 and Smax=39. Generally for the above standard reference sample area and for mixed sediments H values in reference sites are expected to be over 5 and S over 80 and for muddy bottoms H over 4-4.5 and S over 30. However, discrepancies in the values of these indices may arise from sampling methodology differences and habitat particularities. Another point to be considered is that in transitional zones of disturbance (ecotone) Shannon diversity and species richness maybe significantly high leading to misinterpretation of reference conditions; so diversity values should be cross-checked with biotic indices.

Boundary Setting

The Bentix index (Simboura & Zenetos, 2002) is a newly developed biotic index based on the relative percentage of 'sensitive' (GS) and 'tolerant' (GT) species in the fauna weighted analogously to derive a single formula:

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

(http://www.hcmr.gr/english_site/services/env_aspects/bentix.html).

The formula was initially developed as Bentix = [(6 X %GI + 2 X (%GII + %GIII)]/100 where GI includes the sensitive and indifferent taxa, GII the tolerant and second order opportunistic and GIII the first order opportunistic taxa. As the multiplying factor 2 is the same for groups GII and GIII, these groups can be merged to simply represent all tolerant taxa (GT) versus all sensitive taxa (GS). The resulting classification scheme and Ecological Quality Ratio (EQR) which is the universal comparison scale for different metrics is given in table 2.3.2.

The Bentix index was developed in the Mediterranean ecoregion where the benthic fauna is usually very diverse and evenly distributed with no one species naturally dominating over 10 %. Also under slight disturbance conditions there might be the situation where a high diversity of sensitive and opportunistic species may co-exist giving the impression of a high quality status. Given the naturally even distribution of the fauna, the groups are weighted equally in the Bentix formula taking into account simply only the ratio of the groups' occurance in the fauna: the probability of one species randomly picked up from the fauna to belong to a "tolerant" over a "sensitive" group is 3:1. Also the indifferent group is counted with the sensitive. This approach combined with an equal scaling of the ranges of good and moderate classes (2.5-3.5, 3.5-4.5 respectively), results to successfully designate the community health in the Mediterranean benthic ecosystem where, naturally a high number of species are evenly distributed over the benthic population.

Table 2.3.2: The Bentix index classification scheme and EQR.

EcoQS	Bentix values	EQR
High	4.5 < Bentix < 6	>0.75-1
Good	3.5 < Bentix < 4.5	>0.58-0.75
Moderate	2.5 < Bentix < 3.5	>0.42-0.58
Poor	2.0 < Bentix < 2.5	>0.33-0.42
Bad	0	0

* For naturally stressed muddy habitats only, boundary limits 4.5 and 3.5 are reduced to 4 and 3 respectively.

** Also for comparison with the m-Ambi EQR, the EQR scale starts from value 2 corresponding with EQR=0, since the area among 0 and 2 is a non-value area (as for Ambi=6 the EQR=0).

Class boundary values were set by checking community composition of tolerant/sensitive species over the Bentix values for Greek and Cyprus IC data. This ratio is selected as paired metrics to locate class boundaries.

Figure 2.3.1 shows the plotting of the percentage of tolerant and sensitive taxa along the gradient of decreasing Bentix index. Within the range high to poor, changes in the percentage of sensitive and tolerant taxa are gradual and indicated by the smoothing lines.

Based on this plot, it is evident that the cross-line point of the two lines corresponding to the value of Bentix=4, represents the class center of "good" where literally the two ecological groups of tolerant and sensitive share the fauna by 50 % each. This point corresponds with the ecotone point of the transitional zone, middle of good class.

The points at equal distances (0.5) in each side of the crossline represent the high-good boundary limit with value 4.5, and at the other side of the center the boundary between good/moderate with value 3.5. The HG and GM boundaries and the center of good class are indicated by vertical lines.

At the high to good class boundary (Bentix=4.5), the percentage of the sensitive taxa drops to less than 60 % of the fauna and the percentage of the tolerant taxa accounts for more than 40 %. At the good to moderate class boundary (Bentix=3.5), the percentage of tolerant species becomes over 60 % (roughly 2/3 of the fauna) and the sensitive taxa less than 40 % (1/3 of the fauna).

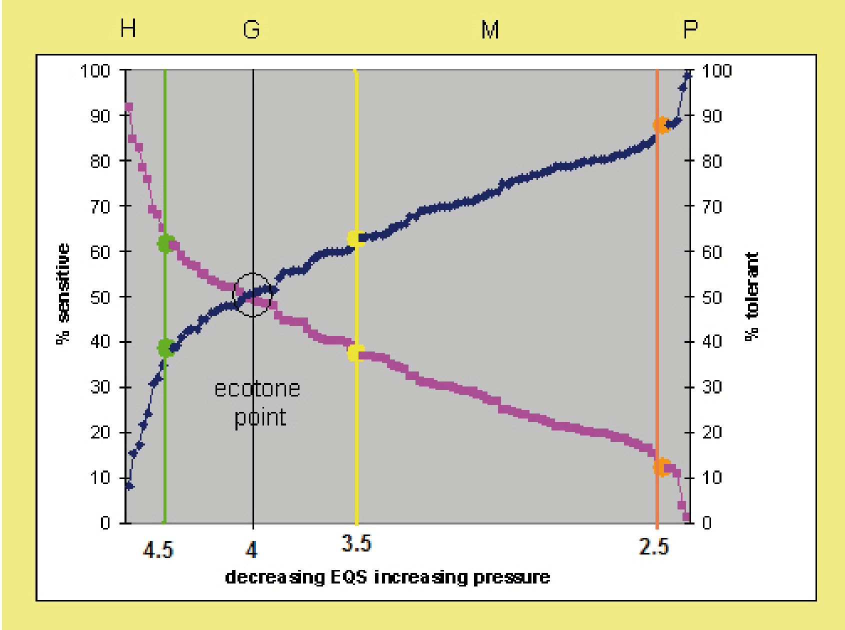


Figure 2.3.1: Bentix values (from Greece and Cyprus IC data) plotted against the percentages of the two ecological groups. P: poor class, M: moderate class, G: good class, H: high class.

Compliance with Normative definitions

Figure 2.3.2 presents the degradation model of the percentage contribution of the three main ecological groups treated initially in the formula: GI (sensitive + indifferent), GII (tolerant + second order opportunistic), GIII (first order opportunistic) in the benthic fauna in relation with the values of the BENTIX index. The sequence of quality classes and class boundaries are interpreted in terms of shifts of ecological group percentages. At the border of good to high status (Bentix=4.5) the sensitive group accounts roughly for more than 60 % or more than 2/3 of the fauna, while the tolerant group as a whole (tolerant plus opportunists) accounts for less than 40 % or less than 1/3 of the fauna. It is important to stress here that for purely muddy habitats where the benthic fauna is normally dominated by some tolerant species, and only in this class border among high and good, a possible refinement of the boundary limit would change 4,5 to 4.
This is to some extent in accordance with the normative conditions, which states that “The level of diversity and abundance of invertebrate taxa is slightly outside the range associated with undisturbed conditions. Most of the sensitive taxa of the type specific communities are present”.At the border of good to moderate status (bentix=3.5) the sensitive group accounts roughly for less than 40 % or less than 1/3 of the fauna, while the tolerant group as a whole (tolerant plus opportunists) accounts for more than 60 % or more than 2/3 of the fauna. This is to some extent in accordance with the normative conditions, which states that “The level of diversity and abundance of invertebrate taxa is moderately outside the range associated with undisturbed conditions. Most of the sensitive taxa of the type specific communities are absent”.

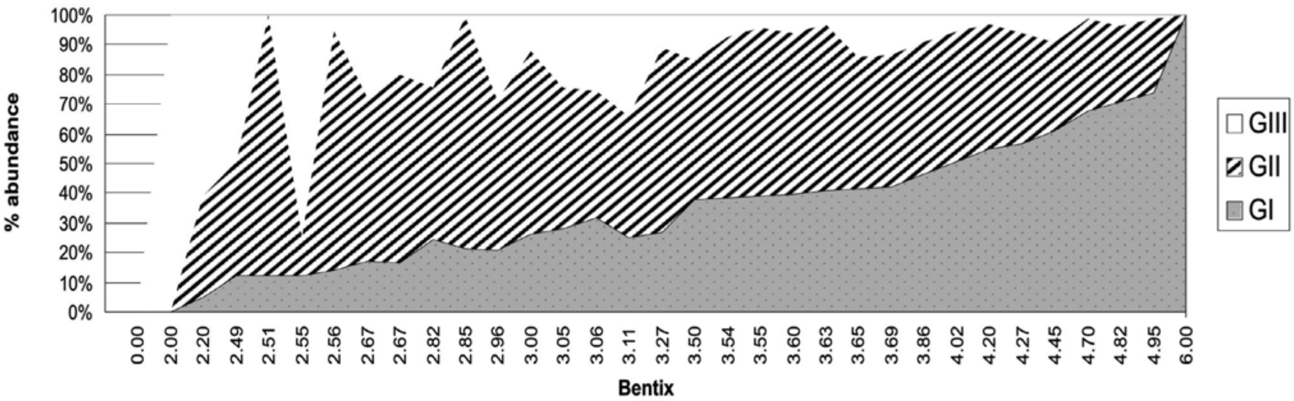


Figure 2.3.2: Degradation model of benthic fauna composition in terms of ecological groups percentages (GI, GII, GIII) from heavily polluted to undisturbed communities in relation to the Bentix index tools.

Index response to the impact gradient

Plotting the Bentix results from Greek IC data with chemical parameters available, the Bentix index showed a linear relationship with the pressure gradient, expressed by chemical pressure factors (nutrients, oxygen and organic carbon content in sediment) with no discontinuities (Fig. 2.3.3).

Using Cyprus data were the pressure gradient is represented by the increasing distance from fish farm areas the results of the Bentix EQS assessment shows a good response of the index in relation with the gradient (Figure 2.3.4).

In the three case areas the EQS is improving (moderate-good or moderate-good-high) with increasing distance. In series C the presence of Posidonia meadows at 300 and 1000 m distance from the cages determine the high EQS status.

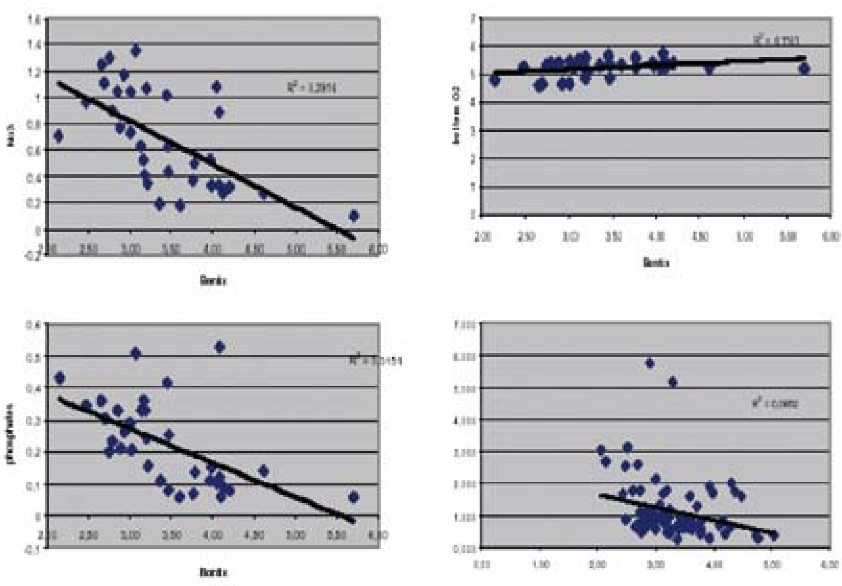
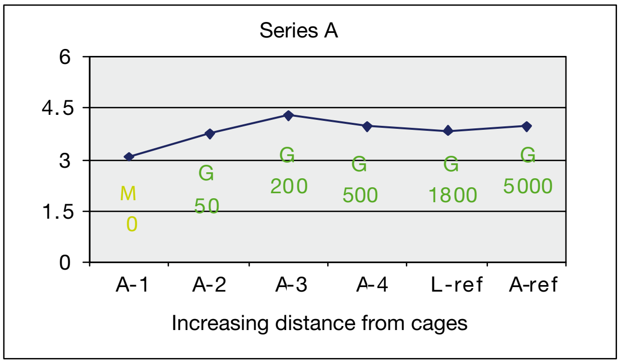
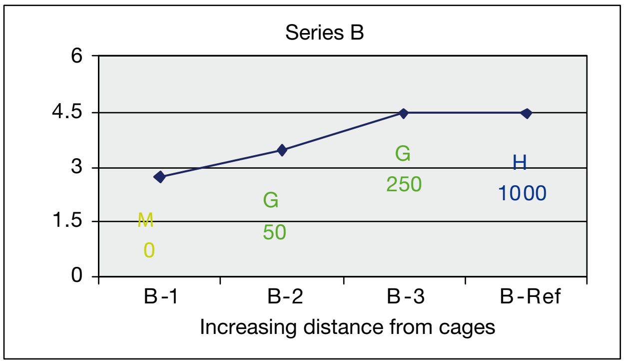


Figure 2.3.3: Correlation of Bentix with chemical parameters.





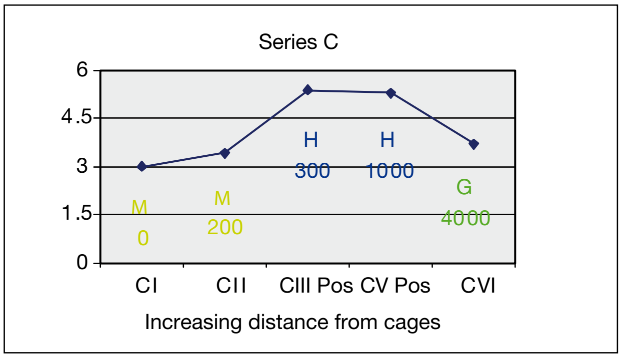


Figure 2.3.4: Variation of the Bentix index and resulted EQS along a gradient of increasing distance from fish farm cages in Cyprus (distances in meters are marked below the EQS).

Results of the comparison

AMBI-BENTIX

The two indices were compared after revising the scores of both indices reaching an agreement on certain ambiguous scores. A direct comparison among the two biotic indices EQRs (Figure 2.3.5) shows the relation among the two indices' Ecological Quality Ratio (EQR) which is linear and statistically significant (R2=61, p=0.000). The lines in the plot define the ranges of each class set by the boundary limits of each method. The Ambi boundary limits were set according to WFD ecological status classification (Muxica et al., 2005). The two indices are linearly correlated, but the EQS assessment rendered by each method differs as shows a Box-and-Whisker plot comparison of the EQS assessment versus the values of the EQRs of the two indices (Figure 2.3.6). This difference is due to a) the differential weight each index puts in the various ecological groups and b) in the different boundary limits of Ambi values and EQRs set among classes as fig. 2.3.5 shows.
In the good class range, the two indices give a common assessment. However, the Ambi classification method gives a wider "good" class range compared with the Bentix, classifying most moderate and often high class sites according to the Bentix to the "good" class. Generally, moderate and high class ranges in the Ambi are more compressed compared to the Bentix scheme.

These differences are structural: in the Ambi method the indifferent group species are weighted separately, while Bentix counts them with the sensitive. Also the Ambi gives different significance to each "tolerant" group of species. In the Bentix method all tolerant species are weighted equally versus the sensitive ones. Besides, the scaling of the distances among classes is different in the two methods. The Bentix sets equal distances for the moderate (2.5-3.5) and good (3.5-4.5) classes, while the Ambi renders a wider good class (1.2-3.3) compared to the moderate (3.3-4.3) and high (0-1.2).

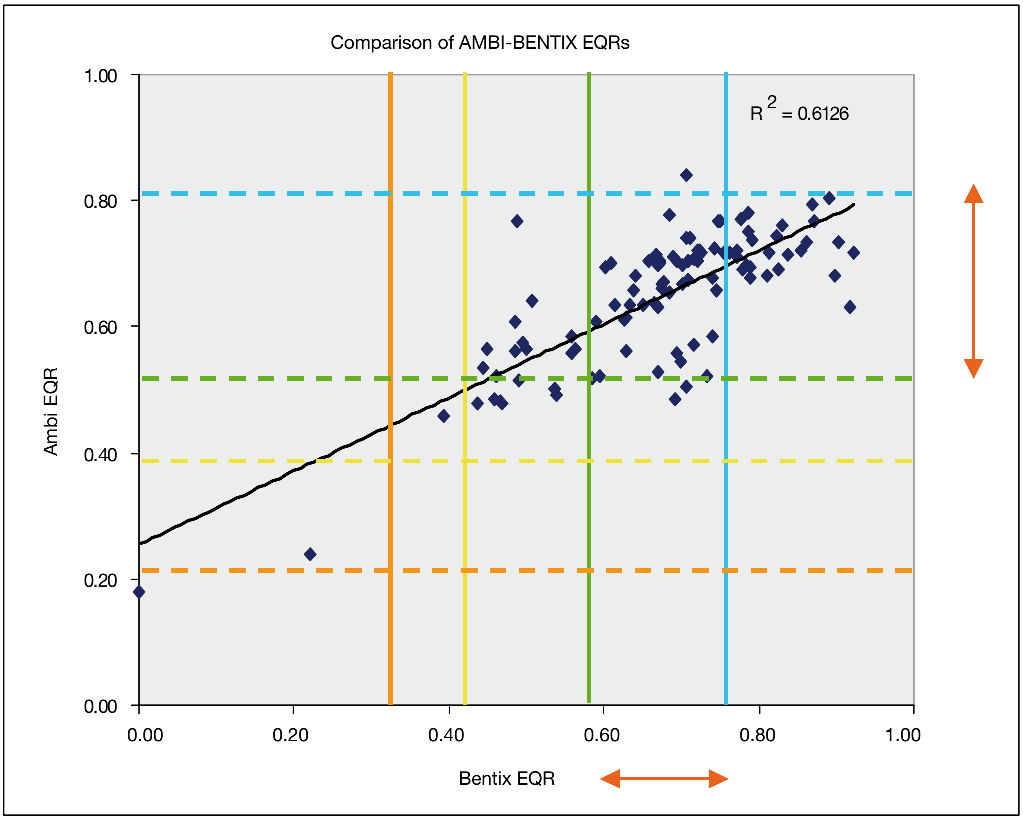


Figure 2.3.5: Comparison among the AMBI and BENTIX EQRs and class boundary thresholds.

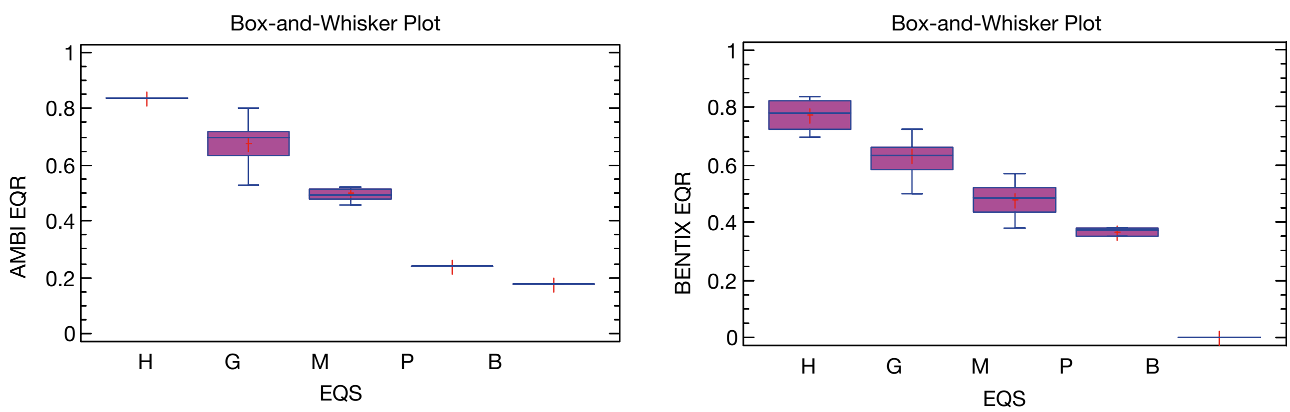


Figure 2.3.6: Box-and-Whisker plot of EQS classification results using AMBI and BENTIX on Greek IC data. For this comparison EQR=0 for Ambi=6 and Bentix=2.

m-AMBI - BENTIX

The Bentix was compared with the combined AMBI-Shannon Diversity-Species Richness multivariate method. In this method a factorial analysis of the three combined indices, using high and bad values for all indices determine the distance of each point-station from the two extremes (high and bad). The threshold values for the EQS classification (EQR determination) were based upon the REFCOND (non-intercalibrated values; Borja et al., 2007) and are referred below (Muxica et al., 2006).

For this comparison two sets of habitats was decided to be treated separately. This is because the indices of Shannon Diversity and species richness vary a lot depending on the type of habitat.

For this reason two main types of habitats were decided to be treated separately: the mixed and vegetated sediments and the purely muddy sediments which is considered a special kind of naturally stressed biotope where normally is inhabited by several tolerant species. Also the Bentix index renders some modifications in the class boundaries for the muddy bottom habitats.
Various sets of “reference” or “highest values” were tested for the indices to run the factor analyis. Finally it was decided to select the ones well above the maximum values encountered in real data. However there is some speculation to use these highest values as reference values in general, as these indices (diversity and species richness) depend much on specific conditions of the substrate, sampling size and methodology, seasonal cycles etc.
In the comparison of Bentix and m-Ambi EQRs the lowest EQR=0 was equated

with AMBI=6 and Bentix=2 because of the lack of numeric values in the area 0-7 for Ambi and 0-2 for Bentix.

a) mixed sediments

Highest values for H (=6) and S (=120) were selected above the maximum values from real data (reference sites) and refer to the standard unit of sampling surface (0.1 m^2) and the mixed sediments habitat. The variability explained by the data is high (69 %) and the agreement in the EQS assessment is moderate (kappa value-0.52), but in the limit with very good (Figure 2.3.7).

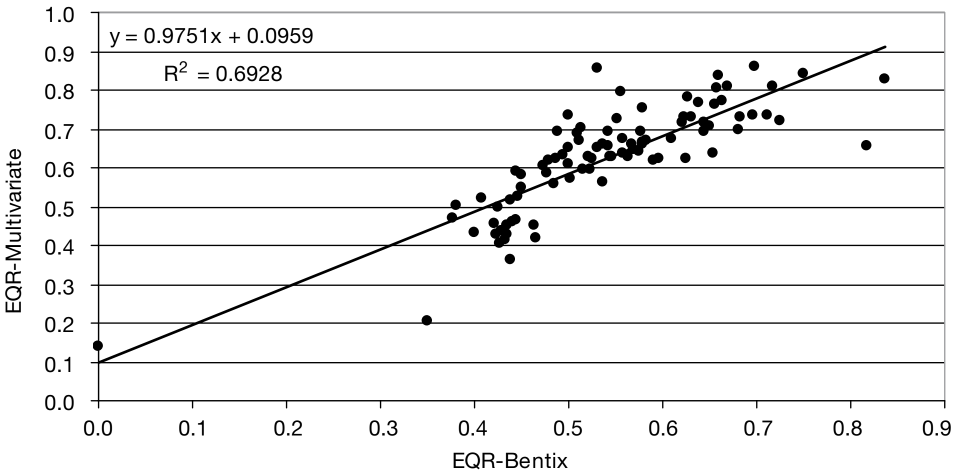


Figure 2.3.7: Correlation among m-AMBI and BENTIX methods’ EQRs in the mixed sediments habitat.

High and Bad values of the indices selected for the factor analysis in the mixed sediments analysis

	HIGH	BAD
H	6	0
S	120	0
AMBI	0	6

Status Class Boundary
REFCOND (non intercalibrated values)

EQS	EQR
B/P	0.2
P/M	0.41
M/G	0.62
G/H	0.83

b) purely muddy bottoms

In this type of habitat are considered those stations sampled in muddy bottoms with silt and clay or mud content of 85 % and over (according to Folk, 1954 classification scheme).

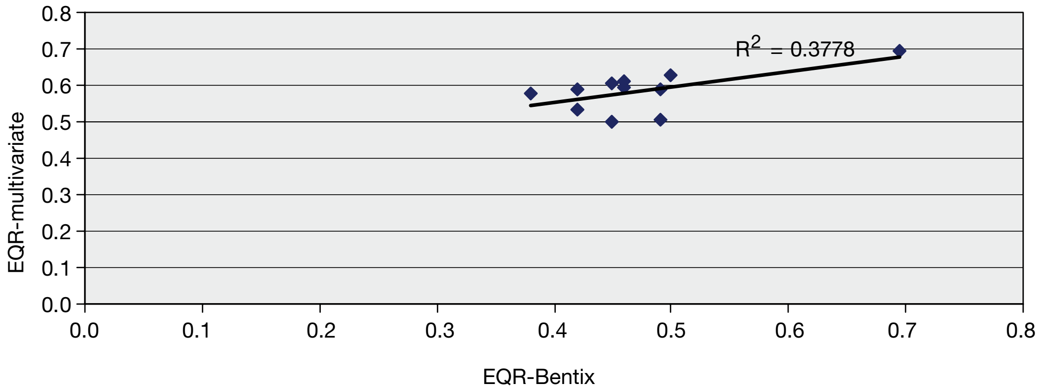


Figure 2.3.8: Correlation among m-AMBI and BENTIX methods' EQRs in the muddy sediments habitat.

High and Bad values of the indices selected for the factor analysis in the muddy sediments analysis

	HIGH	BAD
H	5	0
S	40	0
AMBI	0	6

Status Class Boundary
REFCOND (non intercalibrated values)

EQS	EQR
B/P	0.2
P/M	0.41
M/G	0.62
G/H	0.83

The agreement of m-AMBI and Bentix in this kind of habitat type is almost perfect (kappa value=0.98). The explained variability is moderate (40 %) because of the high aggregation of data in the moderate class. However, again care should be taken in the selection of high values for the diversity and species richness, which could only be considered as a highest threshold for reference conditions setting. Figure 9 shows an overall comparison of EcoQ classification results for all Greek data and separately for muds where different reference values for m-Ambi are used. The agreement between Bentix and m-Ambi is good, especially in the muddy habitats.

Figure 2.3.10 shows the comparison among indices in the Cyprus data. Ambi tends towards higher classes, m-Ambi towards lower classes and the agreement is better among Bentix and m-Ambi.

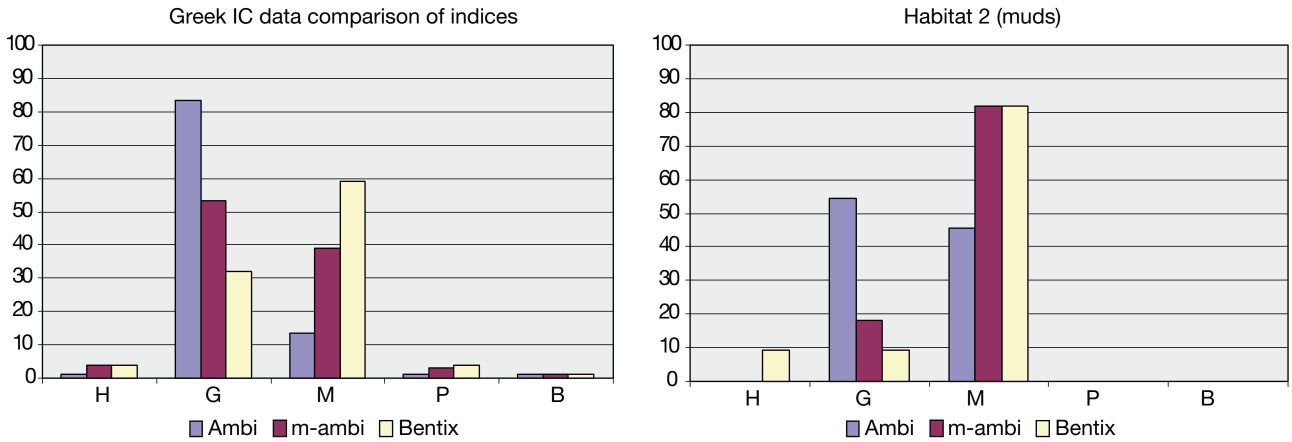


Figure 2.3.9: Relative frequency distribution of EcoQ status of all Greek data and separately for muddy habitat according to the various indices tested.

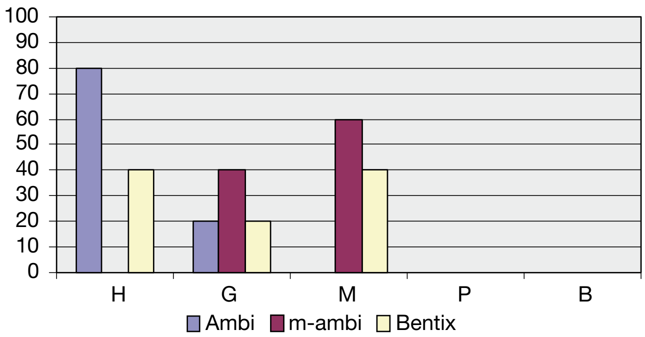


Figure 2.3.10: Relative frequency distribution of EcoQ status of Cyprus data.

FRANCE

The results here presented have to be considered only as an exercise. Data used for the intercalibration exercise was obtained using an identical methodology at all sampling stations:

5 samples per station

use of Van Venn or Smith Mac Intyre bucket

sample size 0.1 m²

sample depth between 20 and 35 metres (according to depth of Posidonia meadow lower limit).

The exercise was mainly based on datasets collected in Languedoc Roussillon by the ARAGO laboratory, situated in Banyuls. Corsican data was supplied by the STARESO station. The company CREOCEAN provided data acquired during numerous impact studies in Languedoc Roussillon. Data collected by CREOCEAN at reference sites (Languedoc Roussillon and Provence-Alpes-Côte d'Azur) during the 2005 cruise was also used (Fig. 2.3.11).

Indices tested in the framework of the intercalibration exercise

Each dataset was processed using H', AMBI, BENTHIX and TI (Trophic Index) index formulas as shown below:

A- Shannon-Weaver (H') Index

The Shannon-Weaver index is a diversity index taking into account both the number of species in a sample as well as their relative abundance, and is hence an efficient tool for assessing the ecological balance of ecosystem populations.

$$H' = -\sum \left[\frac{Ni}{N} \times \log 2 \frac{Ni}{N} \right]$$

Ni = number of individus belonging to the species i; N = total number of individuals per m²

A maximum Shannon index (H'max) is achieved when all species are found in equal numbers. A zero trend indicates that the population is dominated by a single species.

B- AZTI Marine Biotic Index (AMBI) & Biotic Index (BI)

The AMBI (AZTI Marine Biotic Index), also referred to as the benthic coefficient (BC), is based on ecological successions. The 5 ecological groups (GI, GII, GIII, GIV and GV) are defined according to species polluosensitivity. Group I comprises species which are the most sensitive to hypertrophication; group V comprises opportunistic species inhabiting reduced sediment. The reliability of this index also depends on the extent of sampling: if data is too limited, the same average value will be achieved but with a high standard deviation. It has the advantage of being applicable to all environments, as it is based on a predefined list of species.

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

%GI, %GII, %GIII, %GIV, %GV = relative abundance of various trophic groups versus total number.

AMBI values range from 0 to 6. A 0 value indicates an unpolluted environment, whereas a 6 value indicates major pollution and an azoïc environment.

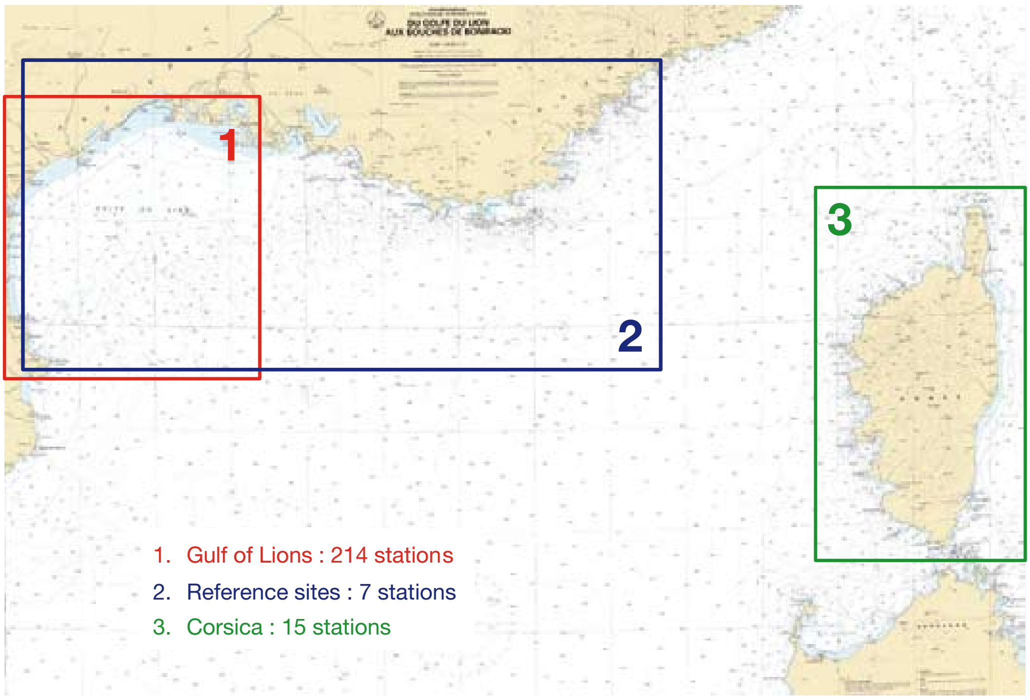


Figure 2.3.11: French Mediterranean coastal regions sampled during the intercalibration exercise on soft-substrate benthic populations.

C- Biological Quality Index (BQI)

The BQI index is used to evaluate the composition of biological communities and hence pinpoint environmental disturbances. BQI values are only valid for the target habitat and the maximum value measured in that particular habitat. Moreover, the reliability of this index depends largely on the extent of sampling. Extensive data is therefore needed to calculate the ES500,05[19] of each species and perform a reliable analysis.

$$BQI = \sum \left[\frac{n_i}{N} ES50_{0,05} \times \log(S+1) \right]$$

Ni = number of individus belonging to the species i; N = total number of individuals per m²; S: Number of species

The BQI index is mainly used to detect physical disturbances to the environment.

D- Trophic Index (TI)

This index assesses the feeding habits of species present in a sample on the basis of the ecological succession principle. Species are classified into 4 trophic groups (1: suspension feeders, 2: detritus feeders, 3: deposit feeders, 4: anaerobic substrate species).

$$IT = 100 - \frac{100 \sum \left(0.n_1 + 1.n_2 + 2.n_3 + 3.n_4\right)}{3N}$$

n1, n2, n3, n4 = number of species from trophic groups 1, 2, 3 and 4
N = total number of species

Index values range from 0 to 100. Values above 60 indicate a normal population, unaffected by sediment enrichment with organic matter. Values between 30 and 60 indicate a population imbalance , slightly affected by sediment enrichment with organic matter. Values below 30 highlight a disturbed population impacted by sediment deterioration due to excessive enrichment with organic matter. The Trophic Index is therefore used to pinpoint environmental disturbances of organic origin.

Using the values calculated at each station with the Shannon, AMBI, BQI and Trophic indices, we can establish ecological status class boundaries per index, as defined in the Water Framework Directive (“High”, “Good”, “Moderate”, “Poor” and “Bad”) (Tab. 2.3.3).

Results of the comparison

LANGUEDOC – ROUSSILLON REGION

Data from 214 stations was analyzed. Thanks to in-depth knowledge of the sampling sites, population counts and surveys conducted at each station, we were able to establish that 170 (79 %) were undeteriorated.

[19] **ES50**: represents the theoretical number of species obtained if this sample contained only 50 individuals. On the basis of this result, we can establish an ES50 distribution curve for this species according to the associated theoretical specific number. The first 5 % of this distribution are referred to as **ES50**$_{0.05}$.

Table 2.3.3: EcoQ values for the Shannon, AMBI, BQI and Trophic indices.

EcoQ	H'	AMBI	BQI Depth. < 20m	Depth. > 20m	
High	H' > 4	AMBI ≤ 1.2	BQI > 18.8	BQI > 26.4	IT > 80
Good	3 < H' ≤ 4	1.2 < AMBI ≤ 3.3	14.1 < BQI ≤ 18.8	19.8 < BQI ≤ 26.4	60 < IT ≤ 80
Moderate	2 < H' ≤ 3	3.3 < AMBI ≤ 4.3	9.4 < BQI ≤ 14.1	13.2 < BQI ≤ 19.8	50 < IT ≤ 60
Poor	1 < H' ≤ 2	4.3 < AMBI ≤ 5.5	4.7 < BQI ≤ 9.4	6.6 < BQI ≤ 13.2	30 < IT ≤ 50
Bad	H' < 1	5.5 < AMBI ≤ 6	BQI ≤ 4.7	BQI ≤ 6.6	IT ≤ 30

The Shannon Index (H') enabled an efficient discrimination of deteriorated stations, 89 % of which obtained a "Moderate" "Poor" or "Bad" score. Moreover, the majority of undeteriorated stations (86 %) achieved a good score.
The AMBI index did not enable discrimination and all stations were classified as "undeteriorated". We noted a drop in the index value at higher depths.
The BQI did not provide satisfactory discrimination between the various stations either. Although all deteriorated stations scored badly, so did half (46 %) of the undeteriorated stations.
The Trophic Index (TI) failed to identify 16 % of the deteriorated stations. The index value dropped at higher depths and 50 % of undeteriorated stations obtained a low score.

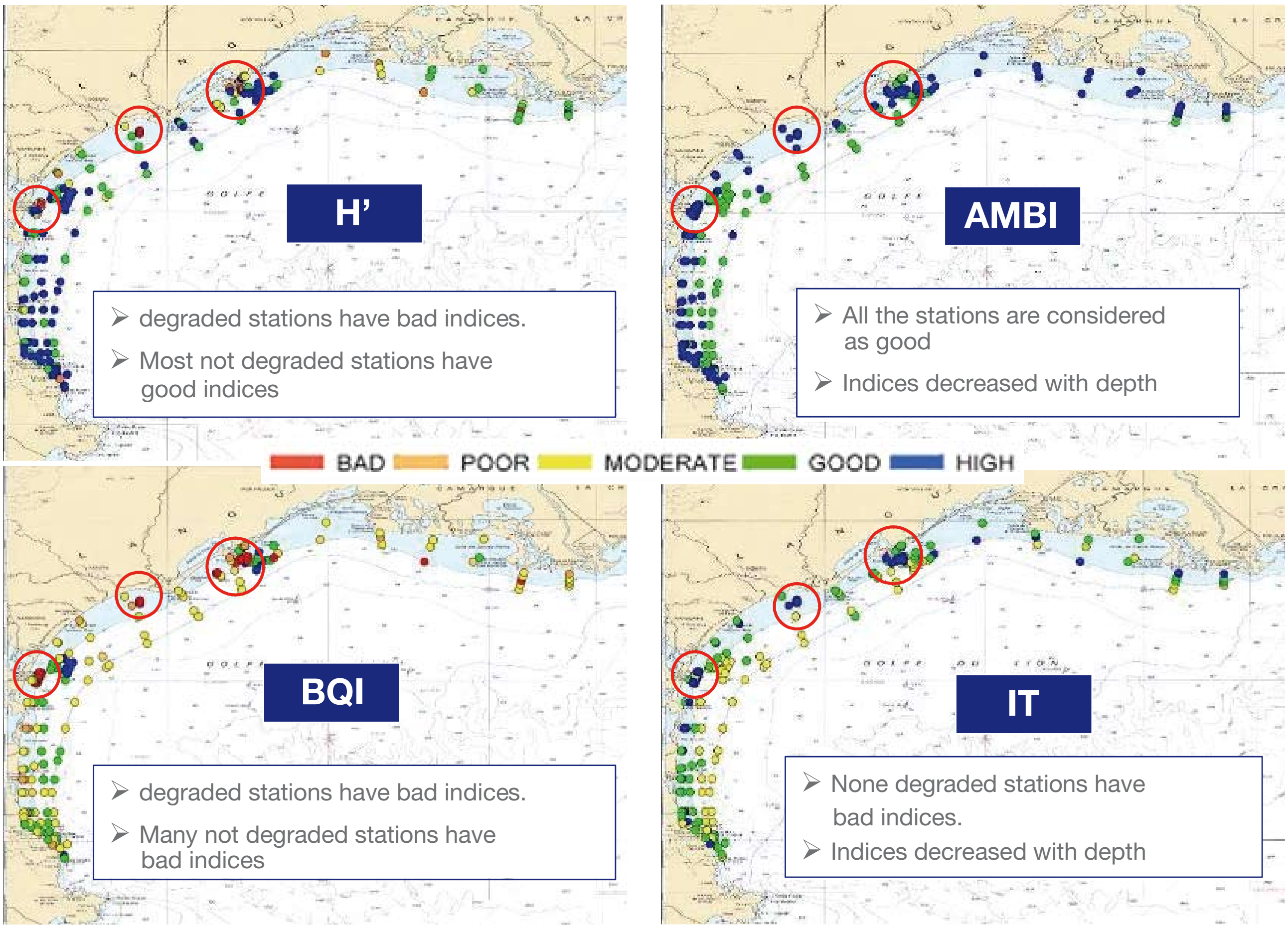


Figure 2.3.12: Comparison of Languedoc-Roussillon site classifications using the AMBI, Shannon-Weaver, BQI and Trophic indices.

Out of the 214 stations sampled in Languedoc Roussillon, 79 % were defined as having a “Good” or “High” status. Application of the various indices to the Languedoc Roussillon stations showed that the H’ index provided the best characterization of deteriorated and undeteriorated sites (Tab. 2.3.3)

Table 2.3.3: Comparison of scores obtained using each index versus actual impacts evaluated at Languedoc-Roussillon sites.

	Eco Q	
Biotic Indices	“High” or “Good”	“Moderate”, “Poor” or “Bad”
Actual Impact	79 %	21 %
Shannon (H’)	69 %	31 %
BQI	26 %	64 %
AMBI	100 %	0 %
TI	57 %	43 %

CORSICA

The dataset included 15 stations sampled for the last 5 years by STARESO.
Generally speaking, the sediment particles found around Corsica are fairly large, hence incurring low species densities. This particular characteristic impedes index reliability, in particular that of the Shannon (H)’ index which indicates a poor ecological status even in undeteriorated environments. We could circumvent this difficulty by calculating the EQRs of each reference site using major community-type sampling (Fig. 2.3.13).

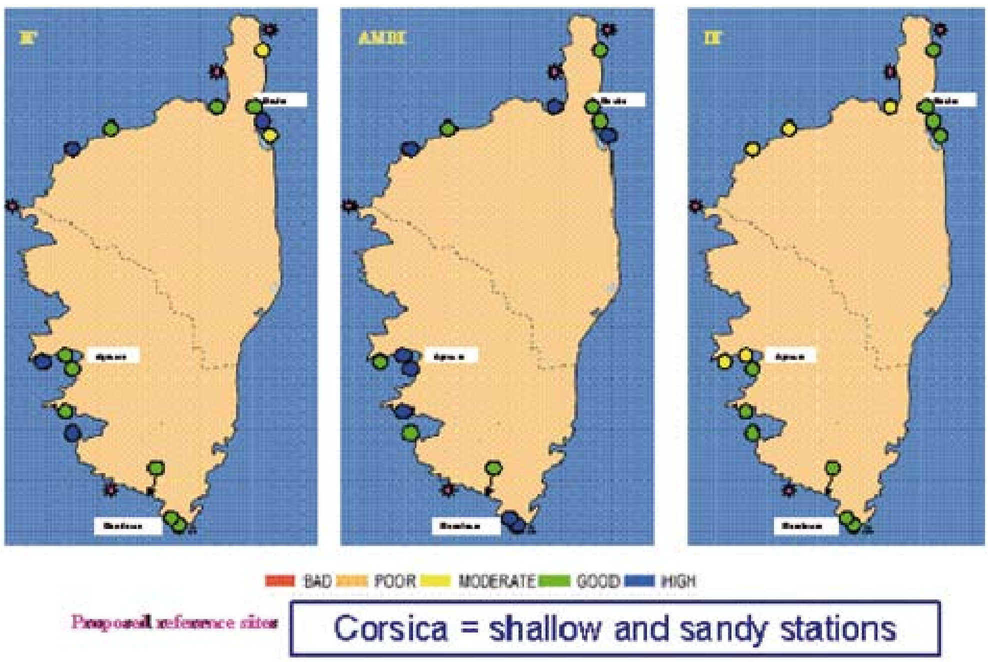


Figure 2.3.13: Application of H’, AMBI and TI indices for the classification of Corsican coastal sites.

Reference sites

7 stations corresponding to reference sites in Languedoc Roussillon (2 stations) and Provence-Alpes-Côte d'Azur (5 stations) were sampled during the 2005 cruise.
Overall, the sampled stations showed balanced populations, with relatively to very high biomasses and densities. Data processed using the H' and BQI indices gave "High" results at all stations; the AMBI index gave "Good" to "High" results and the Trophic Index gave "Moderate" to "Good" results (Fig. 2.3.14). Using BQI calculations, just 27 % of cruise target species were identified using the data collected from over 200 stations. If results obtained with the H' and BQI indices prove similar, the Shannon index would hence appear to be more efficient than the BQI
Analysis of the various results demonstrated that no single index is capable of characterizing environmental disturbances as a whole. None of the indices are exhaustive, and each has its pros and cons. Some are harder to implement than others. However, comparatively speaking, the Shannon index proved more efficient at highlighting environmental disturbances along French Mediterranean coasts.

The Shannon Index could usefully be associated with the AMBI and usual biological parameters (specific numbers, density and biomass).

The BQI requires relatively complicated calculations and an extensive database. The database used to obtain the BQI index (data from over 200 stations) resulted in the identification of just 157 species

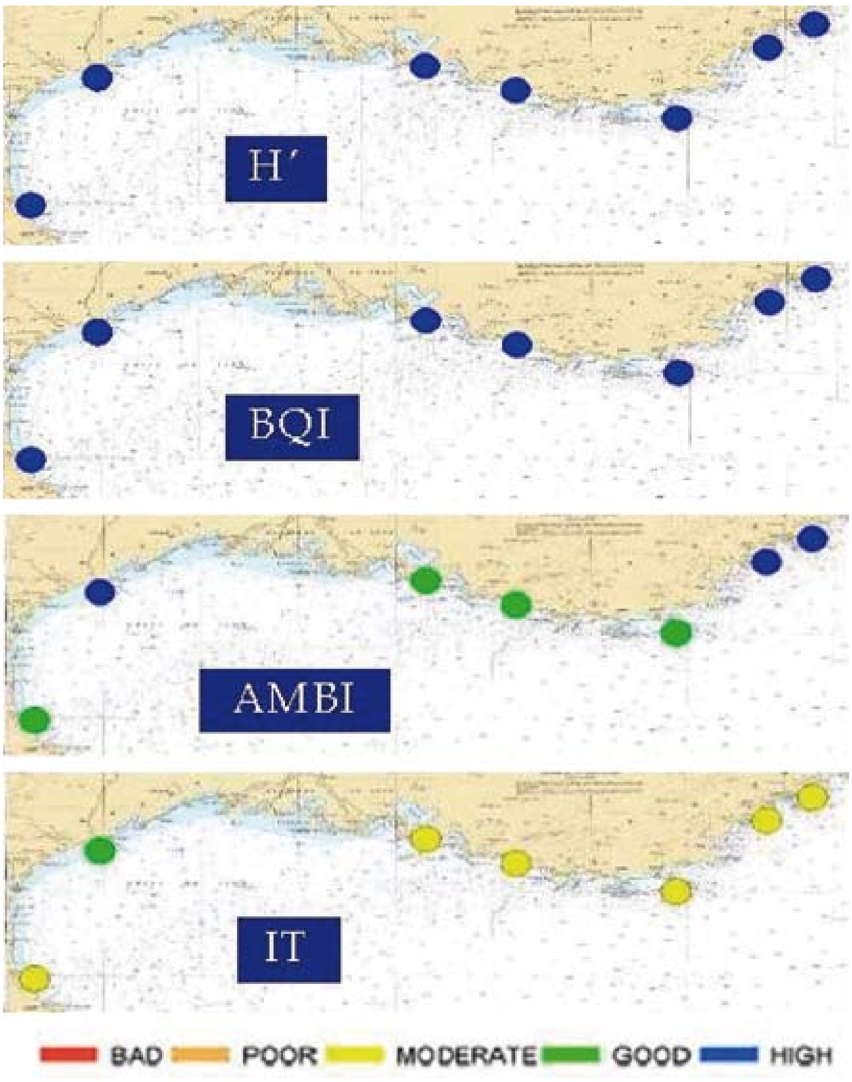


Figure 2.3.14: Classification of reference sites using the H', BQI, AMBI and TI indices.

out of the 586 identified at the reference sites during the cruise. In addition, the BQI and H’ (Fig. 2.3.15) are strongly correlated and are both structural indices, although H’ is very easy to calculate.

Although the TI and AMBI are generally associated (both are organic pollution indicators), they actually appear to differ in several ways due to the AMBI’s weaker range versus the TI, which is more penalizing that the AMBI.

Moreover, the results highlight a close relationship between particle size and population composition,, due to the fact that particle size defines the types of habitat available in the environment.

In view of the heterogeneous character of the sediment particles found in French Mediterranean waters (Languedoc-Roussillon, Provence, Corsica), the experts who took part in this intercalibration exercise recommended using stratified sampling per major community type (4 particle types) and per reference station (undisturbed zone). This would give us a reference notwithstanding the type of sample collected during monitoring and would also offer greater sampling flexibility (samples taken from the predominant substrate of monitored water masses), as, to all intents and purposes, it is very difficult to obtain samples with similar particle characteristics at all French Mediterranean coastal stations.

ITALY

Results presented here have to be considered only as an exercise which is based on the data available till now for the Italian coasts. Data were collected in 50 stations along 11 coastal Regions within the National Monitoring programme (2001-2006) of the Italian Ministry of Environment (still under evaluation).

Major reasons of concerns about this data set and the validation of the ecological classification obtained by their use are related to the following points:

– a high percentage of individuals not identify to species level is present in some samples;
– no chemical-physical parameters (oxygen, organic matter, nutrients, sediment grain size) are available for most of the stations. The chemical-physical parameters are fundamental to interpret the results and to understand the reliability of the quality indices tested.

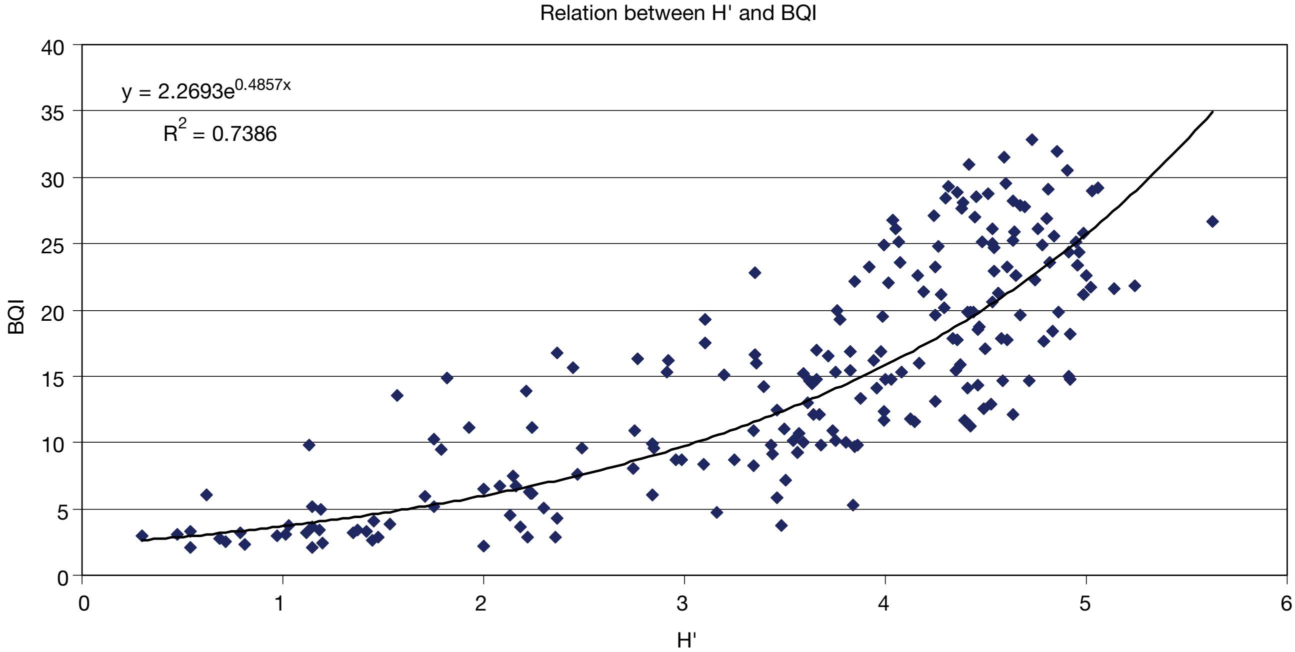


Figure 2.3.15: Correlations between the Shannon (H’) and BQI indices.

The a-priori classification of the intercalibration sites is in some case questionable.
No real reference sites exist.
Having this in mind, the Multimetric AMBI method (M-AMBI) (Muxika et al., 2007) with several reference conditions (RC) and boundaries, adapted to Italian data, were applied and tested. Three different approaches that give similar reference values for AMBI, richness (R) and diversity (H), and that provide the most reliable ecological status classification, are described below.

Reference conditions

The WFD identifies four options for determining reference conditions: 1) existing undisturbed sites, 2) historical data and information, 3) models, and 4) expert judgement. Option 3 and 4 were selected to define different reference values.

1. RC1

In this case, samples with individuals in ecological group I >70 % (sensu Borja et al., 2000) were selected. Then, among those only samples with R> 20, H> 2 were considered as reference. The high percentage of sensitive species is here considered more important than high R and H. The median value of AMBI, R and H in these samples, is used as reference . The mean or median value from the distribution of reference site values are considered the most robust values to be used as reference and relatively few data are needed for sufficient confidence in RC (Wallin et al., 2003).

RC1	AMBI	R	H
	0.5	30	4

2. RC2

In this second attempt, the reference values were calculating on all the data set. For this reason a higher percentile than the median (the 90th percentile) was used for R and H and a lower one (the 10th) for AMBI.

RC2	AMBI	R	H
	0.4	42	4

3. RC3

In this case the Intercalibration sites, which were a-priori classified in High/Good ES and the stations indicated by Regional Agencies as “control sites”, were considered as reference sites . In order to establish the reference values, not being sure of the a-priori classification of these sites, we have considered a higher (lower for AMBI) percentile than the median value: the 75th percentile for R and H, and to the 25th for AMBI.

RC3	AMBI	R	H
	0.8	33	3.3

Boundary Setting

Different boundaries were set on the basis of the different reference conditions considered (1, 2, 3).

1. The 10th percentile of EQR values obtained for the reference samples was selected as the “upper anchor” (Wallin et al., 2003) and the class boundary between high and good. The width of the four remaining classes was evenly spaced over the remaining interval. This has resulted in the following class boundaries.

1	High status	≥ 0.81
	Good status	≥ 0.61
	Moderate status	≥ 0.41
	Poor status	≥ 0.20
	Bad status	<0.20

2. The 75th percentile of EQR values (obtained by applying M-AMBI in all the samples) was selected as the "upper anchor" and the class boundary between high and good. The width of the four remaining classes was evenly spaced over the remaining interval. This has resulted in the following class boundaries.

2	High status	≥ 0.80
	Good status	≥ 0.60
	Moderate status	≥ 0.40
	Poor status	≥ 0.20
	Bad status	< 0.20

3. The 30th percentile of AMBI, R and H calculated in the reference sites has been selected as the "upper anchor" and the class boundary between high and good. The width of the four remaining classes has been evenly spaced over the remaining interval. This has resulted in the following class boundaries.

3	High status	≥ 0.78
	Good status	≥ 0.59
	Moderate status	≥ 0.39
	Poor status	≥ 0.20
	Bad status	< 0.20

Results of the comparison

M-AMBI options 1, 2, 3

Results obtained by the application of M-AMBI with the three different RC and boundaries are reported in the figure 2.3.15. Results of K-analyses are shown in table 2.3.4. M-AMBI applied following option 2, gives the most severe classification of the Italian sites with 19 % of the samples classified in moderate status; with option 3, only the 7 % of the sample results in moderate status. Despite these differences, the K-analyses shows very good agreement between the three methods.

Table 2.3.4: K values and agreement among Ecological status classification obtained by applying M-AMBI with different RC and boundaries (options 1, 2, 3).

	1	**2**	**3**
1		0.82 Very good	0.76 Very good
2	0.82 Very good		0.63 Good
3	0.76 Very good	0.63 Good	

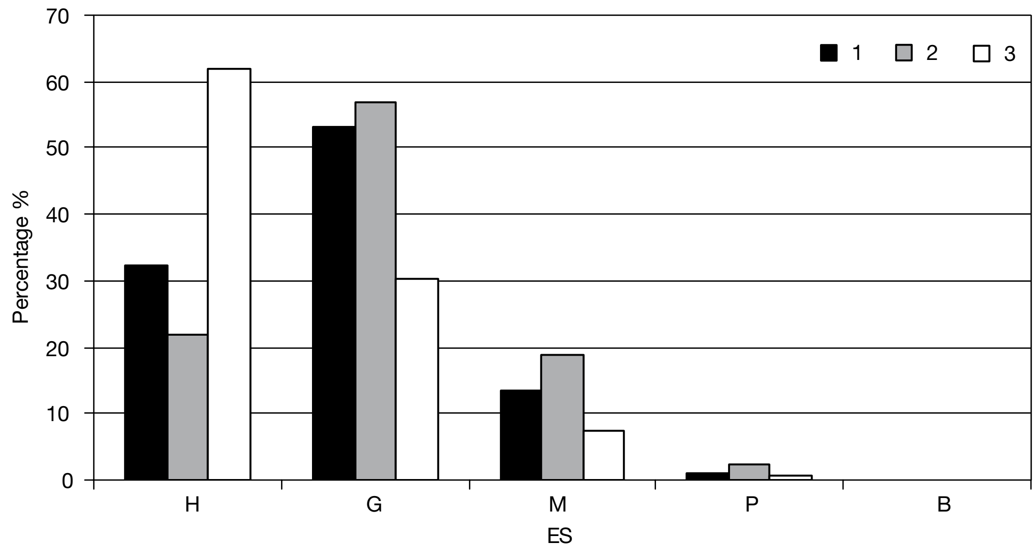


Figure 2.3.15: Percentage of samples classified in High, Good, Moderate, Poor, and Bad Ecological Status by applying M-AMBI with different RC and boundaries (options 1, 2, 3).

M-AMBI options 1, 2, 3/Bentix

Results obtained by applying M-AMBI with different RC and boundaries were compared with those obtained by Bentix Index; the different boundaries are reported in table 2.3.5. The Bentix EQR values for Italian data were calculated by Mika Simùboura.

Table 2.3.5: Boundaries values for Bentix and for M-AMBI (options 1, 2, 3).

Boundaries	Bentix	M-AMBI 1	M-AMBI 2	M-AMBI 3
H/G	0.75	0.81	0.80	0.78
G/M	0.58	0.61	0.60	0.59
M/P	0.42	0.41	0.40	0.39
P/B	0.33	0.20	0.20	0.20

Figure 2.3.16 shows, as an example, the relationship between Bentix EQR and M-AMBI 1 EQR (the M-AMBI option that gives results nearest to those obtained by Bentix). Only a low correlation exists; the major difference is about the H/G classification, the 32 % of the sample classified in high ecological status by Bentix result in Good ES for M-AMBI (Fig. 2.3.17).
Results of Kappa analysis (table 3) confirms low agreement between the two different Ecological Status classifications.

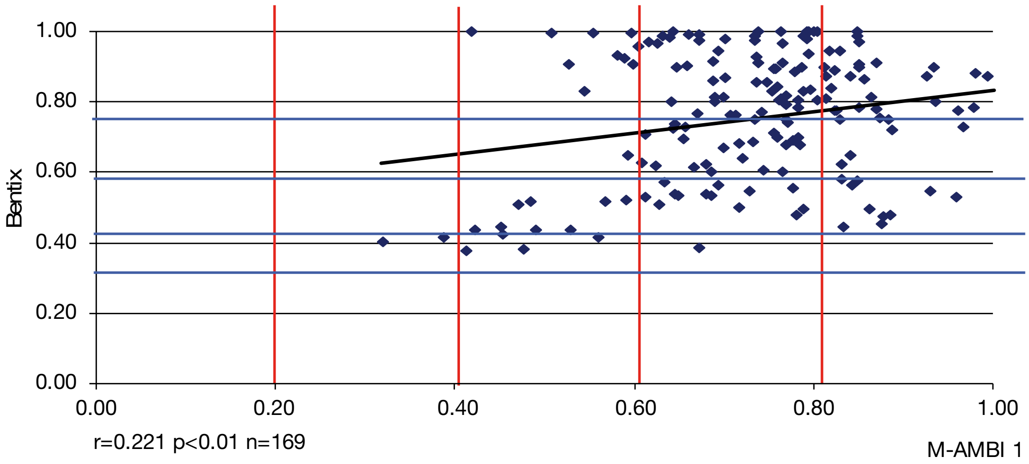


Figure 2.3.16: Correlation between bentix and M-AMBI 1. Vertical and horizontal lines indicate the boundaries.

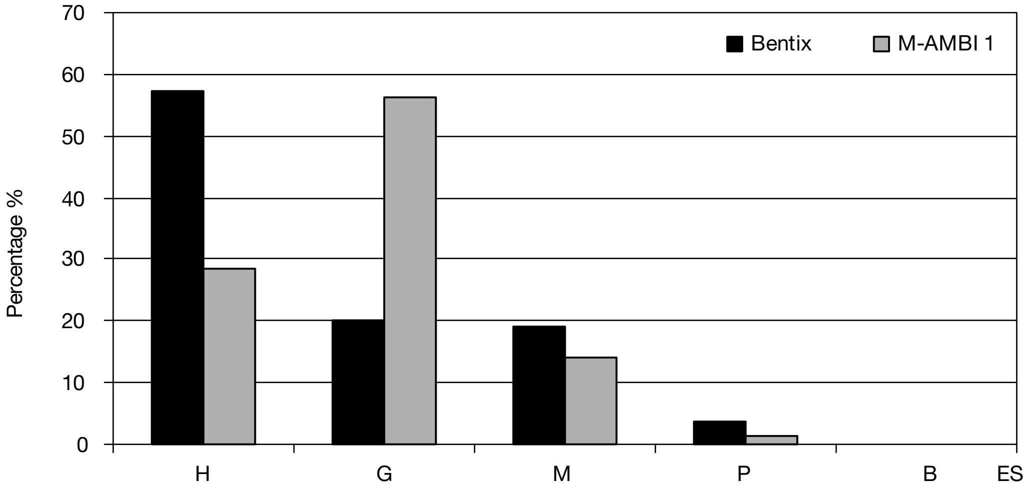


Figure 2.3.17: Percentage of samples classified in High, Good, Moderate, Poor, and Bad Ecological Status by applying Bentix and M-AMBI 1.

K values and agreement among Ecological status classification were obtained by applying M-AMBI with different RC and boundaries (options 1, 2, 3) and Bentix index

	1	2	3
Bentix	0.40 Low	0.30 Low	0.38 Low

SLOVENIA

Reference conditions

Slovenian reference conditions were set on expert judgement, since in the Slovenian sea and whole Gulf of Trieste there aren't any proper reference sites. Two sampling sites from the area with minimal known human impact (SD_VT2_P1 and SD_VT2_P2) were taken into consideration when setting the reference conditions. They were also compared with sites in the Italian part of the Gulf of Trieste and found as suitable. Averaging values from those two stations and adding 15 % on them have set the reference conditions.

SLOVENIA	
Metric	Reference condition
AMBI	1,3/6
Shannon-Wiener (H')	5,8
Richness(S)	110

Setting of Boundaries

Boundaries were determined according to expert judgement. This was the only possible method, since the Slovenian data were not equally distributed over the whole range of EQR values and due to the fact that more than one metrics has been used to calculate EQR.

SLOVENIAN BOUNDARIES	
H/G	0,83
G/M	0,62
M/P	0,41
P/B	0,20

Boundary between High and Good ecological class was set according to the reference conditions and natural variability. Natural variability defines width of High class. In our case natural variability is presumed to be around 20 %, meaning that upper and lower limit of High class differ for 20 %. H/G boundary (lower limit) was calculated by taking median from EQR values of the two stations used in calculating reference conditions (SD_VT2_P1 and SD_VT2_P2) and subtracting additional 5 % on this value. Subtracting of 5 % was done because median of our actual data lays 15 % from upper limit, so to get the lower limit, which differs from upper for 20 % this subtraction must be done.
Other boundaries were set equidistantly from the H/G boundary (0,83): between G/M on 0,62, between M/P on 0,41 and between P/B on 0,20.

Results of the comparison

M-AMBI/BENTIX

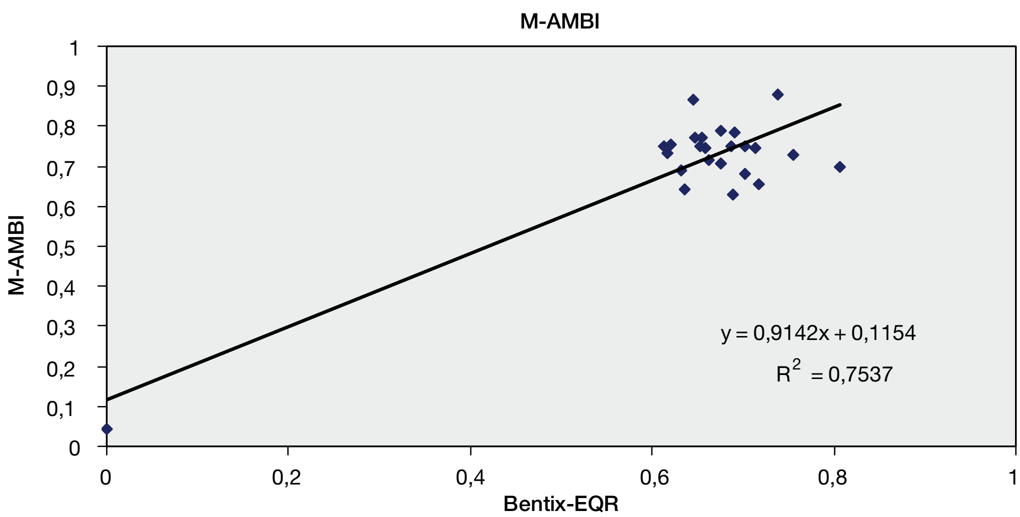


Figure 2.3.18: Correlation among EQR_M-AMBI (SI) and EQR_BENTIX (GR, CY) using Slovenian benthic invertebrate data.

Table 2.3.5: Agreement for ecological status analysis between the methods used in Slovenia (M-AMBI), Greece/Cyprus (Bentix).

	M-AMBI (SI)	BENTIX (GR, CY)
M-AMBI (SI)	1,00	
BENTIX (GR, CY)	0,83	1,00

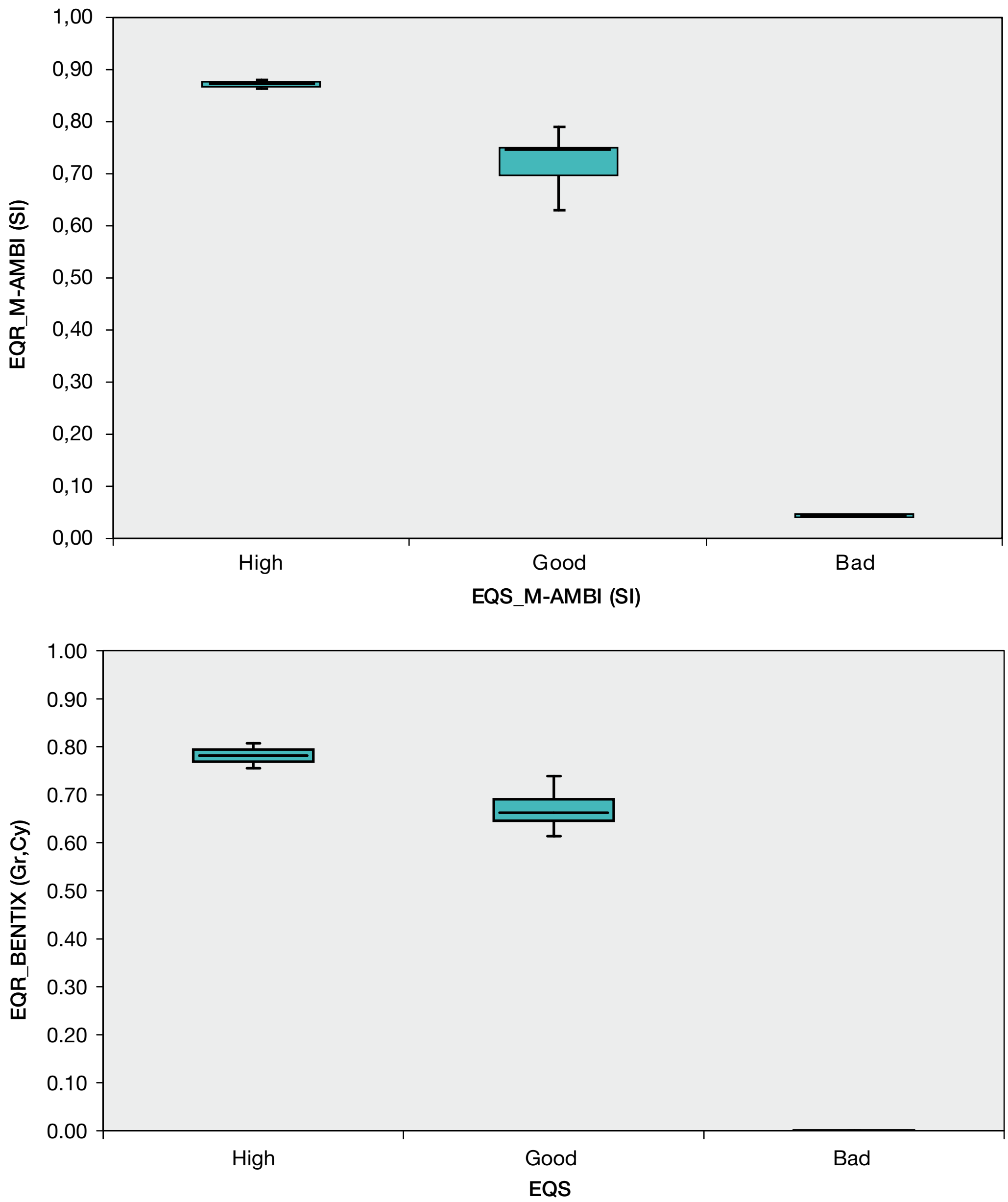


Figure 2.3.19 . Boxplot-and-Whisker plot (75 percentile) of EQS classification results using M-AMBI (SI) and BENTIX (GR, CY) on Slovenian IC data.

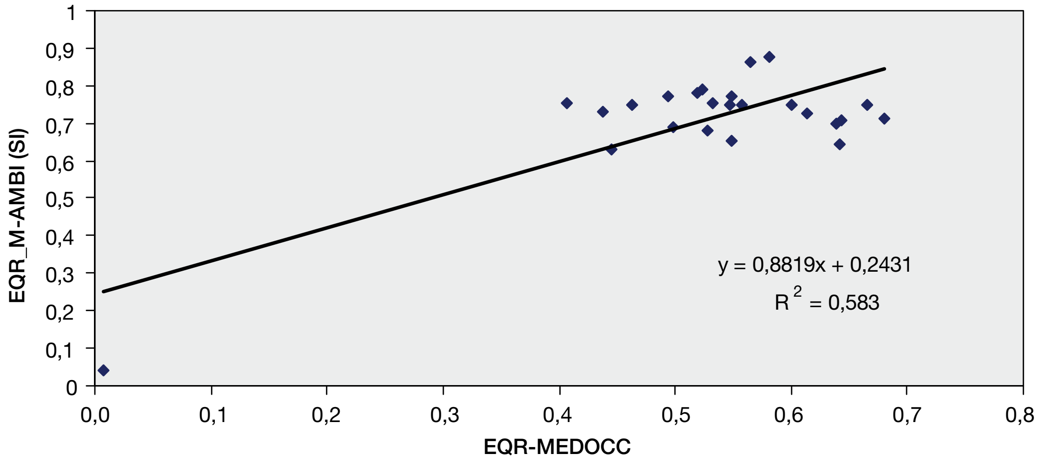


Figure 2.3.20: Correlation among EQR_M-AMBI (SI) and MEDOCC using Slovenian benthic invertebrate data.

Table 2.3.6: Agreement for ecological status analysis between the methods used in Slovenia (M-AMBI) and Spain (MEDOCC) (Slovenian benthic invertebrate data).

	M-AMBI (SI)	MEDOCC
M-AMBI (SI)	1,00	
MEDOCC	0,75	1,00

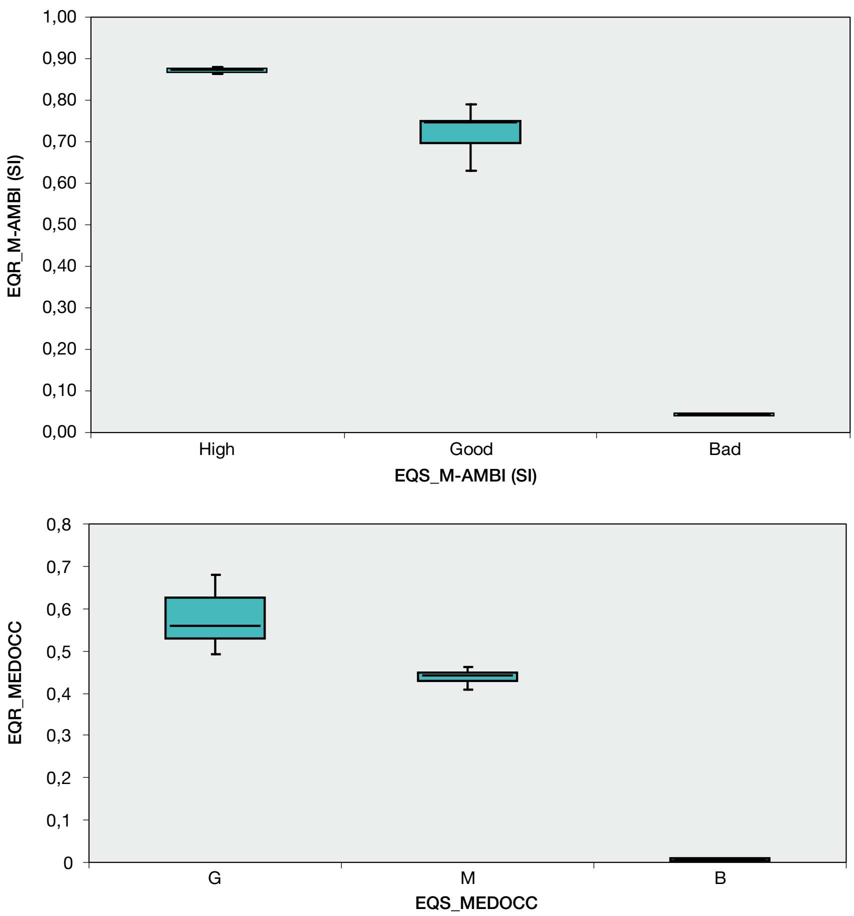


Figure 2.3.21: Boxplot-and-Whisker plot (75 percentile) of EQS classification results using M-AMBI (SI) and MEDOCC on Slovenian IC data.

SPAIN (Catalonia and Balearic Islands)

The MEDOCC index is an adaptation to the western Mediterranean area of the AMBI index developed for the Atlantic coast (Borja et al.,2000) based on sensitivity/tolerance of the species.

Species sensitivity

Species from the dataset have been assigned to one ecological group (EG) in relation to the sensitivity/tolerance of the species mainly to organic enrichment.
Ecological groups have been divided into four categories: sensitive, indifferent, tolerant and opportunistic species, based on available literature, AMBI list (http://www.azti.es), Simboura & Zenetos, (2002); http://www.hcmr.gr/english_site/services/env_aspects/bentix.html), Pinedo (1998) and expert judgment.

MEDOCC index

The MEDOCC index is able to detect organic enrichment following communities succession described by Pearson & Rosenberg (1978) and posterior authors. The main differences with the original AMBI method proposed by Borja et al. (2000) are the following:

Change in the categories of the ecological groups in some species. Although Borja has defended the idea of assigning species to the same ecological group uniquely according to the species' biogeographical latitudinal range, it can be considered that species react differently depending on inter-species interaction and environmental conditions (Rosenberg et al., 2004; Dauvin, 2007). In other way, assigning a species to different ecological groups according to the region could become subjective related to the experience and expertise of the scientist (Dauvin, 2007).

During the intercalibration exercise it was attempted to get an unanimous species scores assignation but we did not arrive to an agreement for some species; thus finally we consider the necessity of changing ecological groups in some species that respond differently to organic enrichment in the Mediterranean and the Atlantic. Carvalho et al. (2006) also suggest different EGs to those assigned in AMBI list for some species. In the following table we include only those species that change EGs comparing with AMBI list and species with no ecological group in AMBI list but assigned to one in MEDOCC index.

Table 2.3.7: Species changing the ecological group and species with no ecological group in AMBI list.

Species	MEDOCC	AMBI
Acanthocardia tubercula	2	1
Ampelisca typica	2	1
Ampharete sp.	2	1
AMPHARETIDAE	2	-
Amphipholis squamata	2	1
Amphiura chiajei	3	2
Anthura gracilis	2	1
Apseudes latreillei	2	3
Aricidea catherinae	2	1
Aricidea suecica	2	1
Bodotria pulchella	1	2
Caecum sp.	1	-
Capitellethus sp.	4	-
Cardium sp.	2	3
Corbula gibba	3	4
Corophium runcicorne	2	3
Cyclichna cylindracea	1	2
Chone longiseta	1	2
Diastilis rugosa	2	1
Euclymene collaris	3	1
Eurydice sp.	1	-
Exogone naidina	3	2
Exogone verugera	3	2
Fabriciola tonerella	4	2
Galathowenia oculeata	2	3
Glans sp.	1	-
Glycera oxycephala	1	2
Goniadella galaica	3	2

Species	MEDOCC	AMBI
Gouldia minima	2	1
Iphinoe trispinosa	2	1
Loripes lacteus	3	1
Lumbrineris latreilli	3	2
Magelona mirabilis	2	1
Marphysa sp.	3	2
Mastobranchus sp.	3	-
Micronephthys maryae	3	2
Pariambus typicus	2	3
Parvicardium ovale	2	1
Parvicardium exiguum	2	1
Parvicardium juvenil	2	1
Pectinaria koreni	2	4
Phtisica marina	2	1
Phyllodoce mucosa	2	3
Plagiocardium papillosum	2	1
Prionospio caspersi	2	4
Pseudolirius kroyeri	3	2
Retusa truncatula	1	2
Retusa umbilicata	1	2
Scolaricia typica	2	1
Scolelepis squamata	2	3
Scoloplos armiger	1	3
Sigalion mathildae	1	2
Sigalion squamosus	1	2
Spiochaetopterus solitarius	2	3
Tellina pulchella	2	1

Four ecological groups have been considered (instead of five in AMBI): sensitive (EGI), indifferent (EGII), tolerant (EGIII) and opportunistic species (EGIV). The reduction of the five EGs to four is proposed as a way to avoid errors in assignation of species to the first or second-order opportunists EG. At the current level of knowledge, we are not able to distinguish between both order of response to disturbance.

The calculation of the index is as following

MEDOCC= **[(0 x % EGI + 2 x %EGII + 4 x % EGIII +6 x % E GIV)]/100**

Index response to the impact gradient

For the development of this methodology to asses the ecological status and to obtain the boundaries required by the WFD we have analysed samples from fine to muddy fine sediments from Catalonia and Balearic Islands. Although the communities' composition in terms of ecological groups is expected to respond to the extent of anthropogenic pressure (sensu lato), this method is developed to detect mainly organic enrichment. Plotting the MEDOCC results from our data with organic matter content in sediment, MEDOCC index shows a significant linear relationship with the pressure gradient (Figure 2.3.22).

Reference conditions and class boundary setting (Catalonia and Balearic Islands, SPAIN)

Reference conditions

One of the problems in deriving reference conditions arises from the absence of unimpacted areas in some European regions. The WFD identifies four options for determinig reference conditions: 1) existing undisturbed sites, 2) historical data and information, 3) models, and 4) expert judgement. Option 3 and 4 have been selected to define reference sites for Catalonia and Balearic Islands, thus selecting the best situation (sample) of our dataset where the most species belong to EGI (sensitive species) and EGII (indiferent species). After choosing this "best of all" situation from our dataset, we have excluded all tolerant (EGIII) and opportunistic species (EGIV) and created a new theoretical situation where the fauna is composed of only sensitive (EGI: 90 %) and indifferent species (EGII: 10 %).

Boundary Setting

Class boundary values are obtained by checking community composition of sensitive/tolerant species over the MEDOCC index for the dataset (Catalonia and Balearic Islands dataset). Figure 2 shows the plotting of the percentage of all EGs considered along the gradient of increasing MEDOCC index in Catalonia and Balearic Islands. The sequence of quality classes and class boundaries are interpreted in terms of shifts of ecological group percentages.

The relation between MEDOCC values and the ecological status classification of the WFD is based on the ecological theory describing the communities succession in an increasing disturbance gradient followed by Glémarec & Hily (1981), Hily (1984), Majeed (1987), Grall & Glémarec (1997), and Borja et al. (2000).
The five levels of ecological status in the WFD are described as below:

High status: "The level of diversity and abundance of invertebrate taxa is within the range normally associated with undisturbed conditions. All the disturbance-sensitive taxa associated

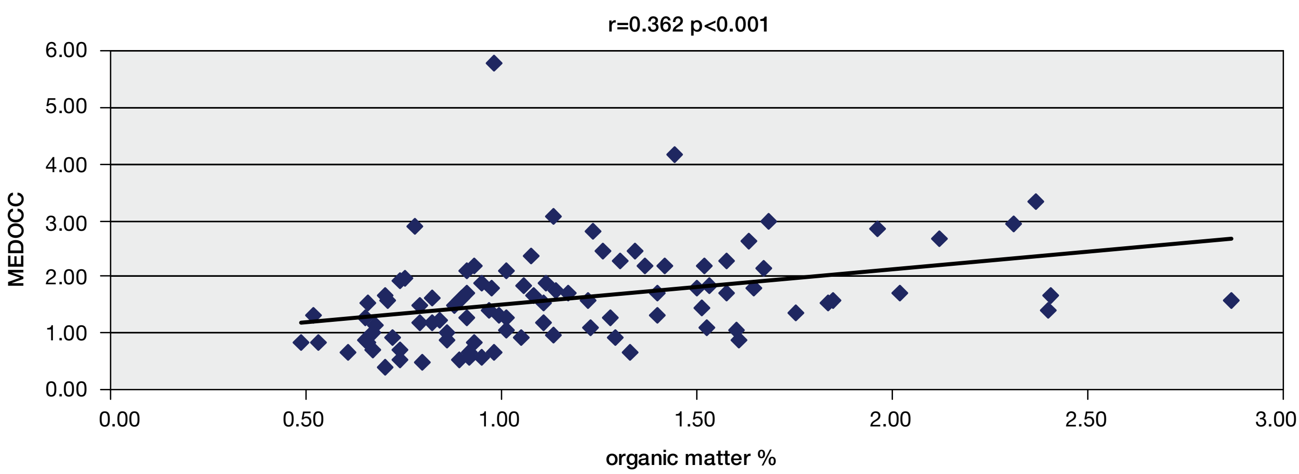


Figure 2.3.22: Pearson correlation of MEDOCC index in Catalonia and Balearic Islands with organic matter (%) in sediments.

with undisturbed conditions are present". This could be associated to normal or unpolluted benthic community, dominated by the Ecological Group I. We propose the border between high and good ecological status for MEDOCC equal to 1.6; that is, sensitive ecological group (EGI) accounting for more than 40 % of total abundances.

Good status: "The level of diversity and abundance of invertebrate taxa is slightly outside the range associated with the type-specific conditions. Most of the sensitive taxa of the type-specific communities are present". This could be associated to unbalanced or slightly polluted benthic community, dominated by the Ecological Group III. The border between good and moderate ecological status is 3.2 for MEDOCC index; in this case tolerant ecological group (EGIII) accounts for 20-50 %, but sensitive taxa (EGI) are also present (10-40 %).
Moderate status: "The level of diversity and abundance of invertebrate taxa is moderately outside the range associated with the type-specific conditions. Taxa indicative of pollution are present". This could be associated to a transitional benthic community, dominated by the Ecological Group III and IV. We propose that tolerant ecological group (EGIII) accounting for nearly 50 % and opportunistic taxa accounting less than 45 %. The border value between moderate and poor ES is 4.77 for MEDOCC.
Poor status: "… the relevant biological communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions". This could be associated to polluted areas, dominated by the Ecological Group IV but still with some presence of species belonging to the ESIII. We propose that the opportunistic ecological group (EGIV) accounts for more than 45 %. The border between poor and bad ecological status is 5.5 for MEDOCC index.
Bad status: "… In which large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent". It includes areas heavily polluted and azoic sediments. We propose that opportunistic ecological group (EGIV) accounting for more than 80 %.

Thus, the ranges of the MEDOCC index and Boundaries obtained for Catalonia and Balearic Islands data following the ecological theory are showed in Table 2.3.8. Boundaries are obtained rescaling MEDOCC values (between 0-6) from 1 to 0.

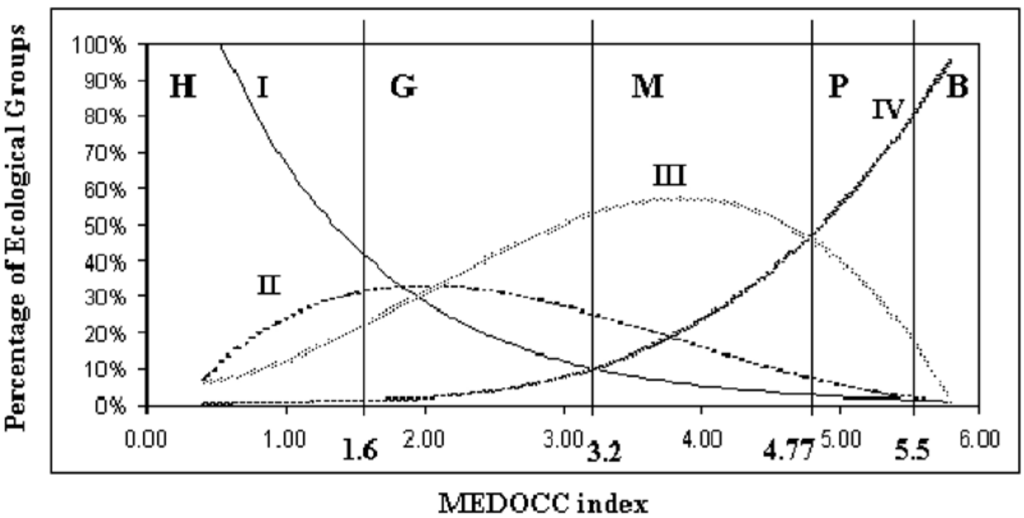


Figure 2.3.23: MEDOCC values relating to the percentages of the ecological groups for the Catalonia and Balearic Islands dataset. Vertical lines show boundaries of the different ecological status. H: High ecological status; G: good ES; M: moderate ES; P: poor ES; and B: bad ES. I: Sensitive species; II: indifferent species; III: tolerant species; IV: opportunistic species.

Table 2.3.8: MEDOCC index and EQRs obtained for Catalonia and Balearic Islands.

Ecological Status	**MEDOCC values (6-0)**	**Boundaries (0-1)**
High	(0<MEDOCC<1.6)	0.73
Good	(1.6<MEDOCC<3.2)	0.47
Moderate	(3.2<MEDOCC<4.77)	0.20
Poor	(4.77<MEDOCC<5.5)	0.08
Bad	(5.5<MEDOCC>6)	0

It is important to note that the assessment of boundaries is an intrinsic part on the development of the MEDOCC methodology. This method is aimed at describing and suggesting the way to establish boundaries for ecological status classes based on the composition of the communities in terms of ecological groups, instead of proposing fixed values for the boundaries between each ecological status. In short, the important part of the method described above is the possibility of creating the relative abundance curves of each EG along a increasing gradient of MEDOCC index adapted to a particular dataset, and delimitate boundaries based on the dominance of EGs, as stated above. In fact for the macroinvertebrates communities from Catalonia and Balearic Islands the distribution of ecological groups over the MEDOCC index (Figure 2.3.22) can not be compared with those obtained for Atlantic waters over the AMBI values (Borja et al., 2000).
The major differences are due to the distribution of species belonging to ecological group III (tolerant species). In the case of Catalonia and Balearic Island tolerant species are more associated to the presence of opportunistic species (EGIV) whereas in Atlantic waters these group of species are more associated to the presence of sensitive taxa (EGI). That means that if we take into account the boundaries considered for Atlantic communities, the "good" ecological status would be dominated by EGIII and EGIV. Those conditions do not agree with the levels of ecological status described in the WFD.

The same pattern of distribution of the ecological groups has been observed for other dataset in the Mediterranean ecoregion (Italia, Slovenia) during the Intercalibration exercise. So, due to differences in benthic assemblages in terms of ecological groups we consider that the boundaries proposed by AMBI index are not applicable to the Catalonia and Balearic Island communities and probably, to other Mediterranean areas.

The EQR values (Table 2) were calculated by rescaling MEDOCC values (between 0-6) from 1 (high) to 0 (bad) and taking into account the reference condition situation. That is, with a reference condition corresponding to a situation where the faunistic assemblage is composed of 90 % of sensitive (EGI) and 10 % of indifferent species (EGII) we obtain a MEDOOC value equal to 0.2. This means that after considering the reference condition, the MEDOCC values for each station have to be corrected by this value. Therefore, after considering the reference condition the MEDOCC values ranges from 0.2 to 6. (Example: MEDOCC value=3.2; MEDOCC corrected by Reference conditions, 3.2-0.2=3.0; EQR (from 0 as High ES to 1 as Bad ES)=3.0/5.8=0.52; EQR (from 0 as Bad ES to 1 as High ES, following WFD)=0.48; Ecological Status=Good).

Results of the comparison

We have run BENTIX and M-AMBI (combination of three indexes: AMBI-Shannon Diversity-Species Richness) in our data applying boundaries established by each method. We then compare the ecological status obtained with MEDOCC with those obtained from the other methods allowing to us to know the percentage of agreement.

BENTIX/MEDOCC

The MEDOCC was compared with BENTIX. The EQR values for our data applying BENTIX index were obtained by Mika Simboura. The BENTIX limits between ES were selected from the boundary setting protocol elaborated by Greece (Table 2.3.9). Figure 2.3.24 shows a linear relationship statistically significant ($r=0.714$; $p<0.001$) between both EQRs values. The lines in the plot define the ranges of each ecological status by the boundary limits of each method.

Table 2.3.9: Ecological Status boundaries for MEDOCC and BENTIX methods.

EQS	Boundary EQR_MEDOCC	Boundary EQR_BENTIX
H-G	0.73	0.75
G-M	0.47	0.58
M-P	0.20	0.42
P-B	0.08	0.33

Figure 2.3.24 shows the relationship between MEDOCC and BENTIX. The 52 % of the samples show an agreement in the Ecological Status (shady areas in the Figure 3). The most significant differences are related to the H/G Ecological status. The 33 % of the stations with "high" ES by one method obtains "good" ES for the other. Anyway, the number of stations in critical situation, thus is "moderate", "poor", and "bad" ES for one of the methods and "high" or "good" for the other is low: 12.4 %. The main problem is that using the "H-G" boundary of BENTIX (Table 2.3.9, EQR= 0.75) in our dataset we are considering that "high" ES accounts for more than 80 % of sensitive and indifferent species. A community with this dominance of species does not agree with the definition of "high" ES according BENTIX (see BENTIX description in this final report) which is taking into account more than 60 % of sensitive and indifferent species to classify a site as "high" ES. When applying BENTIX index in Catalonia and Balearic Islands, if we want to consider "high" ES following BENTIX considerations, the EQR value should be lower than 0.75. Changing the EQR boundary for H-G ES would improve the agreement. Figure 2.3.25 shows the comparison of Ecological Status classification results for Catalonia and Balearic Islands with MEDOCC and BENTIX index.

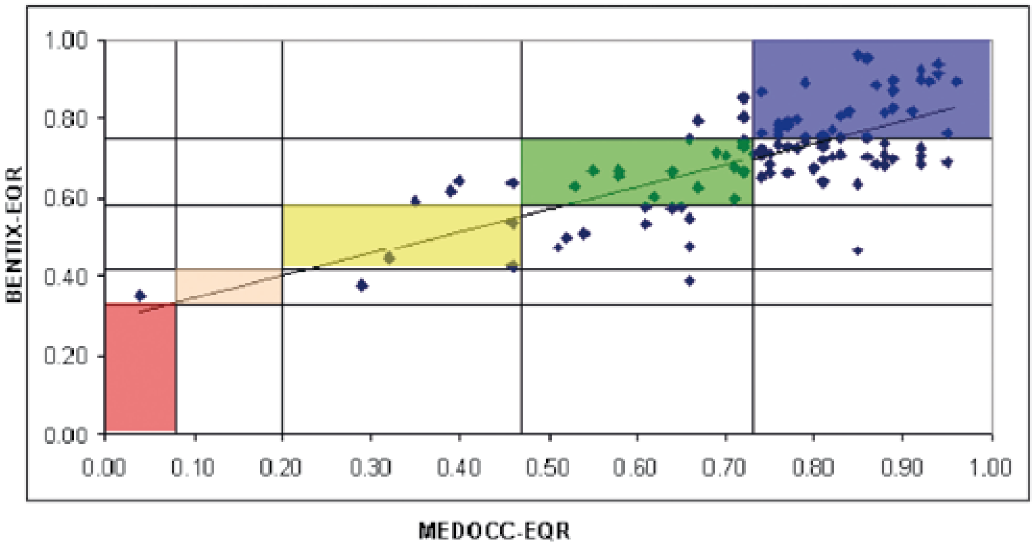


Figure 2.3.24: Comparison between BENTIX and MEDOCC EQRs with the class boundaries. Shady areas show the space where the two methods have the same status classification.

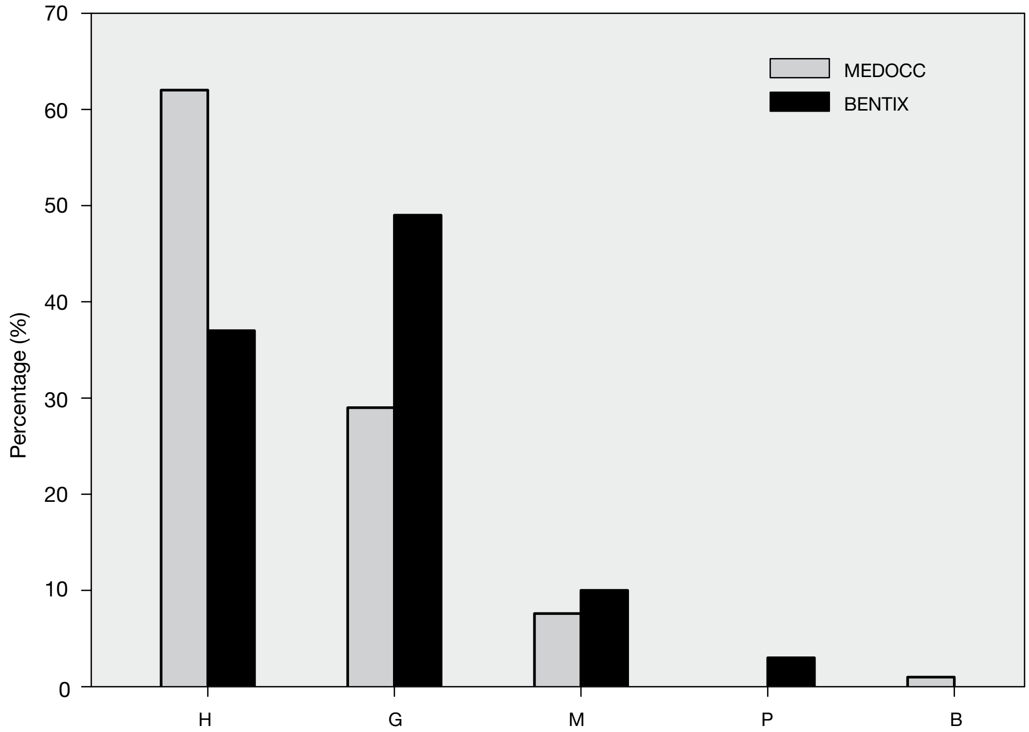


Figure 2.3.25: Relative frequency distribution of Ecological Status (High, Good, Moderate, Poor, and Bad) of Catalonia and Balearic Islands data with BENTIX and MEDOCC indexes.

M-AMBI/MEDOCC

The MEDOCC was compared with M-AMBI. M-AMBI uses a factorial analysis to determine the distance of each station from the two extreme situations (reference conditions bad and high). Various sets of reference conditions were tested for highest values. Finally we decided to select the maximum values encountered in real data, as it was chosen in MEDOCC index. The highest values were: AMBI= 0.14; R=100; H'=5.54. Bad reference conditions were: AMBI=6; R=0; H'=0.

The M-AMBI limits between ES were selected from Borja et al. (2007) (Table 2.3.10). The two indices were compared showing a linear relationship statistically significant ($r=0.517$; $p<0.001$) in Figure 2.3.26. The lines in the plot define the ranges of each ecological status by the boundary limits of each method.

Figure 2.3.26 shows the relationship between MEDOCC and M-AMBI. The 34 % of the samples show an agreement in the Ecological Status (shady areas in the Figure 2.3.26). The most significant differences are related again to the H/G Ecological status. The 59 % of the stations have "high" ES

Table 2.3.10: Ecological Status boundaries for MEDOCC and M-AMBI methods.

EQS	Boundary EQR_MEDOCC	Boundary EQR_M-AMBI
H-G	0.73	0.85
G-M	0.47	0.55
M-P	0.20	0.39
P-B	0.08	0.20

with one index but "good" ES for the other. Anyway, the number of stations in critical situation, thus is is "moderate", "poor", and "bad" ES for one of the methods and "high" or "good" for the other is low: 6.7 %. Figure 2.3.27 shows the comparison of Ecological Status classification results for Catalonia and Balearic Islands with MEDOCC and M-AMBI index.

We believe that the slight relationship between both methods could be related mainly to the assignation of the boundaries, which, as we have explained above, they have to be obtained from each dataset depending on the evolution of the ecological groups considered. This disagreement is understandable if we observe the boundary for "H-G" ES considered for M-AMBI in Table 2.3.10. Selecting an elevated EQR value for H-G ES (=0.85) enhance the high number of stations classified as "good" for M-AMBI in the Figure 5; this result is the main responsible of the disagreement.

Anyway, we also think that the inclusion in the multivariate method of the diversity index and species richness worsen the results, as those indexes change depending on other environmental variables and they are not necessarily related to the degree of habitat disturbance. As an example,

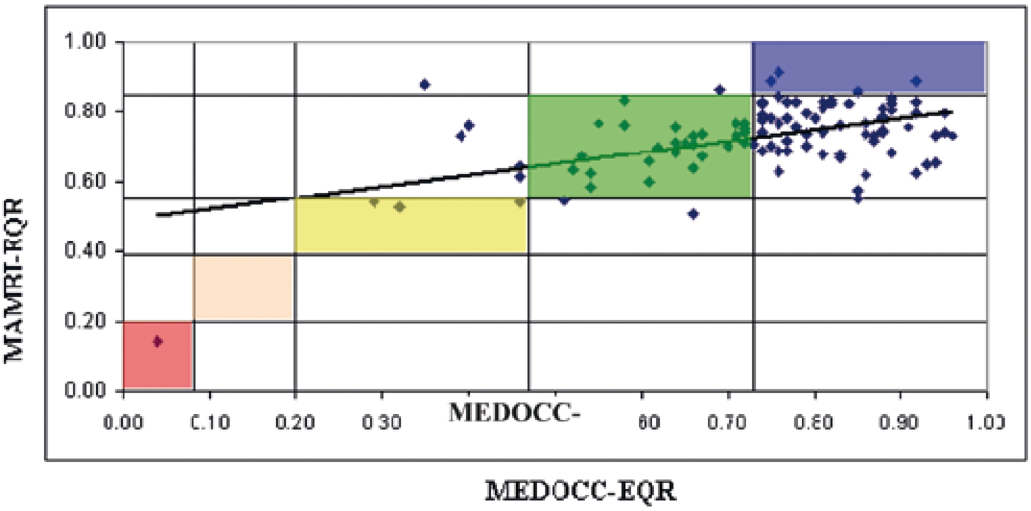


Figure 2.3.24: Comparison between MAMBI and MEDOCC EQRs with the class boundaries. Shady areas show the space where the two methods have the same status classification.

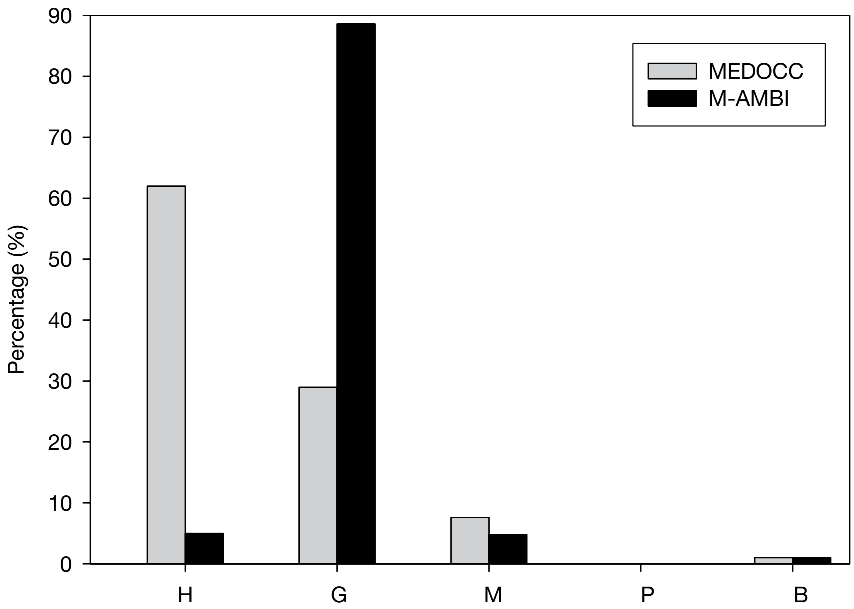


Figure 2.3.27: Relative frequency distribution of Ecological Status of Catalonia and Balearic Islands data with M-AMBI and MEDOCC indexes.

the station with “bad” ES classification using both methods shows similar values in H’ and species richness to that of other stations classified as “high” ES.

2.3.4 Results of the comparison

Each country has tested the different methods with their own data applying boundaries established by each method. Moreover, they compared the ecological status obtained with the different methods in order to know the percentage of agreement. Results are described, for each country.

2.3.5 Results of the harmonisation – Boundary EQR values

Member State	Method	Boundary High/Good (EQR)	Boundary Good/Moderate (EQR)
Cyprus	Bentix	0.75	0.58
France	Multimetric approach (AMBI, Shannon Diversity, BQI Trophic Index)		
Greece	Bentix	0.75	0.58
Italy	AMBI with factor analysis Bentix		
Slovenia	M-AMBI (AMBI, Shannon Diversity, S)	0,83	0,62
Spain - Catalunya	MEDOCC index	0.73	0.47
Spain-Balearic is.	MEDOCC index	0.73	0.47

2.3.6 Open issues and need for further work

For all: need to consider different sub-regions (eastern, western Med), and to develop analysing tools (multimetric indices) for hard bottom substrates.

Still some comparison/harmonization between methods should be done.

For Italy: Application of MEDOCC methods to Italian data, in collaboration with Spanish colleagues. In the attempt of taking into account the variability of Italian coastal areas, a classification of the benthic invertebrates stations using the same method used by the MED GIG phytoplankton group for typologies will be tested. Coastal sites are characterized according to different freshwater influence: Type 1, coastal sites highly influenced by freshwater inputs; Type 2, coastal sites not directly affected by freshwater inputs; Type 3, coastal sites not affected by freshwater inputs. Development of RC for each of these typologies will be assessed.

For Spain: Future work has to be focussed in the harmonization of boundaries. Although a comparison of the results of the ecological status with the different methodologies has been performed, the boundaries obtained by each member state have not been harmonized to get a better agreement. The base of this exercise must to be focused on the agreement around the definition of the conditions of the different Ecological Status.

2 countries (France and Italy) are working on the methods to be used as national: either adopting one method used in other countries or developing their own.

2.4 NE Atlantic GIG

2.4.1 Intercalibration approach

In the NE Atlantic seven basic intercalibration types have been agreed. These are shown in table 2.4.1 below:

Table 2.4.1: NEA GIG Intercalibration Types.

New Type ID	Name	Salinity	Tidal range (m)	Depth (m)	Current velocity (knots)	Exposure	Mixing	Residence time
CW –NEA1/26	Exposed or sheltered, euhaline, shallow	Fully saline (> 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3)	Exposed or sheltered	Fully mixed	Days
CW – NEA3/4	Polyhaline, exposed or moderately exposed (Wadden Sea type)	Polyhaline (18 - 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3)	Exposed or moderately exposed	Fully mixed	Days
CW – NEA7	Deep, low current, sheltered	Fully saline (> 30)	Mesotidal (1 - 5)	Deep (> 30)	Low (< 1)	Sheltered	Fully mixed	Days
CW – NEA8	Polyhaline, microtidal, sheltered, shallow (Skagerrak inner arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Shallow (< 30)	Low (< 1)	Sheltered	Partially Stratified	Days-Weeks
CW – NEA9	Fjord with a shallow sill at the mouth with a very deep maximum depth in the central basin with poor deepwater exchange.	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	Low (< 1)	Sheltered	Permanently Stratified	Weeks
CW – NEA10	Polyhaline, microtidal exposed, deep (Skaggerak outer arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	Low (< 1)	Exposed	Permanently Stratified	Days
TW – NEA11	Transitional waters	Oligo-Euhaline (0 - 30)	Mesotidal (1 – 5)	Shallow (< 30)	Medium	Sheltered or moderately Exposed	Partially- or Permanently Stratified	Days-Weeks

The above types occur in Member States' waters as detailed below in table 2.4.2:

Table 2.4.2: Member States sharing types.

Type	BE	DE	DK	ES	FR	IE	NL	NO	PT	SE	UK
CW – NEA1/26	X	X	X	X	X	X	X	X	X		X
CW – NEA3/4		x					x				
CW – NEA7								x			X
CW – NEA8			x					x		X	
CW – NEA9								x		x	
CW – NEA10								x		x	
TW – NEA11	X	X		X	X	X	X		X		X

For benthic invertebrates, all classification schemes intercalibrated relate only to the soft sediment infauna component. Member States use the same classification schemes in all their types. Differences occur in the reference conditions for the types; these are specific for the habitat type, and for some Member States (NL and DE), sometimes even specific for the water body. However, the basic metrics in each country's scheme remains the same. As Option 3 has been used, the classification schemes are compared at the type level and boundaries set that are specific to particular types. This process is described below.

2.4.2 National methods intercalibrated

The national methods that have been assessed in this intercalibration exercise are shown in table 2.4.3 below:

Table 2.4.3: Member States national methods.

Member State	Method	Status
BE	Benthic Ecosystem Quality Index (BEQI)[1]	Agreed
DE	Multimetric Factorial Analysis (M-AMBI)	Agreed
DK	Danish Quality Index (DKI)	Agreed
ES	Multimetric Factorial Analysis (M-AMBI)	Agreed
FR	Multimetric Factorial Analysis (M-AMBI)	Agreed
IE	Infaunal Quality Index (IQI)	Agreed
NL	Benthic Ecosystem Quality Index (BEQI)[1]	Agreed
NO	Norwegian Quality Index (NQI)	Agreed
PT	Portuguese Benthic Assessment Tool (P-BAT)	Agreed
SE	Biological Quality Index (BQI)	Agreed
UK	Infaunal Quality Index (IQI)	Agreed

NB. BEQI[1] -For the intercalibration exercise, only the level 3 of BEQI (within habitat quality) is applied.

2.4.3 Reference conditions and class boundary setting

Type - NEA1/26 and NEA7

Reference Conditions

DE, DK, ES, IE, NO, PT, UK

As Option 3 has been selected for the intercalibration of this quality element each Member State has derived biological reference conditions for the indices within their classification systems. Reference conditions are specific for habitat sampled and sampling method. For this intercalibration exercise, all Member States used reference conditions that relate to subtidal, mud/sand habitats and these are described below in table 2.4.4. (If different habitats were to be assessed, alternative reference conditions would need to be used.)

General physico-chemical and hydromorphological reference conditions for all types have not been defined.

Table 2.4.4: Reference conditions.

MS	Method	Sample area (m^2)	Component metrics - reference values			Combined reference value
			Taxa no.	Diversity	AMBI	
DK	DKI			Shannon's (logbase2) = 5	0	
ES	M-AMBI		42	Shannon's = 4	1	
IE	IQI	0.1	68	Simpsons = 0.97	1-AMBI/7 = 0.96	
NO	NQI	0.4				0.78
PT	P-BAT	0.1		Shannon's = 4.1 Margarlef = 5	0	
UK	IQI	0.1	68	Simpsons = 0.97	1-AMBI/7 = 0.96	

DE

Reference values used for the Intercalibration of the German M-AMBI classification methods are specific for waterbody and habitat type. Because no regional reference sites or sample data were available DE used reference values from NL database, which is based on similar habitats and shown in table 2.4.5 below. Germany will generate own local reference data within Phase 2.

Table 2.4.5: DE reference conditions.

Method	WaterBody	Component metrics - reference values		
		Taxa no.	**Diversity Shannon's**	**AMBI**
M-AMBI	Vortrapptief, (NEA 1 subtidal 18m), fine sand - sand	31	2.66	0.107
M-AMBI	Hoher Weg (NEA 26), low, littoral sand)	17	2.22	0.393

NL/BE

The BEQI method (used by NL and BE) requires a reference dataset for each habitat within a water body. The requirement of a reference dataset is different from the other MS multimetrics, which only require a (maximal) reference value for a specified habitat. The reference values are also related to sampling surface (determined by a randomisation procedure) and defined for each WFD boundary.

NL: The reference values for reaching good status (good/moderate boundary, sampling surface of $1m^2$) for the fine muddy sand coastal habitat of the Dutch coast (Hollandse kust and Waddenkust) are summarised in table 2.4.6 below.

Table 2.4.6: NL reference conditions.

Habitat type	Sampling surface	No. of species	Similarity	Density (ind/ m^2)	Biomass (gAFDW/ m^2)
Dutch coast (fine muddy sand Q1)	1 m^2	60	0.74	2584 and 7975	14.2 and 52.4

Reference conditions correspond to assessment class Good/Moderate. Reference values are based on the habitat type Q1 cluster of stations within six nautical miles from the coast, using samples between 1983 and 1990.
N.B. The analysis of the benthic communities of Hollandse kust and Waddenkust did not reflect differences related with the current WFD typology: Hollandse kust (NEA3) and Waddenkust (NEA 1). The typology differences between the areas only reflect the polyhaline versus euhaline water characteristics. As such in the intercalibration exercise, the Hollandse kust and Waddenkust and Eems-Dollard kust are taken together in the NEA 1 type intercalibration comparison.

BE: The reference values for reaching good status (good/moderate boundary) for the fine muddy sand coastal habitat (for the minimal sampling surface of 1.5 m^2) are summarised below in table 2.4.7.

Table 2.4.7: BE reference conditions.

Habitat type	Sampling surface	No. of species	Similarity	Density (ind/m^2)	Biomass (gAFDW/ m^2)
Fine muddy sand – *Abra alba* community	1.5 m^2	60	0.84	1516 and 5394	Not yet determined

Too few data were available in the Belgian WFD benthos dataset to determine the reference conditions for the other habitats; this could only be done for the *Abra alba* community. In fact, the habitat typology of the Belgian continental shelf is based on four different macrobenthic communities with typical species associations in between. The reference values for the three other communities will have to be determined in future research on historical data or monitoring in the future. The communities are each characterised by different habitat specifications as briefly illustrated below.

Macrobenthic communities (habitats):

– *Abra alba* community: shallow muddy sand
– *Nephtys cirrosa* community: well-sorted mobile sands
– *Ophelia limacina – Glycera lapidum* community: medium to coarse sand
– *Macoma balthica* community: shallow sandy mud

The macrobenthos status classification will have to be performed for these four communities separately, based on community-oriented monitoring, to come to an overall macrobenthos assessment for the Belgian coastal waters.
Some important issues and difficulties to be highlighted:
The Belgian reference data for the different habitats is gathered in the period 1994 – 2000 and comes not from a \`natural, undisturbed\` reference period, as required by the WFD. However, this reference dataset aims to give a reflection of the spatial and temporal variability within the habitats
Using the BEQI method, reference values for species richness, species composition and density have been given for one community. Reference biomass data is not yet available.

Class Boundary Setting Procedure

As Option 3 has been used, Member States have derived their own initial boundaries for the National classification methodology.
Two examples of boundary setting procedures are as follows for i) the IQI (sample level procedure) and ii) the BEQI (waterbody level procedure incorporating biomass):

i) Infaunal Quality Index (IQI)

The Infaunal Quality Index (IQI) operates over a scale range from 0 (impacted) to 1 (non-impacted). Initially, class boundaries were set at equidistant points along the scale (0.2, 0.4, 0.6, and 0.8). These boundaries were then modified to ensure that the communities conform to the status characteristics as defined in the Normative Definitions. Figure 2.4.1 illustrates the process followed in setting and testing the ecological status boundaries.

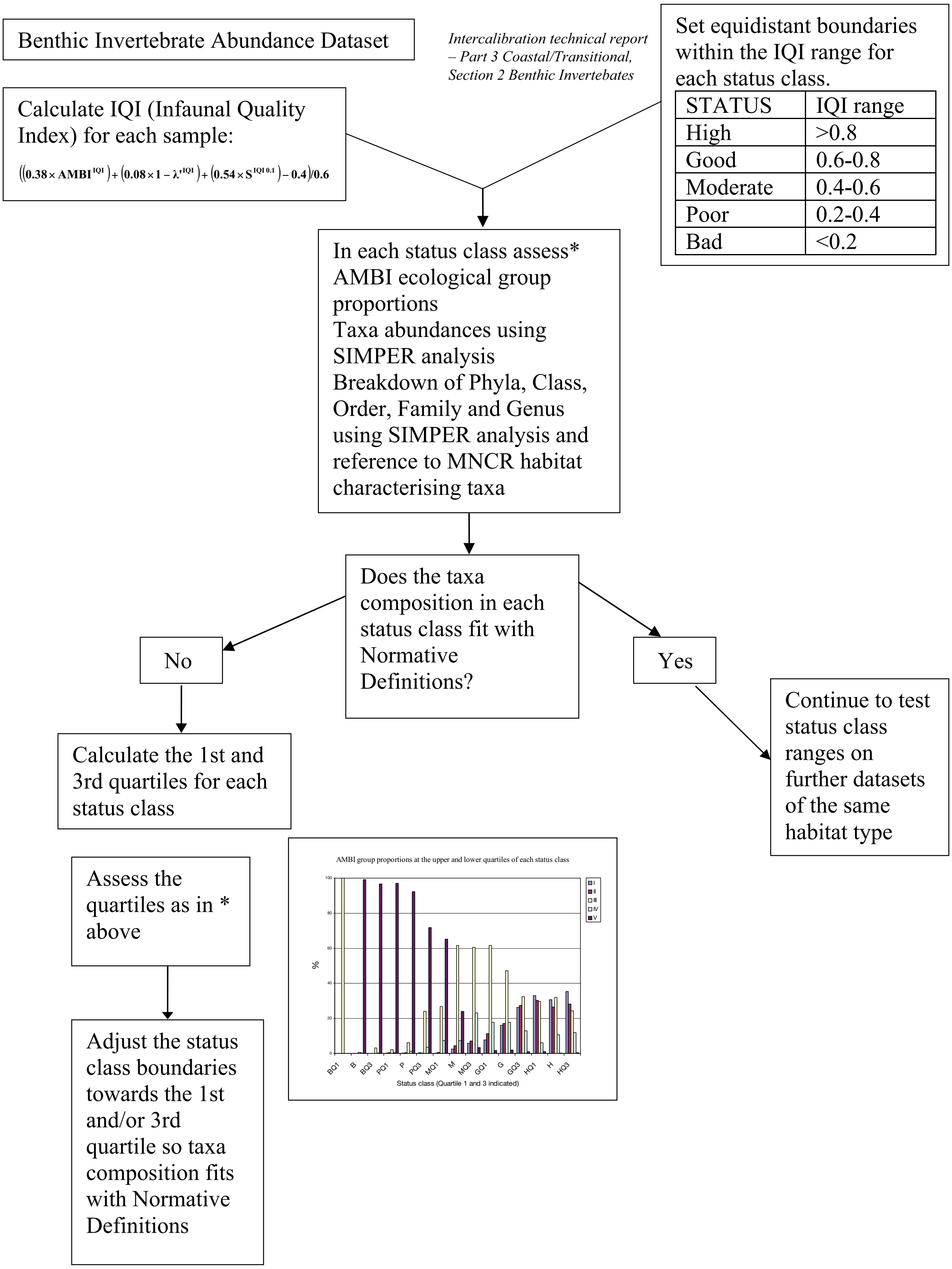


Figure 2.4.1: Flow diagram of stepwise process followed to establish boundaries for each status class which reflect the Normative Definitions.

The class boundaries were set using samples from an organic enrichment gradient; the sewage sludge disposal site at Garroch Head (data courtesy of the Fisheries Research Services, Scotland). Benthic invertebrate abundance data had been obtained across the pressure gradient annually over a 20-year period (total of 186 samples). The samples were taken from habitats classified as type A5.3 (marine sublittoral muds) under the European Nature Information System (EUNIS) habitat classification scheme.
The IQI, which is comprised of three indicators of benthic ecosystem health (Simpson's evenness, taxa number, AZTI Marine Biotic Index (AMBI)), was calculated for all samples.

The IQI is calculated as:

$$IQI = \mathbf{((0.38\times AMBI^{IQI}) + (0.08\times 1 - \lambda'^{IQI}) + (0.54\times S^{IQI\ 0.1}) - 0.4)/0.6}$$

Where:

$AMBI^{IQI}$ = Observed value of (1-AMBI/7) divided by maximum expected value under reference conditions for a given habitat type.

$1\text{-}\lambda'^{IQI}$ = Observed value of Simpsons evenness index (1-λ') divided by the maximum expected value under reference conditions for a given habitat type.

S^{IQI} = Observed number of taxa divided by the maximum expected value under reference conditions for a given habitat type.

AMBI assigns taxa a sensitivity score (ecological group, EG) as follows: EG I – sensitive taxa, EG II – indifferent taxa, EG III – tolerant, EG IV – opportunistic, EG V – indicator taxa. The composition of an assemblage in terms of ecological groups is expected to respond to the extent of anthropogenic pressure (e.g. Figure 2.4.2). The proportions of sensitive taxa, tolerant taxa and taxa indicative of pollution were used to establish class boundaries.

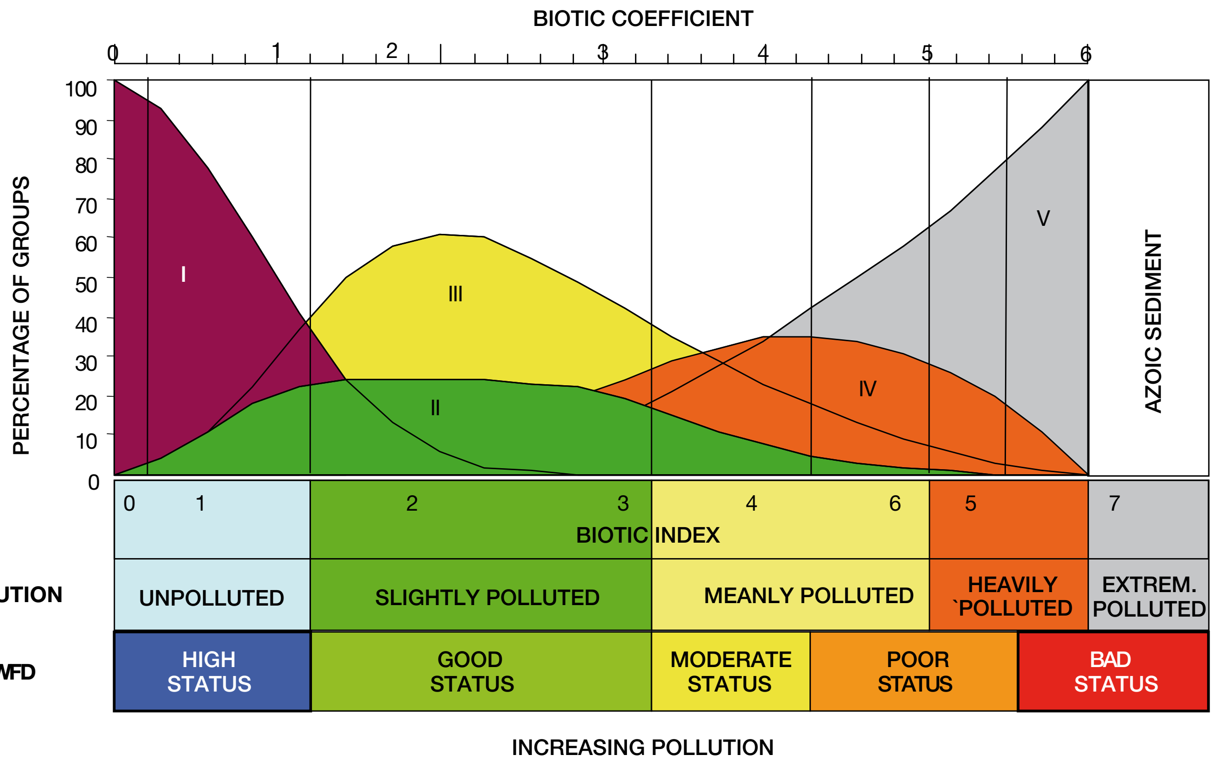


Figure 2.4.2: The AMBI biotic coefficient, relating the ecological groups present in a sample to an assessment of the benthic invertebrate community (Borja et al, 2003).

Using equidistant class boundaries initially, AMBI ecological group proportions in the gradient dataset were assessed as to whether the proportions of sensitive taxa, tolerant taxa and taxa indicative of pollution were in line with the Normative Definition for each status class (Figure 2.4.3).

The boundaries were then adjusted to ensure they captured the sampling occasions that provided the highest agreement with the Normative Definitions. This was achieved by plotting the ecological group proportions within the first and third quartiles of each status (Figure 2.4.4) and adjusting the boundaries until agreement with the Normative Definitions was maximised.

The validity of the adjusted boundaries was then assessed by analysis of the composition of the taxa in each status class. SIMPER analysis (PRIMER©) was carried out, assessing the top 90 %

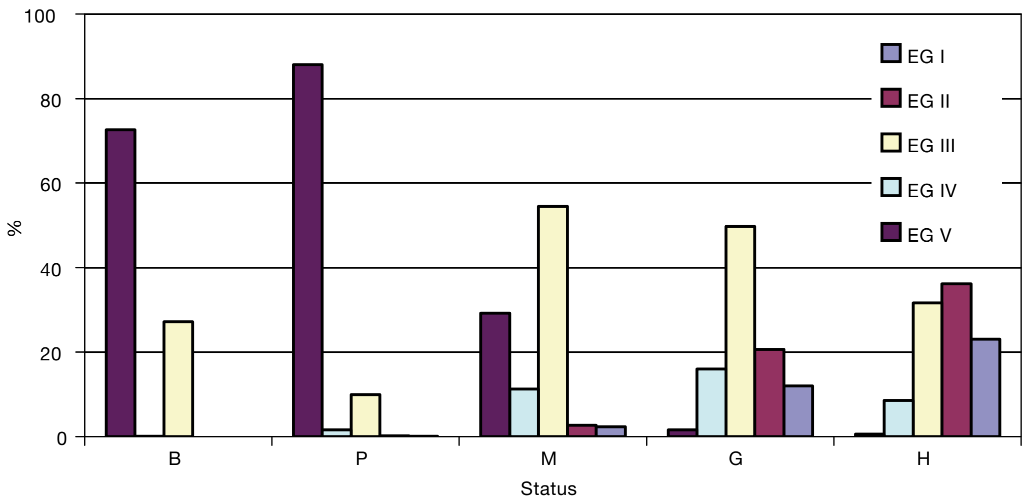


Figure 2.4.3: The proportions of AMBI ecological groups for each ecological status class (B = Bad, P = Poor, M = Moderate, G = Good, H = High) as determined by an equidistant split of the IQI.

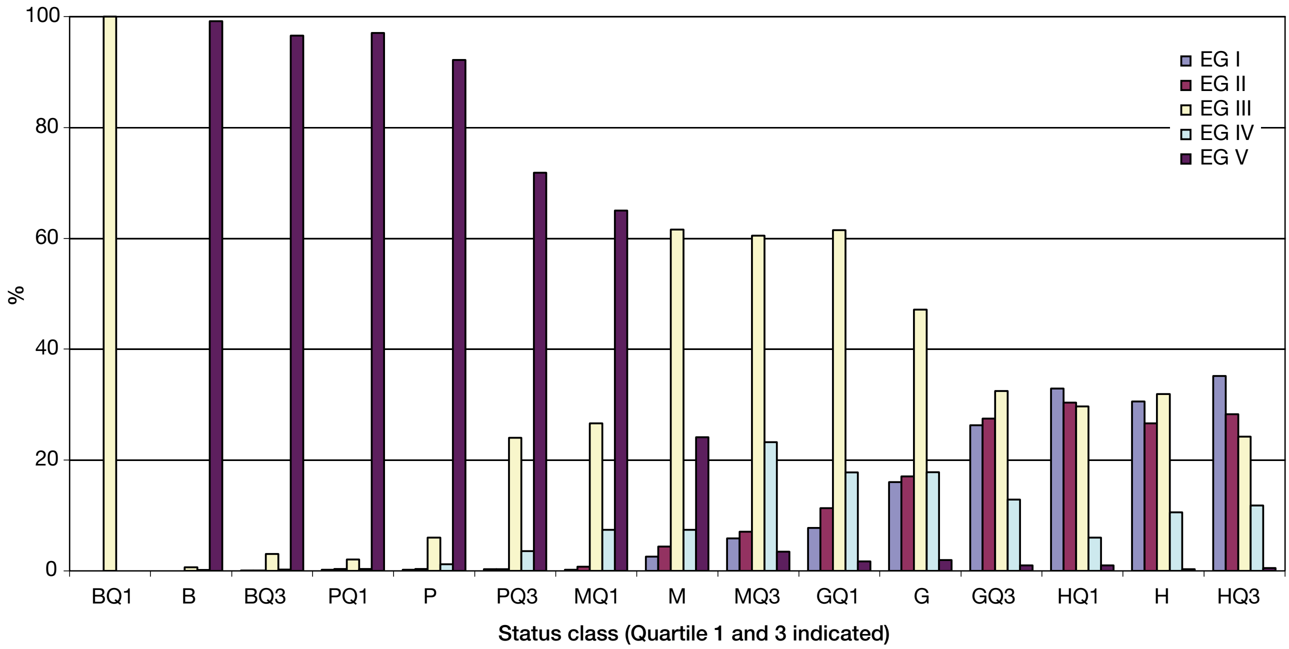


Figure 2.4.4: AMBI group proportions of status classes (B = Bad, P = Poor, M = Moderate, G = Good, H = High) and their upper (Q3) and lower (Q1) quartiles using preliminary equidistant divisions of the IQI.

of contributing taxa (family level taxonomic discrimination). Changes in composition between the status classes were evaluated to determine whether the adjusted ranges mirrored the Normative Definitions in terms of contributing taxa. In addition, the Marine Nature Conservation Review (MNCR) Habitat Directive types were used to ensure an understanding of the characterising taxa and their composition expected for a particular habitat type.

The resulting IQI boundaries and are shown in table 2.4.8 and AMBI group proportions in Figure 2.4.5 respectively.

ii) Benthic Ecosystem Quality Index (BEQI)

The BEQI statistically integrates the risk of misclassification at water body level related to sample size in the methodology and boundary setting. Based on permutation calculations, reference values are determined for each component metric and class boundary.

The reference values are calculated per habitat over increasing sampling surfaces. This allows for the estimation of the reference value for any given sampling surface. The reference for a $1m^2$ sampling surface is based on a set of 2000 artificial random samples out of the reference dataset.

Out of the randomisation procedure, each component metric (indicators: density, biomass, species richness, species composition changes), a 5^{th} percentile value is selected as the value that has to be reached to achieve good status (the value of the good/moderate boundary) (Figure 2.4.6).

Table 2.4.8: Class Status boundaries – IQI assessment
(prior to optimisation through the Intercalibration exercise).

Status Class Boundary	IQI
High/ Good	0.80
Good/ Moderate	0.65
Moderate/ Poor	0.43
Poor/ Bad	0.20

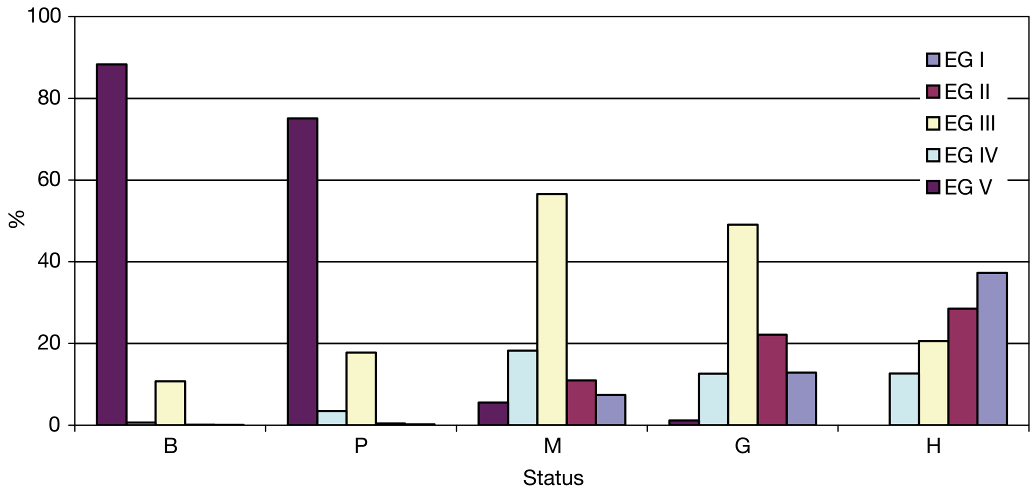


Figure 2.4.5: AMBI group proportions within each status based on the Garroch Head data using the following boundaries: High/Good =0.8; Good/Moderate = 0.65; Moderate/Poor = 0.43; Poor/Bad = 0.2.

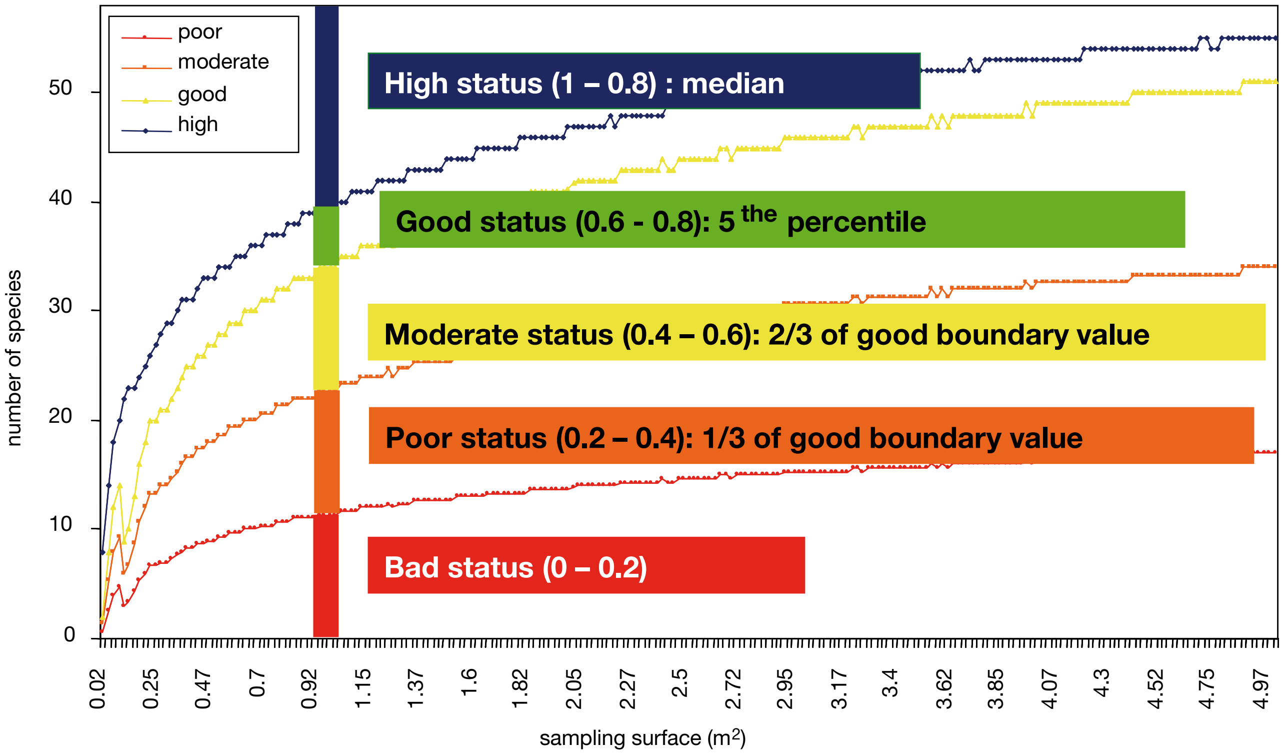


Figure 2.4.6: The boundary settings for number of species in relation to sampling surface. A similar protocol is applied for similarity (species composition changes).

For the parameters density and biomass, a two side deviation from the reference values is scored (Figure 2.4.7). The other boundary values were adopted from this value (equal intervals), except the high/good reference value, which is also directly extracted from the randomisation procedure.

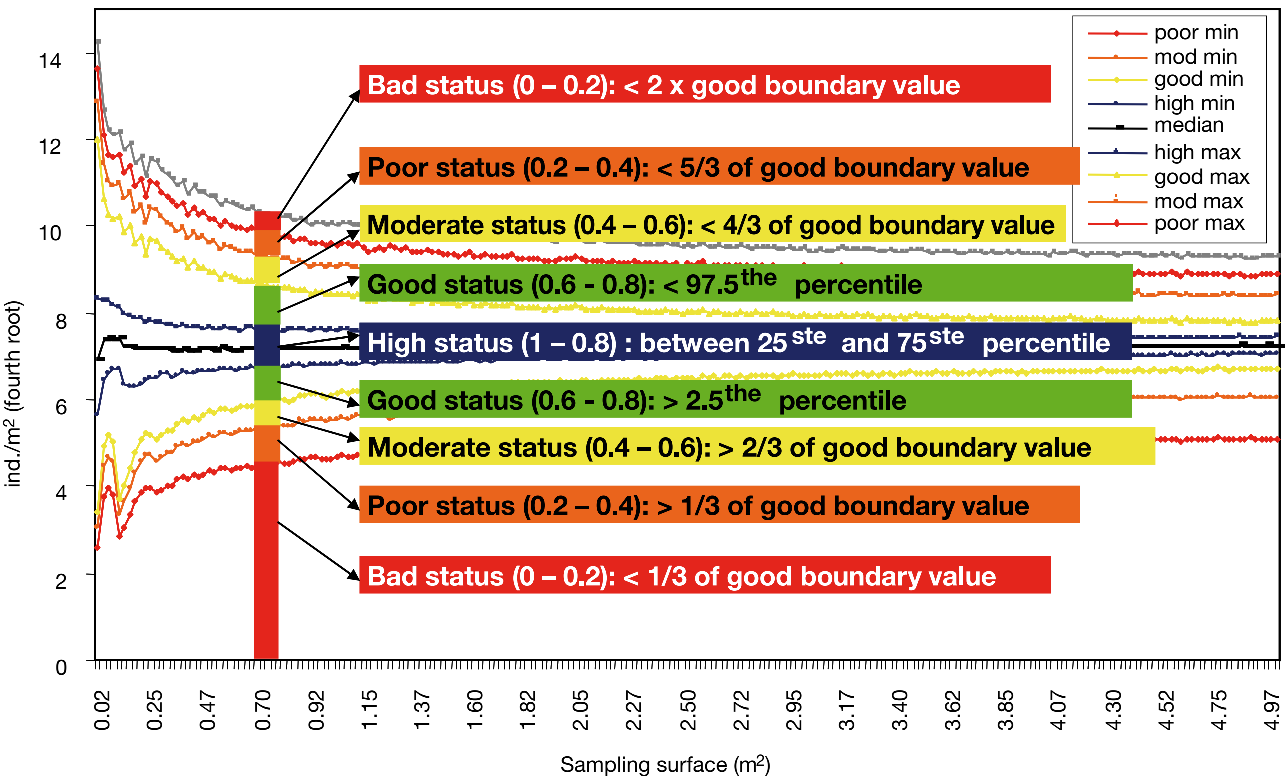


Figure 2.4.7: The boundary settings for density in relation to sampling surface. A similar protocol is applied for biomass.

The boundaries selected from the randomisation procedure, with the corresponding WFD class boundaries are summarised below in table 2.4.9.

Table 2.4.9: NL boundaries.

Boundary	No. species and species composition change	Density & biomass
High/Good: 0.8	Median	25 and 75 percentile
Good/Moderate: 0.6	5th percentile	2.5th and 97.5th percentile
Moderate/Poor: 0.4	2/3 of Good/Mod value	2/3 and 4/3 of Good/Mod value
Poor/Bad: 0.2	1/3 of Good/Mod value	1/3 and 5/3 of Good/Mod value

The boundaries set by the BEQI method are therefore statistical significance levels related to a fixed WFD Class boundary value.

Type - NEA3/4

Reference Conditions

Intercalibration was carried out between the BEQI (Netherlands) and the M-AMBI (Germany). Reference boundary values for four habitats of major importance in NEA3/4 types are shown below in table 2.4.10 (for G/M status).

Table 2.4.10: NL and DE reference conditions.

BEQI	Habitat	Sample surface (m^2)	No. species	Similarity	Density (ind/m^2)	Biomass (gAFDW/ m^2)
	High Littoral Mud	3	13	0.68	448 - 7643	4.1 - 20.6
	Middle Littoral Muddy Sand	3	17	0.7	269 - 12063	18.4 - 58.9
	Low Littoral Sand	3	13	0.6	106 - 7384	4.3 - 24.3
	Brackish Sub Littoral	3	26	0.82	1810 - 103353	18.7 - 88.8
M-AMBI			**No. species**	**AMBI**	**Shannon**	
	High Littoral Mud		18	2.7	2.16	
	Middle Littoral Muddy Sand		23	0.947	2.34	
	Low Littoral Sand		17	0.393	2.22	
	Brackish Sub-Littoral		16	1.541	2.178	

Class Boundary Setting Procedure

Netherlands (BEQI): see section above for procedure described under NEA1/26.

Germany (M-AMBI): The class boundaries to classify the results of the M-AMBI were set after comparison of the assessment results using the boundaries given by Muxika *et al.* (2007) and Spain (Intercalibration Report, 2007) with a classification by expert judgement. The boundaries were then modified to ensure that the communities conform to the status characteristics as defined in the Normative Definitions of the WFD.

The High/Good boundary (0.85) was in agreement with the boundary given by Muxika et al. (2007) but higher than the value of Borja (0.77) (Intercalibration report 2007). The Good/Moderate boundary was set at 0.7, which is higher than the boundaries given by Muxika et al. (2007) (0.55) or Borja (Intercalibration report 2007) (0.53). The reference values chosen for the M-AMBI calculations were determined by NL (van Hoey et al. 2007) on a data basis including the years 1969 to 1983 for the littoral Wadden Sea and the years 1988 to 1990 for the Wadden- and Eemskust. These reference datasets derived therefore from times, in which human impacts on the North Sea and the Wadden Sea were already obvious and first changes in benthic species composition and biomass were detected (e.g. Beukema 1991, Kröncke 1995). Therefore the 'reference data do not represent an unaffected status' and the boundaries needed adjustment. Further calculations should be carried out with references derived from own data sets of the relevant stations. The boundary G/M was set as 0.7, because for all of the chosen intercalibration stations and for many additional stations (Heyer 2007) the M-AMBI results corresponded with the expert judgement.

Type - NEA8/9/10

Reference Conditions

Denmark, Sweden and Norway have these types.

In Sweden when deriving the type-specific class boundaries, the greatest emphasis was placed on the good–moderate boundary. This boundary was primarily determined with the aid of comparative data from high–good areas. Comparative data was chosen for each national type from regions lacking local discharges; in practice areas with the highest mean BQI values existing for that type. Sequential tests identifying the level of BQI (20th percentile) where a water body significantly differs from the comparison material were made to assist in the setting of good-moderate boundary. In those types where the amount of data was insufficient, expert assessments were conducted based on existing data and data from nearby types with similar properties. Once the good–moderate boundary was determined, it was deemed acceptable to consider the area from the good–moderate boundary up to the highest observed index value in the existing type as mainly constituting a status of good. Two-thirds (2/3) of the span exceeding the good–moderate boundary was assigned a status of good, while the upper third was reserved for the status of high. The area below the good–moderate boundary was divided into three equal intervals for the remaining boundaries. It was not possible to describe reference conditions due to a lack of unaffected areas today and historical data were not collected with the same methodology as in use today. As shown above the best available data from unimpacted areas has been used as a reference in setting the boundaries. This data assumed having a status of at least good.

In Denmark the Good-Moderate boundary was determined by the 5th percentile of samples/sites classified as being in at least Good status. The High-Good boundary was set midway between the GM value and the theoretical highest index value of 1. The reference state using this approach is the highest diversity (Hmax) and the lowest

AMBI found in the material having at least Good status from the type in question. In accordance with the normative definition concerning sensitive species the reference state is characterized by high dominance of sensitive species (Ecological group I according to the AMBI system), followed by group II. Group III and the opportunists, groups IV and V are subdominant and virtually absent respectively.
In Norway the 90-percentile (the border value between the 90 % lower and 10 % higher values among all the grab samples from the reference stations) was used to quantify the reference value for the metric.

Class Boundary Setting Procedure

Again as Option 3 has been used Member States have derived their own initial boundaries for the national classification methodology. An example of this from Sweden is as follows:

The performance of Swedish, Danish and Norwegian national methods of assessing status have been compared by Sweden on Swedish national and regional monitoring data from coastal deeper parts (> 20 m depth) of the Kattegat/Skagerrak area. Three steps have been carried out in this intercalibration exercise, comparison of species sensitivity classifications, comparison of index values in different environments and comparison of status classification on water body level.

Species classifications

A major part of the Swedish (BQI), Danish (DKI, Borja et al. 2006) and Norwegian (NQI) method for classification is based on species sensitivity classifications. In the Swedish method an objective method was used to classify species sensitivity to environmental degradation. In general, few species appear in degraded areas. Thus, a species that frequently appears in species-poor environments are given a low index value compared with species that only appear in species-rich environments. We have used Hurlberts diversity index as a measure of the number of species in a sample. The species sensitivity value has been calculated as the 5th percentile of that species diversity-value frequency distribution, where each observation in the frequency distribution represents an individual's diversity value from the sample in which that individual appeared. We have used a large amount of both Swedish and Danish data when calculating the sensitivity values.

The Danish and Norwegian method uses AMBI species classifications (see Borja et al 2000). AMBI classifies species into five ecological groups, I = Sensitive, II = Indifferent, III = Tolerant, IV = Second order opportunists and V = First order opportunists.

The correspondences between the two classifications are not that good. There are only small differences between distributions of sensitivity values in the ecological groups I, II, III and IV.

Index correlations

To see how the indices correlates the BQI, DKI and NQI was calculated in each grab sample (~0.1 m^2) and the results where plotted against each other. The Swedish good and moderate boundary lies around a BQI value of 12 for this type. Up to this value there is an almost linear relationship but over this value DKI and NQI tends to level off while BQI continues to increase. Still there is a rather good correlation between BQI, DKI and NQI. The relationship between NQI and DKI is nearly perfect, not unexpectedly because they both use the same components i.e. AMBI.

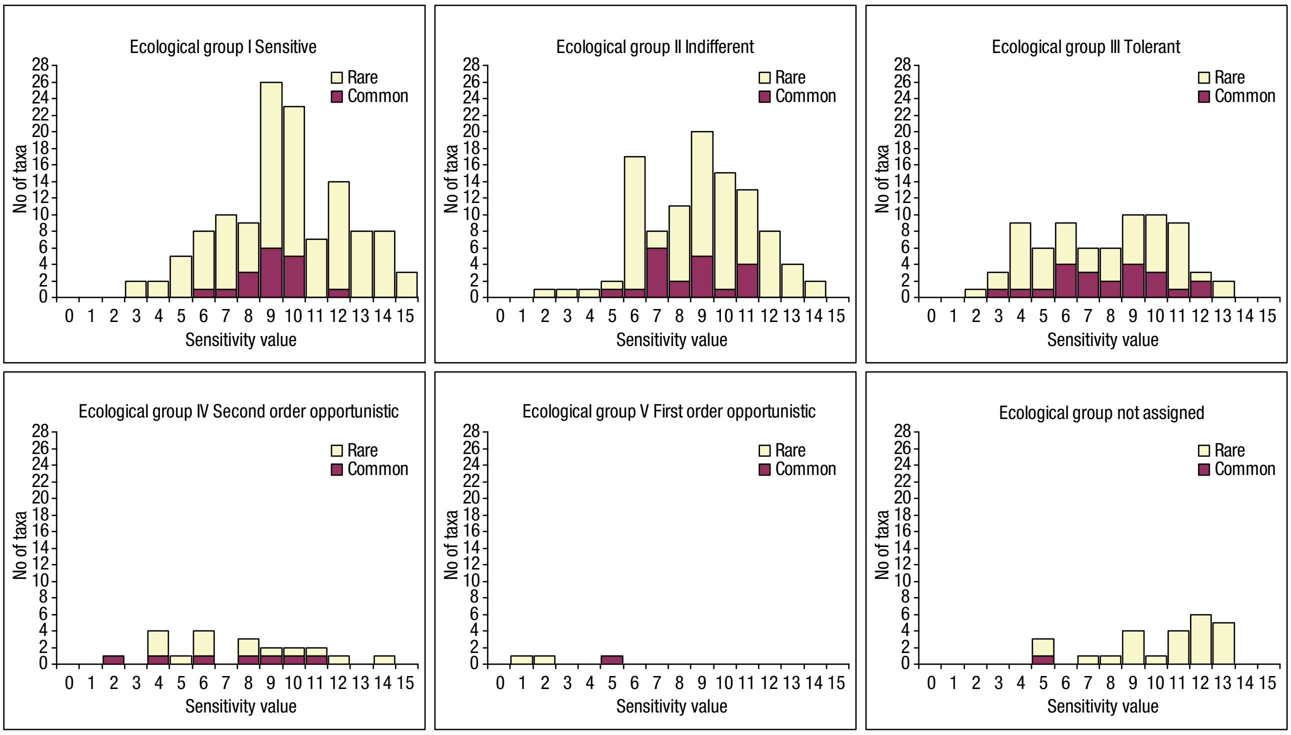


Figure 2.4.8: Swedish sensitivity values in different ecological groups. Only species found in the Swedish coastal part of deeper (> 20 m depth) areas in the Kattegat/Skagerrak are included in the comparison. Common taxa, found in more than 500 samples, are indicated with purple color.

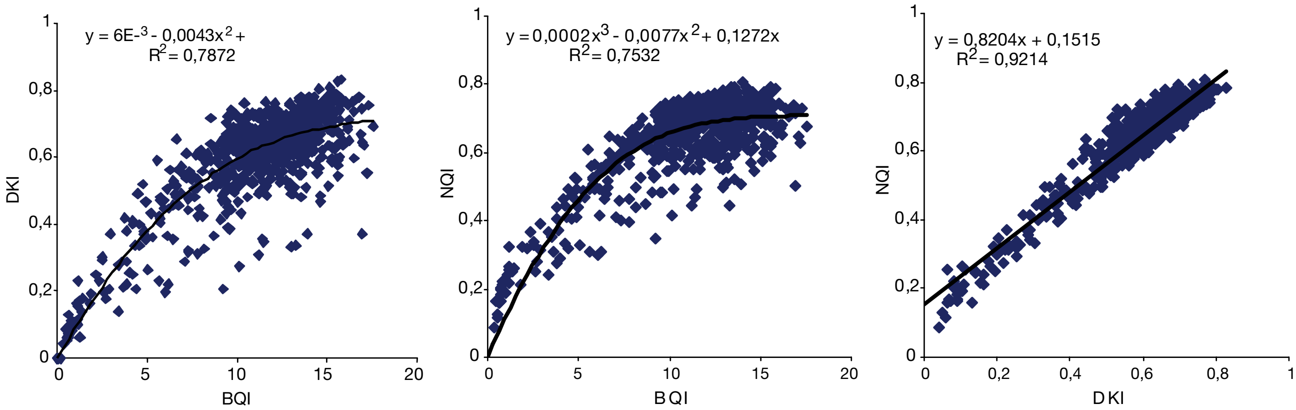


Figure 2.4.9: Correlation between BQI, DKI and NQI.

To compare how the indices behaves in different disturbed situations plots were made on data from the deepest part of the Gullmar fjord where low oxygen levels have affected the fauna at several occasions and data from the Saltkälle fjord where organic loading have decreased and fauna recolonized after closure of a sulphite mill factory in 1966.

In both of these examples the BQI, DKI and NQI behaves similarly, only minor differences can be seen.

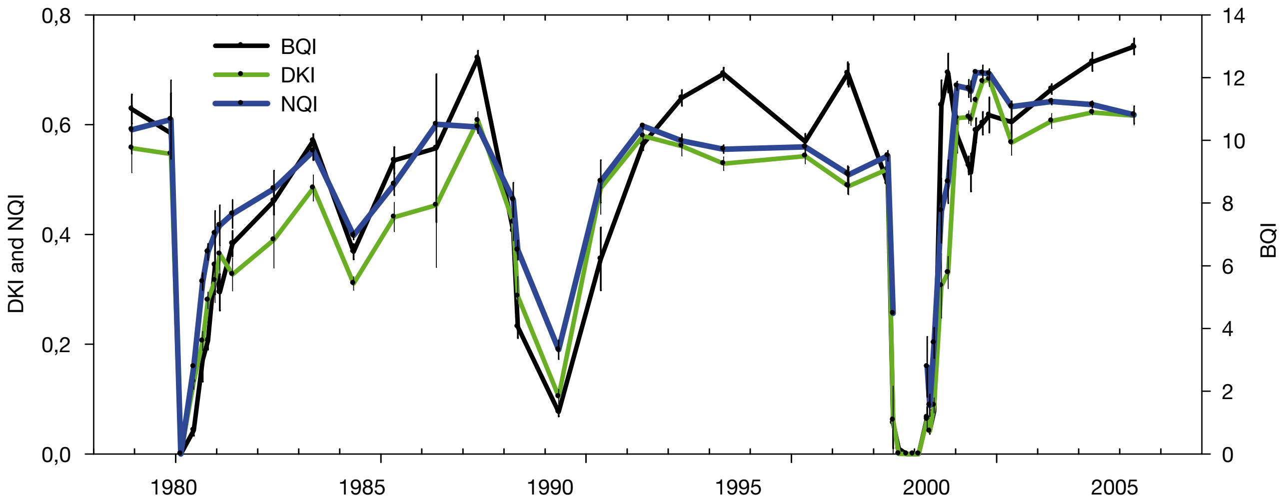


Figure 2.4.10: Comparison of index response to low oxygen content at Alsbäck 118 m depth in Gullmar fjord on the Swedish west coast. The indices are shown with standard error of the mean. Data from monitoring programmes and Agrenius pers comm.

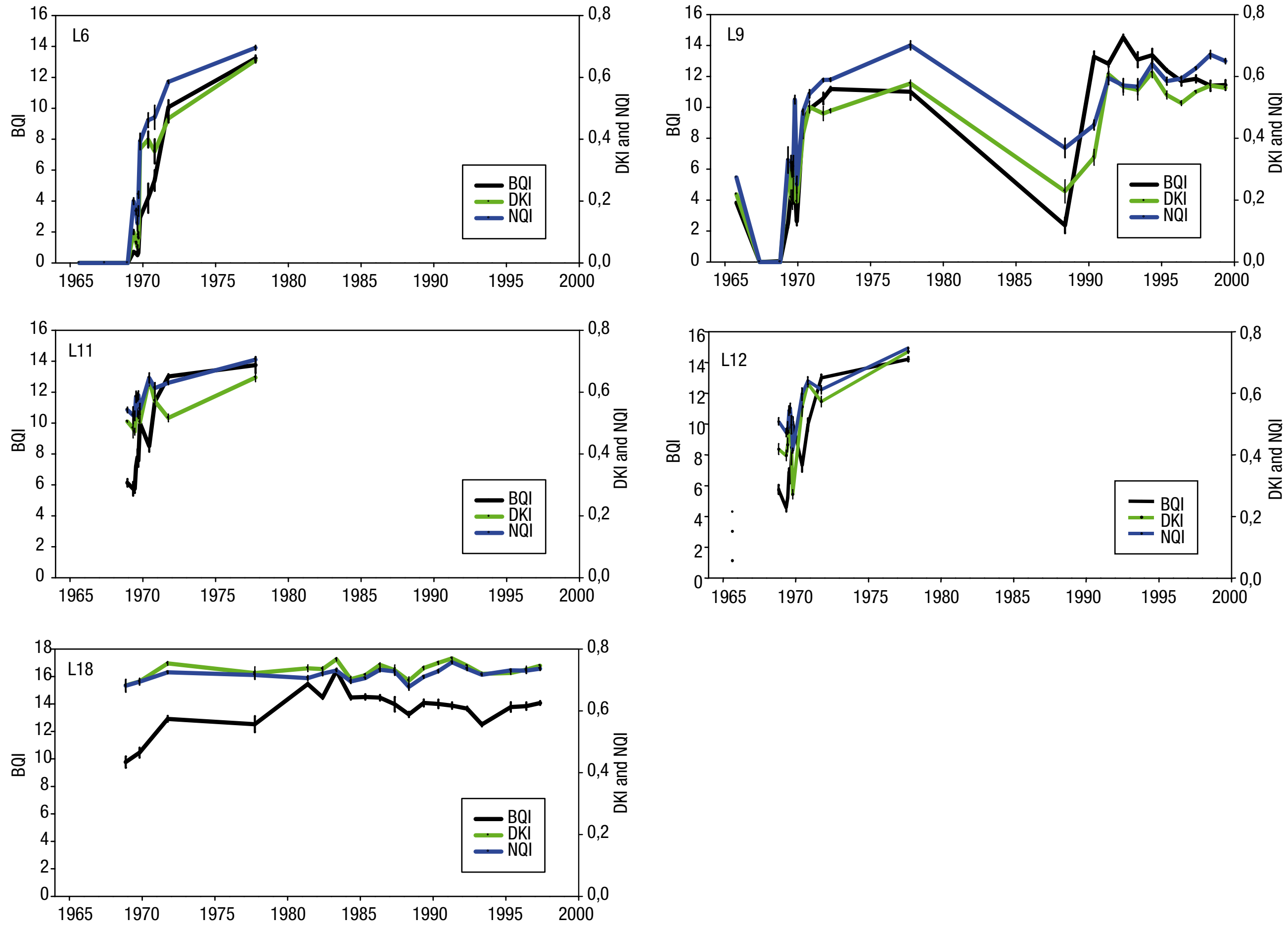


Figure 2.4.11: Comparison of index response to decreased organic load after closure of sulphite mill factory in 1966. Stations L6, L9, L11, L12 and L18 are shown with increasing depth (25 – 44 m) and increasing distance to discharge from river Örekilsälven in the inner part of the Saltkällefjord on the Swedish west coast. Station L9 had a low value in 1988 due to a temporary drop in oxygen concentration. The indices are shown with standard error of the mean. Data from Bagge 1969, Leppäkoski 1975, Rosenberg 1972, 1973, 1976 and monitoring programmes.

Water body status

To compare the assessment of status of water bodies' national indices and boundaries were applied on data from a selection of Swedish water bodies with at least 5 stations sampled in a year. Denmark and Norway has not yet presented any method for how to go from sample to water body level so for comparison the mean value of DKI and NQI were compared with the 20th percentile of BQI according to the Swedish method.

Comparisons on time series from three water bodies have been made. The results of these comparisons are shown. The agreement between the three methods are good, only in a few instances does the status differ slightly between them.

Type - NEA11

This is the generic single transitional water type covering all transitional waters in eight countries. As such it is very wide ranging and demands methods that cover all the salinity ranges in the different transitional waters.

Reference Conditions

Reference conditions derived for coastal waters may be used in the euhaline parts of transitional waters assuming equivalent habitats (sediment types) are assessed. The reference conditions from the coastal water type into which the transitional water drains may therefore be applicable. For the

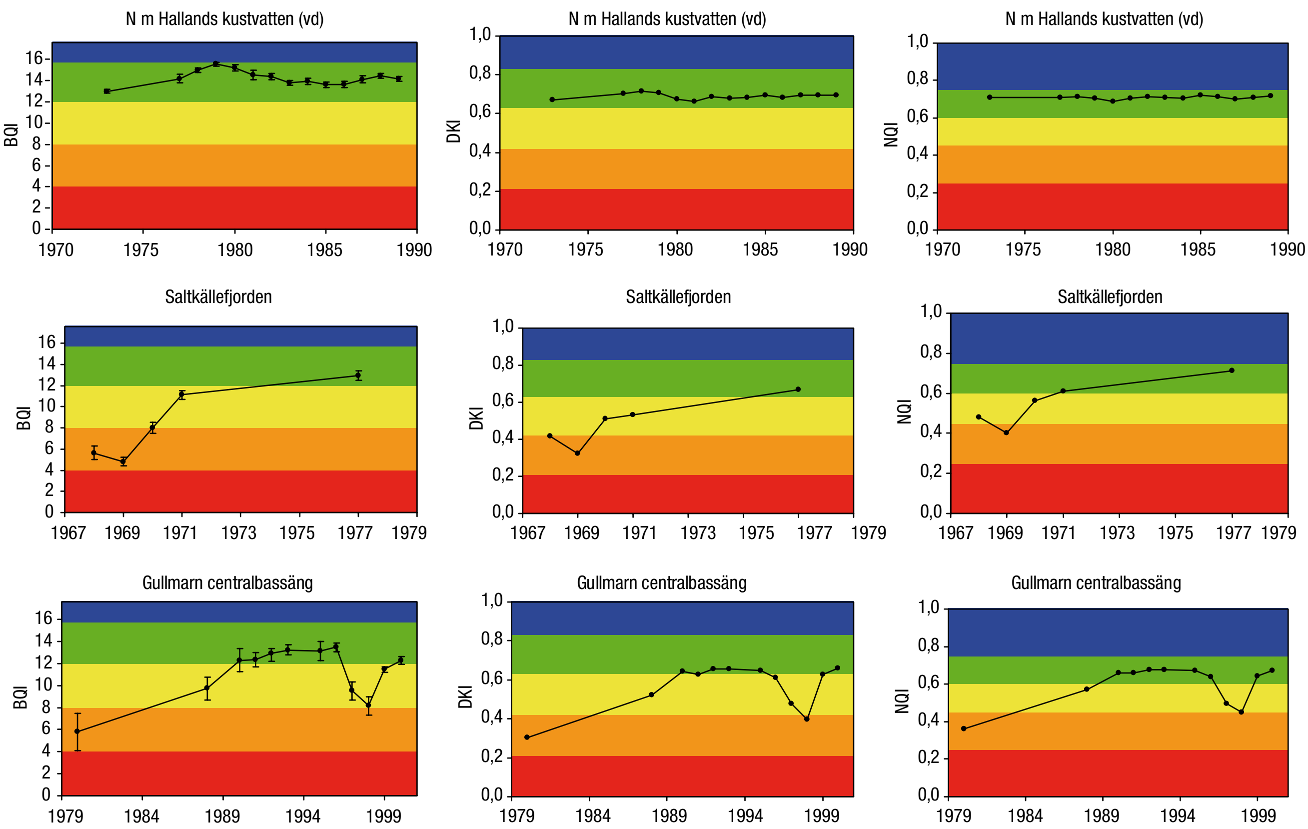


Figure 2.4.12: Comparison of water body status assessed with Swedish (left column), Danish (middle column) and Norwegian (right column) methods. Error bars in Swedish graphs denotes 20th and 80th percentile. Note that the Swedish method uses the 20th percentile for assessment. The agreements between the methods are good. Data from Bagge 1969, Leppäkoski 1975, Rosenberg 1972, 1973, 1976 and monitoring programmes.

polyhaline and mesohaline stretches within transitional waters different reference criteria may be required for use in the specified classification scheme. At this time, these reference conditions are still under development. All required reference conditions are not expected to be fully developed before June 2007 therefore postponing intercalibration in transitional waters until Phase II, 2006 to 2009.

Class Boundary Setting Procedure

If assessments are made in the euhaline areas of transitional waters (or with a similar salinity regime to the adjoining coastal waterbody) then the classification tool for coastal waters would be used in the same habitats to which that tool applies. As such the boundary setting procedure would be the same as described above for the coastal waters types. The same principles would be used in setting boundaries in other areas of transitional waters with different salinity regimes but the classification would be anchored by different reference condition specific to those areas. At this time, there are no classification systems developed that allow a comparison between Member States, therefore postponing intercalibration in transitional waters until Phase II, 2006 to 2009.

Any future approach should build on the lessons learnt from the coastal water intercalibration exercise. It has been suggested that as many transitional waters have been identified as ‘heavily modified water bodies, it may complicate the simple aggregation of present metrics adapted to cover salinity ranges and differences in habitats, for some classification assessments.

2.4.4 Results of the comparison

Type - NEA1/26 and NEA7

DK, ES, IE, NO, PT, UK

Data have been exchanged by Member States and a standardised Intercalibration dataset collated. This dataset has been run through each proposed national classification scheme to determine the matches in status obtained when assessed by the different methods. For example, Danish data were assessed using the UK classification scheme, and vice versa, and the matches in status assessed.

Five classification schemes (M-AMBI, IQI, NQI, DKI and P-BAT) were available for this assessment. The assessment indicated the agreement of sites that have been assigned similar status (High/Good or Moderate/Poor/Bad) by the different assessment methods. The EQR boundaries in the ecological status assessment were then altered, to achieve the highest agreement between Member States (i.e. optimisation of boundaries).

Following optimisation of the boundaries, agreement between boundaries was assessed according to guidelines issued by Ecostat to compare the agreement in classes both for all five classes and only three, high, good and moderate or worse. In addition further evaluation was made using harmonised boundaries and allowing a 0.05 EQR range of deviation around the boundaries. These results are presented in the table 2.4.11 below.

Table 2.4.11: Percentage agreement between classification methods. (Following optimisation of boundaries).

	Agreement (5 classes)	**Agreement (3 classes)**	**Agreement (5 cl.+0.05 EQR)**	**Agreement (3 cl. +0.05 EQR)**	**Average class difference**	**Absolute average class difference**
Average A (DKI –DK)	77.098	80.303	97.918	98.13	0.035	0.068
Average B (M-AMBI – FR/ES)	71.77	75.01	95.098	95.273	0.013	0.078
Average C (NQI – NO)	72.635	76.298	94.033	94.173	0.01	0.085
Average D (PBAT – PT)	70.995	74.448	94.958	95.205	0.018	0.085
Average E (IQI – UK/IE)	71.578	75.275	96.55	96.69	-0.035	0.08
Overall average ABCDE	72.815	76.267	95.711	95.894	0.008	0.079

Table 2.4.12: Overall summary data for this comparison.

Number of classes	**3**	**5**	**3**	**5**
EQR deviation	**No deviation**	**No deviation**	**0.05 EQR**	**0.05 EQR**
Percentage of classification agreement	76.3	72.8	95.9	95.7

In the case of three classes the percentage of classifications that differ by 2 classes is 0.479 for no deviation allowance and 0.028 for the 0.05 EQR deviation allowance situations.

Table 2.4.13: Average and absolute class differences.

Average of class difference	0.008
Absolute average of class difference	0.079

This is a high level of agreement bearing in mind that the data was collected by participating countries independently, in many cases before intercalibration really started and the inherent variability of biological data. This is deemed by the GIG to be an acceptable level of agreement and validates the national boundaries.

DE

In the intercalibration report of Heyer (2007) a comparison of the methods (BEQI, M-Ambi) showed following results:

Comparison of the BEQI, M-AMBI, and IQI were carried out for two sites in NEA1/26. (This differs from the above exercise where a standardised Intercalibration dataset was used by all the Member States involved.)

Table 2.4.14 shows the compilation of the assessment results for different assessment methods for the German intercalibration stations: blue =’high status’(‘I’), green = ’good status’(‘II’), yellow = ’moderate status’(‘III’) and orange = ’poor status’(‘IV’).

Table 2.4.14: DE comparisons.

Waterbody Type	Station	Sediment	Expert judgement	M-AMBI Assessment, DE boundaries	BEQI Assessment, NL boundaries	IQI Assessment, UK boundaries (pre-harmonisation)
NEA1	NS2, Vortrapptief, mean 2002 to 2004	Fine sand to sand	'II' to 'III'	0.77	0.36	0.78
NEA26	Hoher Weg 1988	Low littoral sand	'II' to 'III'	0.46	0,53	0,57
	Hoher Weg 2004			0.52	0,79	0,68

Type NEA1: The assessments of the ecological status of station 'NS2' by three different methods showed following results: 'high status' assessed by the 'IQI, 'good status' assessed by the 'M-AMBI' and 'poor status' assessed by the 'BEQI'. The assessment by the M-Ambi reflected the status given by expert judgement better than the other methods (status 'good' to 'moderate')

Type NEA26: For the year 1988, the ecological status of the stations was assessed as 'moderate' by the three methods. For 2004, the status of the stations was assessed as 'good' by the IQI and the BEQI, while the M-AMBI led again to the assessment 'moderate'. The results of the different methods were in good agreement, also with the expert judgement (status 'good' to 'moderate').

NL

This intercalibration/comparison of methods is on a habitat level, as the BEQI evaluates directly at a habitat level within a waterbody. This approach to intercalibration at a water body level may be completed in Phase II Intercalibration.
Comparison of the BEQI, M-AMBI, IQI, DKI and NQI on Dutch data was done for the habitat Q1 (fine muddy sand) in two water bodies in the North Sea coastal zone, Waddenkust and Hollandse Kust. DKI, IQI and M-AMBI scores were calculated with reference settings suggested by the member states for their defined habitats in the earlier exercise (fixed reference) and with reference values determined from the Q1 reference dataset of the North Sea coastal zone (Table 2.4.15). The reference setting is the same for Hollandse Kust and Waddenkust. NQI does not allow for use of local reference values.
The assessment period is 3 years (2002-2004) with a total of 15 samples per habitat in the two water bodies. Except for the BEQI method the average of assessment station scores within a habitat was taken as the overall habitat status.

Assessment results were very similar for both water bodies (Figure 2.4.13). DKI, IQI and NQI are hardly different between water bodies. BEQI and M-AMBI show more variation between Hollandse Kust and Waddenkust. In all cases, the outcomes were higher when the local determined reference settings were used, in three out of six cases use of the local reference resulted in a shift

Table 2.4.15: Local reference values used for index calculation. Reference values based on the Q1 cluster of stations within six nautical miles from the coast, sampled between 1983 and 1990.

	AMBI		Shannon-Wiener		# Species		Simpson
	Bad	High	Bad	High	Bad	High	Max
Waddenkust	6	0.107	0	2.66	0	31	0.91
Hollandse kust	6	0.246	0	2.83	0	31	0.92

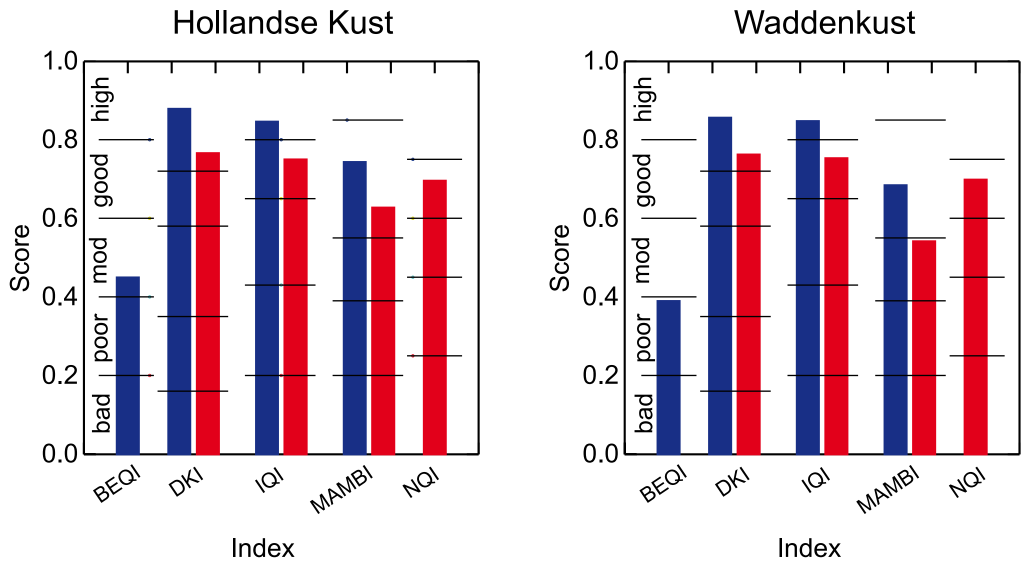


Figure 2.4.13: Bar graph of five metrics calculated for two water bodies of the Dutch coast, Hollandse kust and Waddenkust. Calculations are done using a fixed reference (in red) suggested by the member states and a local reference (blue) determined from a reference data set (Q1 cluster). Boundaries of the indices are indicated with horizontal lines.

to a higher quality status. Of the five compared methods the BEQI method assigned both water bodies to a lower quality class than the other methods, regardless of the reference used (fixed or local). Differences in assessment ranged as much as between poor and high status. These differences are caused by differences in the applied methodology. In the first place, total biomass of the macrobenthic community is included in the BEQI but not in the other assessment methods. Biomass has increased considerably in the North Sea coastal zone since the period of reference setting, causing very low scores for the sub-metric biomass and subsequent low overall scores. Also the measure of similarity between reference and assessment was rated not more than moderate at the Hollandse Kust and Waddenkust, as it detects the changes in species composition caused by an invasive species.

Besides the use of different input and sub-metrics, the BEQI relates assessment and reference conditions differently. The BEQI explicitly takes spatial and temporal variability into account. The class boundaries are determined by the variance in the reference data.

To increase the number of data for comparison, index scores are calculated for several time intervals (four periods: 91-95, 96-98, 99-01 and 02-04). BEQI responds strongest to the temporal and spatial differences with a coefficient of variation (cv) of 0.12. M-AMBI showed less variation in time and space with a cv of 0.09. The other indices DKI IQI and NQI responded least with cvs of 0.06, 0.05 and 0.05 respectively. M-AMBI, DKI, IQI and NQI are all positively correlated. This is not really surprising as the indices rely on the same or similar sub metrics. The BEQI correlates poorly (not significant) with the other methods.

Based on this very limited comparison between the BEQI and classification methods, the BEQI seems to rate water bodies at a lower ecological class than the other methods. Further comparisons with a larger dataset with a larger variation in conditions are needed to further evaluate the performance of BEQI relative to the other indices.

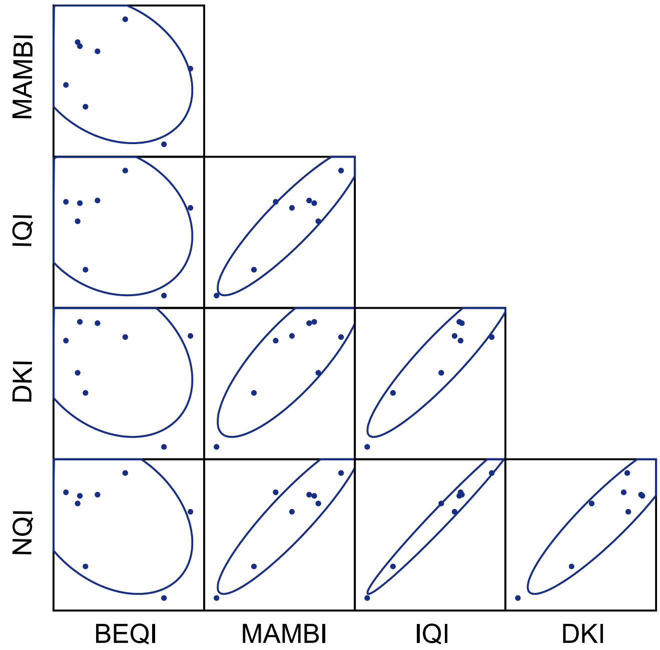


Figure 2.4.14: Scatterplot matrix of the index scores of the different methods calculated for two water bodies and four time periods in the coastal zone of the Netherlands, Waddenkust and Hollandse Kust. Sample ellipses are plotted together with the data.

Type – NEA3/4

NL

At present Germany applies the M-AMBI to the Wadden Sea, which is assigned to Intercalibration type cluster NEA 3/4. A comparison is made between the results of the BEQI method for the different habitats of the Dutch Wadden Sea, and M-AMBI values calculated for the same assessment data. Additionally the BEQI and M-AMBI are calculated for benthos datasets from Norderney and the Leybucht, supplied by Germany for the intercalibration exercise. Norderney data cover a five-year period from 1998 to 2002. For Leybucht, data from a longer period are available and there the six most recent years from 1998 to 2003, are selected for the BEQI and M-AMBI calculation. Information on biomass of the macrobenthic species is not available, so only three of the four sub-metrics are calculated.

Results of the BEQI calculations are presented in Table 2.4.16. For Norderney reference conditions of Middle Littoral Muddy Sand are used. Leybucht results are relative to the reference for High Littoral Mud. Total densities of the macro fauna are higher than the reference. Number of species is very large, in both areas more species are found than described in the reference data. Similarity is rated poor in both areas. The overall score is good for Norderney and moderate for the Leybucht. This difference is due to the larger deviance of total density in the Leybucht. This very strong divergence from the reference conditions is caused by high numbers of *Hydrobia ulvae, Tubificoides benedeni* and *Corophium* sp. This could indicate a disturbed situation, however it is questionable if the reference description is appropriate in this case. No *T. benedeni* or other Oligochaetes are present in the reference set, which might suggest that this group was neglected in the Dutch monitoring, or alternatively this ecotype was not well covered. However, excluding *T. benedeni* in the calculations still leads to a poor density status.

M-AMBI scores are calculated for the same six assessment samples as are used for the BEQI scores, four habitats of the Dutch Wadden Sea are presented and the two German sites discussed above. M-AMBI scores are calculated per station and then averaged per habitat. Reference values are outlined in Section 2.3.3 (reference conditions NEA 3/4).

Table 2.4.16: BEQI results of three sub-metrics for Norderney (years 1998 to 2002) and Leybucht (years 1998-2003) in the German Wadden.

Norderney (Middle Littoral Muddy Sand)

	Assessment		EQR	
Parameters	surface	value	score	status
density	14.34	8206	0.51	moderate
species	14.34	47	1	high
similarity	14.34	0.54	0.38	poor
Overall			0.63	good

Leybucht (High Littoral Mud)

	Assessment		EQR	
Parameters	surface	value	score	status
density	4.98	21784	0.16	bad
species	4.98	32	1	high
similarity	4.98	0.47	0.37	poor
Overall			0.51	moderate

Table 2.4.17: BEQI and M-AMBI assessment (Spanish pre-optimisation boundaries) results for the Dutch and German Wadden Sea habitats. The samples in the Dutch Wadden Sea consist of stations in several areas.

Country& period	Habitat	Area	BEQI		m-AMBI	
			EQR	status	**EQR**	status
		Netherlands				
2003-2005	Brakish Sub-Littoral	Western Wadden Sea	**0.57**	moderate	**0.79**	good
	High Littoral Mud	Balgzand				
2003-2005		Groninger Wad	**0.56**	moderate	**0.767**	good
		Balgzand				
	Middle Littoral Muddy Sand	Groninger Wad				
2003-2005		Piet Scheveplaat	**0.73**	good	**0.683**	good
2003-2005	Low Littoral Sand	Balgzand	**0.795**	good	**0.8**	good
		Germany				
1998-2002	Middle Littoral Muddy Sand	Norderney	**0.63**	good	**0.66**	good
1998-2003	High Littoral Mud	Leybucht	**0.51**	moderate	**0.85**	high

A comparison between the BEQI and M-AMBI scores for the Dutch and German Wadden Sea habitats is made in Table 2.4.17. In three out of six cases the status classification is the same for both metrics. In the other three cases BEQI gives a lower quality status, twice moderate versus good and once moderate versus high for the Leybucht. In the Leybucht the difference is mainly caused by the very high abundances of three species with much stronger effect on BEQI than M-AMBI. Moderate BEQI scores in the Dutch Brackish Sub-Littoral and High Littoral Mud habitats are caused by large biomass values relative to the reference and moderate similarities. This partly explains the difference in status score.

A more detailed analysis of the comparison between BEQI and M-AMBI results for the Wadden Sea does not seem adequate until the number of cases is substantially larger. In the next section data from the Coastal zone of the North Sea, the Wadden Sea and the Eems-Dollard are combined to increase sample size and variation in assessment conditions for a comparison between the two indices. This generates a larger dataset with a larger range of variation in conditions reflected in the indices. M-AMBI scores are calculated per assessment station and averaged per habitat. M-AMBI

reference values are selected from the same reference datasets as are used for the BEQI reference description. Calculation of BEQI scores and results are presented in previous sections of this report.
The comparison was made based on 20 observations from the combined water bodies. In Figure 2.4.15 the AMBI scores are plotted against the BEQI scores. The range of M-AMBI scores is 31 % and the range of BEQI 43 % of the scale from 0 to 1. In this limited comparison most M-AMBI assessments fall within a single category, good and a few in high. The BEQI status assessments mainly fall within two categories moderate and good. Overall the two indices are positively correlated with a Pearson correlation coefficient of 0.45 ($P<0.05$). Within water bodies scores are not related. In five out of twenty cases the status is judged similar by BEQI and M-AMBI, in the fifteen remaining cases the M-AMBI status was higher than the BEQI status.
BEQI and M-AMBI are indices with several differences in methodology. This leads to differences in the outcomes when applied to the same data. On a large scale there is agreement in the direction of the response of the two methods, shown as a positive correlation. However BEQI is more sensitive to changes in the assessment conditions relative to the reference than M-AMBI.

DE

The assessments for the monitoring stations 'T1' to 'T8' by the BEQI and M-AMBI were in good agreement: the ecological status of these stations was assessed by both methods as 'good' and these assessments are also in agreement with expert judgement.
The assessments for the stations of the 'Leybucht' from the BEQI and M-AMBI were different: the ecological status of these stations was assessed as 'moderate' by the BEQI and as 'good' and 'moderate' by the M-AMBI. The BEQI method was in full agreement with expert judgement, which assessed the area as 'moderate' ecological status.

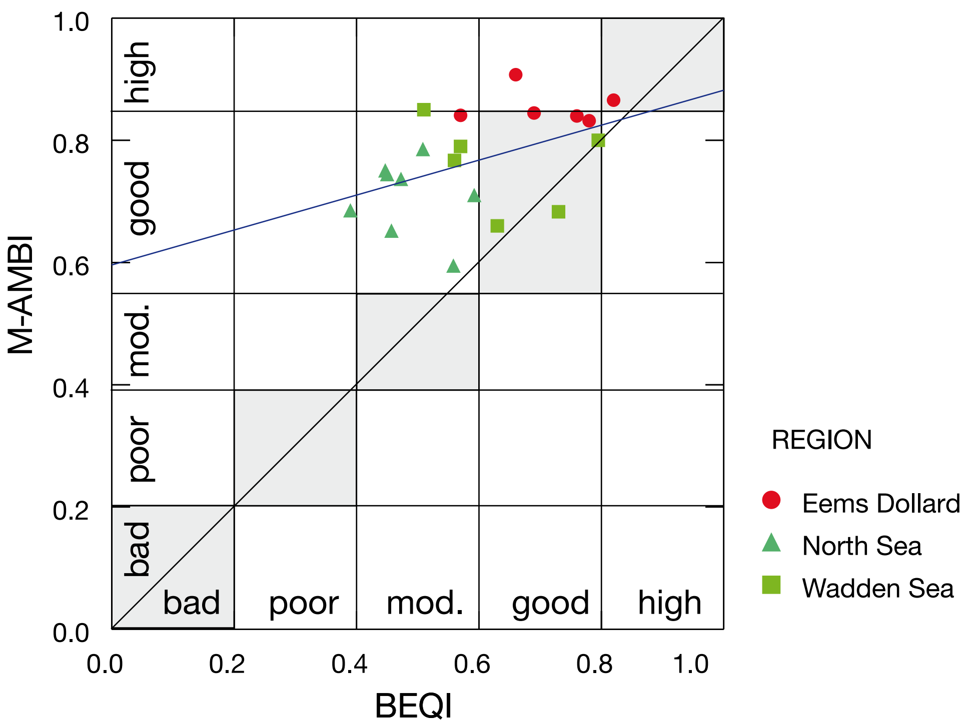


Figure 2.4.15: M-AMBI scores (Spanish pre-optimisation boundaries) plotted against BEQI scores from different water bodies. Quality boundaries are included. Points in the shaded regions have the same status for both indices. The black line is the x = y relationship. The blue line is the linear relationship between M-AMBI and BEQI.

Compilation of the assessment results for different assessment methods for the German intercalibration stations, blue = 'high status'('I'), green = 'good status'('II'), yellow = 'moderate status'('III') and orange = 'poor status'('IV') ecological as shown in table 2.4.18 below.
The boundaries (H/G and G/M), that were proposed by Borja et al. (2007) would result in much too high ecological assessments for the German intercalibration sites, since in comparison to historical papers (eg. Hagmeier & Kändler 1927, Hagmeier 1925, Linke 1939) - at all stations more or less changes in species composition and numbers were detected. Therefore the boundaries (H/G and G/M) had to be adjusted, which was done by expert judgement under consideration of the Normative Definitions of the WFD. The assessment results with the adjusted German boundaries resulted in assessments, which were, at all stations, in better agreement with expert judgement and in most cases in agreement with the other assessment methods.

Table 2.4.18: DE assessments in type 3/4.

Waterbody type	**Station**	**Sediment**	**Expert judgement**	**M-AMBI Assessment,** DE boundaries	**BEQI Assessment,** NL boundaries
NEA ¾	Norderney, Mean T1 to T8, 1998 to 2003	Middle littoral muddy sand	'II'	0.75	0.63
NEA ¾	Leybucht, mean autumn 2003, mud stations	High littoral mud	'III'	0.71	0.51

BE

The comparability between the BEQI (Benthic Ecosystem Quality Index) and the other benthic evaluation methods is tested on a temporal dataset (1995-2003) at a slightly organically enriched station in the Belgian coastal waters. The BEQI evaluates the benthic status on habitat/water body level (by directly pooling the samples), whereas for the other benthic evaluation methods, the average of the EQR (Ecologocial Quality Ratio) of the samples within the habitat/water body is used as the habitat/water body assessment. This way of habitat/water body level assessment has been accepted at the NEA-GIG benthos workshop in Lisbon (February 2007) in anticipation of the final acceptance of the habitat/water body level assessment methods, which are currently in development in other European countries. For an appropriate comparison, only the third level of the BEQI method is applied. The parameter biomass could not be included due to lack of sufficient data. This analysis delivers 22 assessment cases (based on 200 samples) for testing the comparability between the different methods at habitat/water body level. Through the use of the average of the EQR's of the samples as habitat/water body assessment, the comparison best takes into account a standard deviation, in order not to consider variability of the result within sample-based methods as a real difference with another methodology. Therefore, the average of the standard deviations of the sample EQR's of each method has been taken into account. A small deviation from the class boundaries less than 0.05 (IQI, NQI) or 0.06 (DKI, m-AMBI, PT) EQR units, is not considered as a real misclassification. The same principle is applied in other GIG's (Alpine and central-Baltic GIG) for the intercalibration, but they arbitrarily selected 0.05 EQR units. The results of the comparison between the BEQI and the other methods are shown in table 2.4.19.

Agreement between boundaries of the BEQI and the other methods was further assessed according to guidelines issued by ECOSTAT to compare the agreement in classes, both for all five classes and for only three classes (being (1) high, (2) good and (3) moderate or worse). In addition, a comparison was also made between allowing a 0.05 EQR range of deviation around the boundaries and not allowing any deviation. The results of the comparison between the BEQI index (whole water body assessment) used in BE and NL and the other national methods are presented in figures 2.4.16, 2.4.17 and tables 2.4.20, 2.4.21, 2.4.22.

Table 2.4.19: The percentage of cases in which a certain class difference is found between the BEQI and the other methods (a deviation of 0,05 taken into account).

class difference	IQI	DKI	m-AMBI	NKI	PT	Total
-4	0	0	0	0	0	0
-3	4,5	18,2	0	0	0	4,5
-2	22,7	40,9	9,1	9,1	4,5	17,3
-1	50,0	31,8	50,0	45,5	45,5	44,5
0	22,7	9,1	40,9	45,5	50,0	33,6
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
equal or 1 class	72,7	40,9	90,9	90,9	95,5	78,2

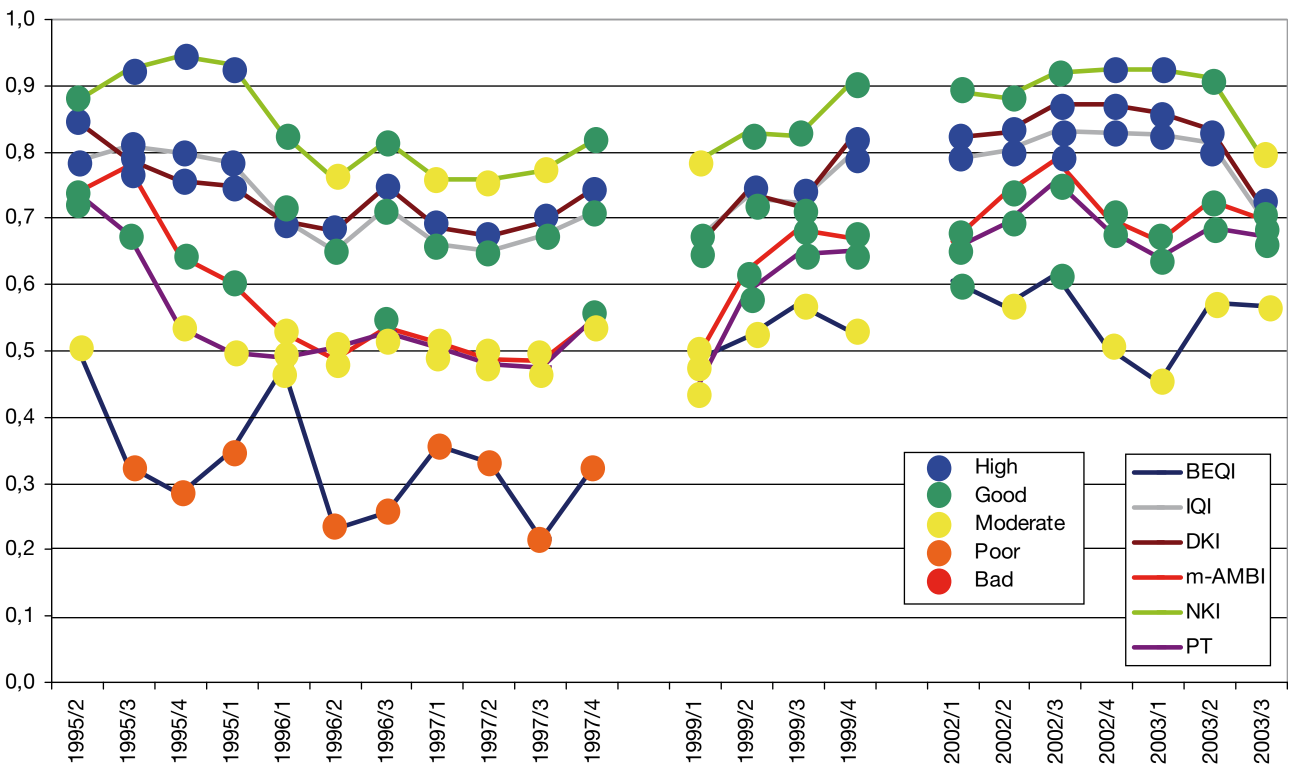


Figure 2.4.16: Comparison of the results from the BEQI and the other national methods (no standard deviation of 0,05 EQR taken into account) for 22 assessments of one site that is subject to some organic enrichment in the Belgian coastal waters. One assessment is a cluster of a series of samples per season (indicated by number 1 to 4).

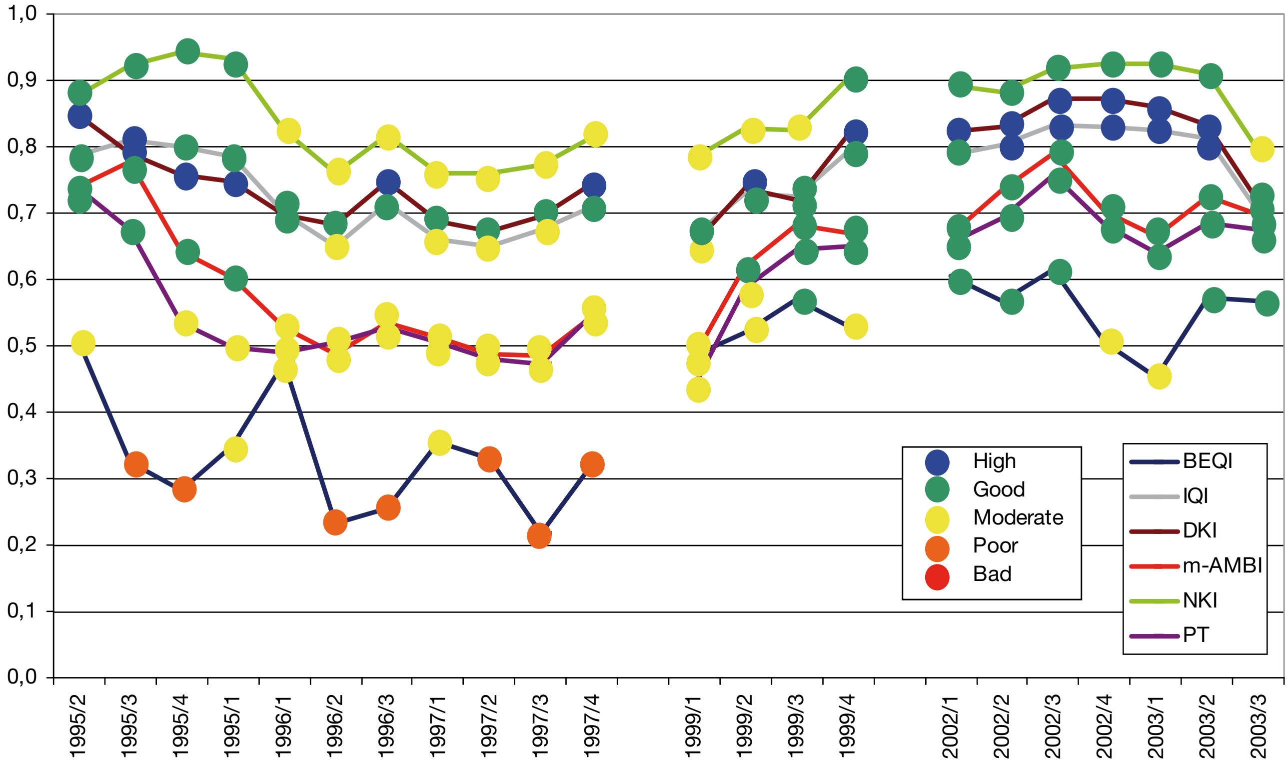


Figure 2.4.17: Comparison of the results from the BEQI and the other national methods (standard deviation of 0,05 EQR taken into account) for 22 assessments of one site that is subject to some organic enrichment in the Belgian coastal waters. One assessment is a cluster of a series of samples per season (indicated by number 1 to 4).

Table 2.4.20: Percentage of classification agreement between the BEQI and the other benthic methods for the four different comparison options.

	3 classes no deviation	5 classes no deviation	3 classes 0,05 EQR deviation	5 classes 0,05 EQR deviation
IQI	0 (40,9)	0,0	36,4 (13,6)	22,7
DKI*	0 (86,4)	0,0	9,1 (45,5)	9,1
m-AMBI	31,8 (4,5)	13,6	63,6 (0,0)	40,9
NKI	36,4 (22,7)	18,2	68,2 (0,0)	45,5
PT	54,5 (0,0)	18,2	77,3 (0,0)	50,0
Total	24,5 (30,9)	10,0	50,9 (11,8)	33,6

*DKI comparability results were very low; more investigation is needed to clarify this and meanwhile caution is needed by the interpretation of this result.
For the option of using three classes, the percentage of classifications that differs 2 classes is given between brackets.

Table 2.4.21: Average of class difference between the BEQI and the other benthic methods.

	3 classes no deviation	5 classes no deviation	3 classes 0,05 EQR deviation	5 classes 0,05 EQR deviation
IQI	-1,4	-1,8	-0,8	-1,1
DKI*	-1,9	-2,3	-1,4	-1,7
m-AMBI	-0,7	-1,1	-0,4	-0,7
NKI	-0,9	-1,3	-0,3	-0,6
PT	-0,5	-0,9	-0,2	-0,5
Total	-1,1	-1,5	-0,6	-0,9

Table 2.4.22: Absolute average of class difference between BEQI and other benthic methods.

	3 classes no deviation	5 classes no deviation	3 classes 0,05 EQR deviation	5 classes 0,05 EQR deviation
IQI	1,4	1,8	0,8	1,1
DKI*	1,9	2,3	1,4	1,7
m-AMBI	0,7	1,1	0,4	0,7
NKI	0,9	1,3	0,3	0,6
PT	0,5	0,9	0,2	0,5
Total	1,1	1,5	0,6	0,9

Figures 2.4.16 and 2.4.17 illustrate that all the methods show similar trends, but still significant differences remain. The conclusion is that, for the current state-of-the-art, the BEQI is generally one class more stringent than the other methods. The PT shows the highest comparability with the BEQI, followed by the NKI and the m-AMBI. The lowest comparability is observed with the IQI and especially the DKI, with which a classification difference of about 2 classes is observed.

The reasons for the deviations can be described as follows:

- different approaches are applied within the BEQI and the other methods concerning statistical confidence (requirements sufficiently sampled area) and water body level assessment versus sample level assessment
- the multimetric set-up of the BEQI includes community species composition, which is more sensitive than a classification based on assigning species to specific categories (sensitive, tolerant etc.)
- the multipressure set-up of the BEQI detects the impact of a combination of pressures on the benthos, not only eutrophication or enrichment with organic matter or specific substances, but also physical disturbances or impacts of invasive species
- significantly higher densities in relation to the reference are considered as a negative impact within the BEQI and contribute to an assessment result with a lower classification
- the amount of reference data used within the BEQI is still very limited and not ideal yet. This has an influence on the reliable estimation of the natural reference situation and the derived statistical boundaries. Further elaboration of the reference dataset and improvement of the method is necessary and will enhance comparability. However, this cannot be done on the very short time and will be performed as part of the monitoring contracts.

The comparability at the level of the habitat/water body will be improved in the second intercalibration round, when every country has its definitive assessment method at habitat/water body level.

Class Boundaries – Member States *(in summary)*

Type NEA1/26

DE, DK, ES, FR, IE, NO, PT, UK

Despite differences in index construction, classifications (M-AMBI, DKI, IQI, NQI and P-BAT) for DK, ES, IE, NO, PT, and UK behave in a similar way. Class boundaries were harmonised through the Intercalibration exercise and status classifications are in good agreement between the Member States.

NL/BE

As the BEQI method evaluates directly at the water body level, and in the current intercalibration exercise no water body intercalibration was done, an adjustment of class boundaries is not considered appropriate. Adjustment of the class boundaries in the harmonisation of ecological status classification of the water body will affect the statistical significance levels for the individual parameters.
Status classification between Belgium and the Netherlands is in full agreement as the BEQI is used by both Member States.

The assessment results comparing the BEQI (BE, NL) with M-AMBI, DKI, IQI, and NQI (DK, ES, FR, IE, NO, UK) clearly result from the differences in methods (as described earlier).

Type - NEA3/4

Adjustment of the class boundaries for the German m-AMBI, as a result of the comparison with the BEQI, is fully consistent for larger (5 year) datasets and sufficient using a one year comparison and expert judgement.
Status classification between DE and NL is in good agreement.

Type - NEA8/9/10

Despite differences in index construction and species classifications indices behave in a similar way and status classifications are in good agreement between Sweden, Denmark and Norway.

Type - NEA11

As described above the boundaries agreed for coastal waters could be used in transitional waters in areas with a similar salinity regime. However, as NEA11 intercalibration will be postponed until Phase II, class boundaries for habitats within varying salinity regimes are not available at this time.

2.4.5 Results of the harmonisation – Boundary EQR values

Type - NEA1/26 and NEA7 (EQRs) (Site level assessment, soft sediment habitat)

	Good/Moderate	*High/Good*
Denmark	0.53	0.67
France	0.53	0.77
Germany	0.70	0.85
Ireland	0.64	0.75
Spain	0.53	0.77
Portugal	0.58	0.79
Norway	0.81	0.92
United Kingdom	0.64	0.75

Type - NEA1/26 (EQRs) (Waterbody level assessment, all habitats)

Belgium	0.60	0.80
Netherlands	0.60	0.80

Type - NEA3/4 (EQRs)

	Good/Moderate	*High/Good*
Germany	0.70	0.85
Netherlands	0.60	0.80

Type - NEA8/9/10 (EQRs)

	Good/Moderate	*High/Good*
Denmark	0.63	0.82
Norway	0.81	0.92
Sweden	0.68	0.89

Type - NEA11 (EQRs)

Boundaries cannot be agreed by June 2007. Boundaries are to be completed in Phase II of the Intercalibration process.

2.4.6 Open issues and need for further work

There are a high number of issues remaining to be resolved in Phase II so clear prioritization must take place.

The intercalibration process to date has concentrated on the assessment of classification methods for the subtidal soft sediment infauna within waterbodies. Classifications relate to general disturbance pressures (hazardous substances, organic enrichment, eutrophication, etc) but not necessarily to physical disturbance (method dependent). (Data used by Member States, however, may relate to multi-pressure environments where physical disturbance may play a role.) Hydromorphological pressures may require a different assessment methodology which takes more account of spatial variation (e.g. habitat extent tools). May also need to revisit the coastal waters types to see if any subdivision is necessary.

The key work area in the next phase will be to develop intercalibration of assessment methods that are applicable in the polyhaline, mesohaline and oligohaline habitats of transitional waters. Both intertidal and subtidal habitats need to be considered.

The majority of developed classifications for the coastal soft sediment infauna, deal with samples grouped within specifically defined habitat types. There is now a general need to assess classifications at a water body level, incorporating sampling design and risk of misclassification. The Dutch/Belgian methodology (BEQI) has made a start with such an approach.

There is also a need to further investigate the intercalibration of methods for other habitats (e.g. maerl, mobile sands, zostera beds, gravels) where they will be sampled by Member States. Reference conditions and methods are still under development and would need to be assessed in a further stage of the Intercalibration process.

The development of reference species lists for all habitats to be considered and agreement on the designation of sensitive species, the role of invasive species in classification, and community changes in habitats where there is little occurrence of “sensitive” species is required.

Methods applicable for hard substratum and subtidal epibenthos assessments are yet to be developed.

Results of ongoing FP6 research could not be taken fully into account in this phase of Intercalibration but should be addressed in starting a second phase of Intercalibration. Better communication is required between the scientists involved in FP7 research related to WFD and the people involved in the application of the science (according to the CIS process). This requires new ways of interactive bridging, which should be made transparent in programming both processes.

All future work is dependent on adequate funding being made available by countries within the GIG. We should also consider using the FP7 framework if possible.

3 Discussion

3.1 Comparability between GIGs

Table 2.4.23: Summary of intercalibration topics per GIGs.

	NEA GIG	MED GIG	BAL GIG
INDICES/Metrics	BEQI, M-AMBI, DKI, IQI, NQI, P-BAT	BENTIX, Multimetric approach, AMBI, M-AMBI, MEDOCC	Shannon, AMBI, M-AMBI
N^{O}. TYPES	7	4 not relevant	1
OPTION	3	3	3
BOUNDARY SETTING PROCEDURE	Equal classes revised by expert judgement	Expert judgement on indices values, Percentage from reference sites	percentage from average reference values; ecological status boundaries equally scaled (80 % for good, 60 % for moderate, 40 % for poor and 20 % for bad status), H/G, G/M.
REFERENCE CONDITION	Expert judgement on indices values,	Expert judgement on indices values, Percentage from reference sites	Expert judgement, unaffected areas or good status sites
OPEN ISSUES	Refine typology, relate to pressure,	consider different sub-regions, introduce new multimetric indices	Refine typology

Metrics

Intercalibration for the benthic invertebrates quality element has been successfully performed by all of the four Coastal Waters GIGs. AMBI and its multivariate version (M-AMBI) results to be the favourite experts choice (or at least the more common), together with Shannon index (in some cases aggregated with other indices) and a few highly specific national methods.

Types

Most of the types result to be covered by the Intercalibration results, even though there is a common view (e.g. MEDGIG) stating that the a-priori types are definitely not relevant for the quality element ecological assessment.

Option

Option 3 has been chosen by all GIG, thus Member States have developed their own classification schemes, these have been assessed against each other through the exchange and evaluation of data and the boundaries in each scheme harmonized to give an acceptable level of agreement.

Boundary Setting Procedure

All Coastal Waters GIGs are providing results for the H/G and G/M boundaries (at least), applying similar approaches based on the expert supervised selection of appropriate percentiles as boundaries best fitting with evident changes in ecological status.

Reference Condition

The expert judgment has been selected as the favorite option in setting Reference Condition.

3.2 Open issues and need for further work

To refine typology going down to a level of details that include the sub-ecoregion in the process has been highlighted as a major task for the next phase.

4 References

Anger, K. 1975. On the influence of sewage pollution on inshore benthic communities in the south of Kiel Bay. Part 1. Qualitative studies on indicator species and communities. Merentutkimuslait. Julk./Havsforskningsinst. Skr. 239:116-122.

Anger, K. 1977. Benthic invertebrates as indicators of organic pollution in the Western Baltic Sea. Int. Revue ges. Hydrobiol. 62:245-254.

Bäck, S., Kauppila, P., Kangas, P., Ruuskanen, A., Westberg, V., Perus, J. & Räike, A. 2006. A biological monitoring programme for the coastal waters of Finland according to the EU Water Framework Directive. Environmental research, engineering and management 4 (38): 6-11 (ISSN 1392-1649)

Blomqvist, M., Cederwall, H., Leonardsson, K. & Rosenberg, R. 2007. Bedömningsgrunder för kust och hav. Bentiska evertebrater 2006. Rapport till Naturvårdsverket 2007-04-11. 70 pp. (in Swedish with English summary)

Borja A., Muxika I., Franco J. (2003). The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts. *Mar. Pollut. Bull.* 46 : 835 – 845

Borja, A., A. B. Josefson, A. Miles, I. Muxika,, F. Olsgard, G. Phillips, J. G. Rodríguez and B. Rygg. 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. *Mar. Poll. Bull.*, 55: 42-52.

Borja, A., Franco, J., Muxika, I., 2003. Classification tools for marine ecological quality assessment: the usefulness of macrobenthic communities in an area affected by a submarine outfall. ICES CM 2003/Session J-02, Tallinn, Estonia, 24–28 September.

Borja, A., J. Franco & V. Pérez, 2000. A marine biotic index to establish the ecological quality of soft bottom benthos within European estuarine and coastal environments, Marine Pollution Bulletin, 40(12): 1100-1114.

Carvalho, S., M. Barata, F. Pereira, M. B. Gaspar, L. C. da Fonseca, and P. Pousão-Ferreira. 2006. Distribution patterns of macrobenthic species in relation to organic enrichment within aquaculture earthen ponds. *Mar. Poll. Bull*., 52: 1573-1584.

Clarke, K. R., R. M. Warwick, 1994. Change in marine communities: an approach to statistical analysis and interpretation. Plymouth, Plymouth Marine Laboratory, 144 pp.

Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance Document № 13, Overall Approach to the Classification of Ecological Status and Ecological Potential, 2005, EC, 53 pp.

Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance Document № 5, Transitional and Coastal Waters Typology, Reference Conditions and Classification Systems, 2003, EC, 116 pp.

Dauvin, J-C. 2007. Paradox of estuarine quality: Benthic indicators and indices, consensus or debate for the future. *Mar. Poll. Bull*., 55: 271-281.

Diaz, R. J. & Rosenberg, R. 1995. Marine benthic hypoxia: a review of its ecological effects and the behaviour responses of benthic macrofauna. Oceanogr. Mar. Biol. Ann. Rev. 33: 245-303.

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy, 72 pp.

Fauvel, P., 1923. Polychètes errantes. Faune de France, Vol. 5, Paris.

Fauvel, P., 1927. Polychètes sédenteires. Faune de France, Vol. 16, Paris.

Glémarec, M. and C. Hily. 1981. Perturbations apportées à la macrofaune benthique de la baie de Concarneau. *Acta Œcologica*, 2: 139-150.

Grall, J. and M. Glémarec. 1997. Using biotic indices to estimate macrobenthic community perturbations in the Bay of Brest. *Estuar. Coast. Shelf Sci*., 44: 43-53.

Gray, J. S., Shiu-Sun Wu, R. & Ying Or, Y. 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. Mar. Ecol. Prog. Ser. 238: 249-279.

Hansson, M. & Håkansson, B. 2004. Indelning av Svenska Övergångs- & Kustvatten i typer enligt Ramdirektivet för Vatten. Dnr: 2002/1796/1933 SMHI (http://www.vattenportalen.se/

Helawell, J.M. 1986. Biological Indicators of Freshwater Pollution and Environmental Management. Pollution Monitoring Series. Elsevier Appl. Sci. Publ., London.

Heyer, K. 2007; German Intercalibration Report for the NEA-GIG (May 2007) ' Assessment of German Coastal Waters (NEA1/26, NEA3/4) by benthic invertebrates with the 'M-AMBI' assessment method' for the Intercalibration Report- Coastal GIGs for the 6th milestone report for CIS

Hily, C. 1984. Variabilité de la macrofaune benthique dans les milieux hypertrophiques de la Rade de Brest. *Ph D thesis*, Úniversité Bretagne Occidentale. Vol 1, 359 pp; Vol 2, 337 pp.

Järvekülg, A. 1970. Bottom fauna as an indicator of pollution of the marine benthal in the vicinity of Tallinn. Estonian Contribution to the International Biological Programme 1: 158-193.

Kangas, P., Byholm, L., Stigzelius, J. 2001: Changes in zoobenthos communities. In: Kauppila, P. & Bäck, S. (eds.). The state of Finnish coastal waters in the 1990s. Helsinki, Finnish Environment Institute. The Finnish Environment ; 472: 79-88.

Kangas, P., Bäck, S. & Kauppila, P.(eds) 2003. Suggestions for a Typology of Coastal Waters for the Finnish Coast According to the European Union Water Framework Directive (2000/60/EC). Mimeograph series of Finnish Environment Institute 284, 51 p. (in Finnish with an English summary)

Karlson, K., Rosenberg, R. & Bonsdorff, E. 2002. Temporal and spatial large-scale effects of eutrophication and oxygen efficiency on benthic fauna in Scandinavian and Baltic waters – a review. Oceanogr. Mar. Biol. Ann. Rev. 40: 427–489.

Labrune C., 2006. Utilisation de la macrofaune benthique en tant qu'indicateur des changements environnementaux. PhD, Université de Perpignan: 1-250 + annexes.

Labrune C., Amouroux J.M., Sarda R., Dutrieux E., Thorin S., Rosenberg R., Gremare A., 2006. Characterization of the ecological quality of the coastal Gulf of Lions (NW Mediterranean). A comparative approach based on three biotic indices. *Mar. Pollut. Bull.* 52: 34-47.

Landner, L., Nilsson, K. och Rosenberg, R. 1977. Assessment of industrial pollution by means of benthic macrofauna surveys along the Swedish Baltic coast. Vatten 3:324-379.

Leppäkoski, E. 1975. Assessment of degree of pollution on the basis of macrozoobenthos in marine brackish-water environments. Acta Academiae Aboensis. 35: 1-96.

Licari M.L, 1998. Mise au point d'un système d'aide à l'interprétation des données benthiques en milieu marin et lagunaire. PhD, Ecole Pratique des hautes Etudes : 1-280 + annexes.

Lipej, L., P. Mozetič, M. Orlando Bonaca, B. Mavrič, M. Šiško & N. Bettoso (2006). Opredelitev ekološkega stanja morja v skladu z Vodno direktivo (Water Framework Directive, 2000/60/ EC). Poročila 85. Morska Biološka Postaja, Nacionalni Inštitut za Biologijo, Piran, 180 str.

Majeed, S. A. 1987. Organic matter and biotic indices on the beaches of North Brittany. *Mar. Poll. Bull.*, 18: 490-495.

Mandaville, S.M. 2002. Benhic Macroinvertebrates in Freshwaters – Taxa Tolerance Values, Metrics, and Protocols. Project H-1, Soil and Water Conservation Society of Metro Halifax. http://chebucto.ca/Science/SWCS/SWCS.html, 48 s.

Marinov, T., 1977. Fauna of Bulgaria, 6. Polychaeta. Sofia, Publishing house of the Bulgarian Academy of Sciences, 258 pp. (In Bulgarian).

Morduhay-Boltovskoy, M. D., (Ed.), 1968. A key to Black Sea and Azov Sea fauna, vol. I, Kiev, Naukova Dumka, 437 pp. (In Russian).

Morduhay-Boltovskoy, M. D., (Ed.), 1969. A key to Black Sea and Azov Sea fauna, vol. III, Kiev, Naukova Dumka, 340 pp. (In Russian).

Morduhay-Boltovskoy, M. D., (Ed.), 1972. A key to Black Sea and Azov Sea fauna, vol. II, Kiev, Naukova Dumka, 536 pp. (In Russian).

Muxika I., Borja A., Bald J., 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European water framework Directive. *Mar. Poll. Bull*., 55: 16-29.

Muxika, I., Borja, Á., Bonne, W., 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. Ecological Indicators, 5: 19-31.

NFS 2006:11 http://www.naturvardsverket.se/sv/Lagar-och-andra-styrmedel/Lag-och-ratt/Foreskrifter-och-allmanna-rad/Foreskrifter-utgivningsordning/#2006

Pearson, T.H. and Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Ann. Rev. 16: 229–311.

Perus, J., Bäck, S., Lax, H-G., Westberg, V., Kauppila, P. & Bonsdorff, E. 2004. Coastal marine zoobenthos as an ecological quality element: a test of environmental typology and the European Water Framework Directive. Coastline Reports 4: 27-38.

Perus, J., Bonsdorff, E., Bäck, S., Lax, H-G., Westberg, V. Villnäs, A. 2007. Zoobenthos as indicator of ecological status in coastal brackish waters: A comparative study from the Baltic Sea. Ambio 36 (2-3): 250-256.

Pinedo, S. 1998. Structure and dynamics of Western Mediterranean soft-bottom communities along a disturbance gradient. Natural and man-induced variability in the Bay of Blanes. *Ph D thesis*, Universidad de Barcelona, Spain. 177 pp.

Rosenberg, R., Blomqvist, M., Nilsson, C.H., Cederwall, H., Dimming, A., 2004. Marine quality assessment by use of benthic species-abundance distributions; a proposed new protocol within the European Union Water Framework Directive. Mar. Poll. Bull. 49, 728-73

Shannon, C. E. & W. Weaver, 1963. The mathematical theory of communication, University Illinois Press, Urbana, 117 pp.

Simboura N, Panayotidis P, Papathanassiou E., 2005. A synthesis of the Biological Quality Elements for the implementation of the European Water Framework Directive in the Mediterranean Ecoregion: the case of Saronikos Gulf. *Ecol. Indicators,* 5: 253-266.

Simboura, N. and A. Zenetos. 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic index. *Med. Mar. Science*, 3(2):77-111.

Simboura, N., E. Papathanassiou & D. Sakellariou, 2007.The use of a biotic index (Bentix) in assessing long term effects of dumping coarse metalliferous waste on soft bottom benthic communities. /Ecological Indicators, 7(1): 164-180.

Todorova V. & Konsulova T., 2005. Manual for collection and treatment of soft bottom macrozoobenthos samples. Online: http://bsc.ath.cx/documents/ExpertNetwork/docs/

Expert %20Network %20- %20Zoobenthos/Todorova %20Manual_zoobenthos.doc

Vuori, K-M., Bäck, S., Helssten, S., Karjalainen, S. M., Kauppila, P., Lax, H-G., Lepistö, L., Londesborough, S., Mitikka, S., Niemelä, P., Niemi, J., Perus, J., Pietiläinen, O-P., Pilke, A., Riihimäki, J., Rissanen, J., Tammi, J., Tolonen, K., Vehanen, T., Vuoristo, H., Westberg, V. 2006. The basis for typology and ecological classification of water bodies in Finland. The Finnish Environment. 807. 151 pp. (in Finnish with English summary)

Wallin M., Wiederholm T., Johnson R.K., 2003. Guidance on establishing reference condition and ecological class boundaries for inland surface waters. Produced by CIS Working Group 2.3 – REFCOND: 86 pp.

Wiederholm, T. 1973. Bottom fauna as an indicator of water quality in Sweden´s large lakes. AMBIO 2:107-110.Warwick R (1986) A new method for detecting pollution effects on marine macrobenthic communities. Mar.Biol. 92: 557-562.

5 Annexes

Annexes can be downloaded from the following address:

http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library?l=/intercalibration_2&vm=detailed&sb=Title

Section 2 – Benthic Invertebrates – Overview of Annexes

A – Baltic GIG

Annex 2.1: Comparison of the Finnish and Swedish macroinvertebrate classification systems using Finnish coastal data from the Baltic Sea

Section 3 – Phytoplankton

1 Introduction

All four coastal water GIGs have been able to produce results for the phytoplankton quality element. NE Atlantic, Baltic and Mediterranean GIG have intercalibrated single metrics based on chlorophyll *a* concentration, while Romania and Bulgaria in the Black Sea GIG agreed on the phytoplankton boundaries based on seasonal average biomass (mg/m^3). Only NEA GIG intercalibrated also other metrics (besides chlorophyll a) indicative for phytoplankton composition. Those were Indicator Taxa (Frequency of Phaeocystis Cell counts) and Taxa Cell Counts (Frequency of phytoplankton taxa cells counts).

All the GIGs have chosen option 2 or a hybrid between option 2 and 3 in this phase of the intercalibration process. Member States have developed metrics as part of their own classification schemes. All Selected metrics have been chosen by each GIG for assessment and agreement of boundaries in this phase of the intercalibration process. At this stage not all the metrics that make up Member States' schemes can be intercalibrated, so it is not possible to produce EQRs for the whole quality element. Boundaries have been agreed for the selected metrics.

2 Methodology and results

2.1 Baltic GIG

2.1.1 Intercalibration approach

The Baltic Sea Geographical Intercalibration Group (GIG) includes the whole or parts of the coastline of the following countries: Germany, Denmark, Estonia, Finland, Latvia, Lithuania, Poland and Sweden (Table 2.1.1).

The common coastal water types are characterised by the descriptors of the System B typology. The typology factors are based on the common typology framework presented in the guidance on the typology for the coastal and transitional waters[20]. In the Baltic Sea GIG, the common intercalibration types were characterized using basic salinity and exposure with further delineation based on depth and number of ice cover days (Table 2.1.1). One transitional water type (TW B 13) was identified. All countries agreed to focus the intercalibration on the quality elements that are sensitive to eutrophication pressures.

The common intercalibration types were characterized by the following descriptors:

- – Salinity (using practical salinity scale): low (0,5-3) and high (3-6) oligohaline, mesohaline (6-22)
- – Depth: all shallow (<30 m)

[20] Guidance document No. 5 'Transitional and Coastal Waters - Typology, Reference conditions, and Classification systems'. Common Implementation Strategy of the Water Framework Directive, Available at: http://forum.europa.eu.int/Public/irc/env/wfd/library

– Exposure (using agreed Pan-European scale[21]): exposed, sheltered and very sheltered
– Duration of ice cover: >150 days/ year, 90-150 days/ year, no or very short ice cover

For phytoplankton a hybrid between option 2 and 3 was used. A common metric – summer mean of chlorophyll *a* from May/June to September[22] - was agreed. Intercalibration was performed by comparison of the results of the national assessments. Within each type member states essentially agreed on common reference and classifications values for the whole type or for sub areas in the type.

Details of the national methods, reference condition and boundary setting, as well as references to earlier literature and reports within each country, are available in Annex 3.1-3.7.

Table 2.1.1: Description of Baltic Sea Common intercalibration types that have included in the intercalibration exercise.

Type	Salinity psu	Exposure	Depth	Ice days	Other Characteristics
CW B0	0.5- 3	Sheltered	Shallow	> 150	Sites in Botnian Bay (Northern Quark)
CW B2	3-6	Sheltered	Shallow	> 150	Sites in Bothnian Sea
CW B3 a	3-6	Sheltered	Shallow	90-150	Sites in the area extending from the southern Bothinian Sea to the Archipelago Sea and the western Gulf of Finland
CW B3 b	3-6	Exposed	Shallow	90 -150	
CW B12 a Eastern Baltic Sea	5-8	Sheltered	Shallow	-	Sites in the Gulf of Riga
CW B12 b Western Baltic Sea	8 - 22	Sheltered	Shallow	-	Sites at the Southern Swedish coast and the South western Baltic Sea open coast along Denmark and Germany
CW B13	6-22	Exposed	Shallow	-	Sites along the coast of the Estonia, Latvia and Lithuania, the Polish coast and the Danish island “Bornholm”
CW B 14	6-22	Sheltered	Shallow	-	Lagoons
TW B 13	6-22	Exposed	Shallow		Transitional water. Sites along the coast of Lithuania and Poland

Countries sharing types that have been intercalibrated:
Types CWB0, CWB2, CWB3a, CWB3b: Finland, Sweden.
Type CWB12a: Estonia
Type CWB12b: Germany, Denmark, Sweden.
Type CWB13: Denmark, Estonia, Lithuania, Latvia, Poland.
Type CWB14: Denmark, Poland
Type TWB13: Lithuania, Poland.

21 According to the definitions of the common European exposure categories; Guidance document No. 5
22 The definition of the length of the summer period depends on the latitude and area of the Baltic Sea.

2.1.2 National methods that were intercalibrated

In the Baltic Sea GIG average summer chlorophyll *a* concentration was chosen as the metric for the intercalibration of the phytoplankton element. All countries used the monitoring data available in their national data bases to set the reference conditions for their national types. At the time of the intercalibration, final national metrics were not yet available. Chlorophyll *a* values for summer growth season were averaged to obtain reference conditions and boundary values for seasonally defined phytoplankton metric. The period of aggregation varied depending of the latitude. Finland and Sweden used average chlorophyll *a* values from data sampled between June and August for the types in the Northern Baltic Sea. For the types in the Baltic Proper (B13) and Gulf of Riga (B12) Estonia used growth period between June-September to calculate average values. In the Southern Baltic Sea, Denmark and Poland used values averaged over May-September. In Germany chlorophyll *a* values were originally averaged for the period between March-October in their national reference conditions setting.

Further information on national methods and assessments can be found in Annexes 3.1-3.7**.**

2.1.3 Reference conditions and boundary setting:

Denmark

Two methods have been used to establish reference conditions for phytoplankton biomass expressed as chlorophyll *a* in Danish waters:

1. Development of reference conditions using historical Secchi depth measurements and relationships between Secchi depth and chlorophyll *a* obtained from recent monitoring data from Danish coastal waters (more detailed presentation of the method and procedure is presented in the Annex 3.1) Reference conditions for chlorophyll *a* are calculated as predictions from the relationships corresponding to an average secchi depth around the beginning of the 20th century.
2. Reference conditions were estimated from a combination of 1) hind-casted nutrient inputs (loading of total nitrogen) to the Danish straits based on estimates of the nitrogen surplus from Danish agriculture and estimated changes in point sources, 2) characterization (expert judgment) of reference loading using the hind-casted estimates, and 3) historical nitrogen inputs projected into total nitrogen (TN) levels and related to chlorophyll *a* levels in coastal waters (see Annex 3.1 for detailed presentation)

Two different approaches were used to set the chlorophyll *a* boundaries between good and moderate ecological status for the selected Danish intercalibration sites (described in detail in Annex 3.2):

1. Historical secchi depth observations are compared to chlorophyll *a* -secchi depth relationships established from recent data (as described above). Boundaries between good and moderate status for chlorophyll *a* are then defined as reference conditions plus 50 % in accordance with the HELCOM Eutro approach.
2. Relationships between nitrogen loading and total nitrogen (TN) as well as between TN and chlorophyll *a* are established using recent monitoring data. Site-specific boundaries for TN and chlorophyll *a* were predicted from modelled time series of nutrient inputs to the Danish straits. The boundary values of nutrient inputs for different time periods were been selected using expert judgement.

Comparing the results from the two approaches indicated that the use of historical nitrogen inputs (method 2) generally resulted in lower reference conditions and boundaries than the use of historical secchi depths (method 1). Confidence intervals for the historical secchi depth method are also wider, suggesting that the use of historical nitrogen input is giving more precise and unbiased estimates of reference conditions (Table 2.1.2).

Table 2.1.2: Comparison of good - moderate boundary values of the summer mean chlorophyll *a* (μg l^{-1}) from a number of Danish coastal water bodies, based on two different approaches: 1) Historical secchi depths and chlorophyll *a* - secchi relationships, and 2) historical nitrogen inputs projected into total nitrogen levels and subsequently to chlorophyll *a* levels. In the second approach median values are shown in order to obtain a comparable value to the geometric mean of the first approach.

Area	1) Historical secchi depths			2) Historical nitrogen inputs		
	Geometric mean	Lower confidence	Upper confidence	Median	Lower confidence	Upper confidence
Northern Kattegat	2.28	1.72	3.01	1.69	1.45	1.96
Fakse Bay	2.06	1.51	2.81	1.62	1.04	2.34
Hjelm Bay	2.32	1.75	3.06	1.53	0.86	2.37
Hirtshals	2.42	1.65	3.54	2.84	2.62	3.07
Århus Bay	2.40	2.11	2.73	1.84	1.60	2.09
West of Bornholm	3.66	2.57	5.23	1.31	1.01	1.68
Outer Wadden Sea	3.76	2.82	5.01	6.13	5.57	6.74
North of Funen	2.79	2.42	3.22	2.25	1.97	2.56
Northern Sound	2.55			1.55	1.05	2.17
Dybsø Fjord				1.31	0.65	2.55
Inner Wadden Sea				6.10	5.25	7.11

The recommended boundaries for the Danish intercalibration sites, based on the relationship between total nitrogen and chlorophyll *a* (as method 2), are presented in Table 2.1.3. The good-moderate boundaries are calculated as the range between the median value and the upper confidence value from the Table 2.1.2. The high-good boundary and the reference conditions are established by expert judgement based on historical nitrogen loading and corresponding TN and chlorophyll *a* relationships.

Table 2.1.3: Recommended chlorophyll *a* boundaries (arithmetic mean for summer values between May –September, as µg l^{-1}) for the Danish intercalibration sites in the Baltic Sea.

Intercalibration sites	Reference conditions	High/good boundary	Good/moderate Boundary
Fakse Bay Hjelm Bay	1.4	1.5	1.7 – 2.6
Bornholm west	1.2	1.3	1.5 – 1.9
Dybsø Fjord	0.9	1.1	1.6 – 3.1

Estonia

In Estonian waters reference conditions were obtained by setting empirical relationships between nutrient concentrations, water transparency and phytoplankton parameters. The time series data from the period 1993-2005 was used to assess average concentrations levels and trends for summer period (between June to September) in each national coastal type. Reference conditions were set by using frequency distributions and assuming that 20 % deviation from the lowest concentrations as reference conditions. For the Secchi depth transparency, 20 % deviation from the maximum values was proposed as reference conditions, respectively.

Expert judgement based on analysis of the monitoring data from the period 1993-2005 was used in the boundary setting. The areas belonging to the Baltic GIG common type B13 have been monitored only in 2005. The time series data were considered to assess average concentrations levels and trends in each coastal type. The high-good and good-moderate boundary values for chlorophyll *a* were derived as 20, and 50 % deviation from the reference conditions (Table 2.1.4).

Table 2.1.4: Recommendations for the reference conditions and classification boundaries for the Estonian coastal waters belonging to the common intercalibration type B13. The boundary value for the high status class represents 20 % deviation and the good-moderate boundary 50 % deviation from the reference conditions. The two lower boundaries are derived as 70 % and 90 % deviation from the reference conditions.

Parameter	Reference value	unit	% deviation from reference conditions			
			20 %	50 %	70 %	90 %
Total nitrogen (annual mean)	10.6	μM	12.27	15.90	18.02	20.14
Total phosphorus (annual mean)	0.28	μM	0.336	0.42	0.476	0.532
Secchi depth	8	m	7.20	6.00	4.40	2.40
Chlorophyll *a*	1.1	mg m^{-3}	1.32	1.65	1.87	2.09
Biomass	0.29	mg l^{-1}	0.348	0.43	0.493	0.551

Finland

In Finnish coastal waters, the reference values of chlorophyll *a* were estimated based on three methods: (1) empirical modeling using the relationships between chlorophyll *a* and Secchi depth, (2) statistical analyses on frequency distribution data by using 5 % deviation from the lowest values as the boundary between high and good status and (3) trend analyses of chlorophyll *a* in the intercalibration sites or in the vicinity of them (detailed presentation in Annex 3.4).

Type specific reference conditions for phytoplankton have been estimated using empirical relationships between concentrations of chlorophyll *a* and Secchi depth of the present monitoring programs and the historical Secchi values of the early 1900s (Annex 3.4). The reference chlorophyll *a* concentrations represent mean values which were estimated from the empirical equations using the historical Secchi values covering the Finnish intercalibration types. Following the Swedish model, the 95 % confidence limits of variation were estimated to represent the boundary between high and good at its maximum.

Scientific grounds are required to justify the boundaries between good and moderate status. Therefore, changes in eutrophication, revealed by other ecological variables, were used to find out if there is an ecologically reasonable base to estimate the chlorophyll boundary between good and moderate status in the Finnish coastal types. The variables included were the depth limit of *Fucus vesiculosus* and the occurrence of cyanobacteria in phytoplankton assemblages in relation to Secchi depth and chlorophyll *a* concentrations.

Germany

In the German coastal waters, modelling was used to calculate loading of total nitrogen (TN) from rivers (see Annex 3.7). The marine value of 10 µM TN was taken from the literature was considered to be consistent with pristine coastal data. This value was also taken for the Baltic Sea due to the long time period between pristine and recent conditions. References for all salinities were estimated using mixing diagrams. For local calculations of references, recent mean salinity data and the freshwater reference of the next eastern located river were used, assuming a residual current moving westward along the surface of the German Baltic coastline. The reference chlorophyll *a* values were calculated using regression between total nitrogen (TN) and chlorophyll *a* concentrations during the growth season (months March- October).

Boundary values were calculated from summer values of recent monitoring data. The analysis is based on 438 data sets sampled during 1978 and 2004 on 8 outer coastal sites along a salinity gradient from 7 to 17 PSU, which represent the extended intercalibration sites. A significant correlation between TN and chlorophyll *a* could not be found for these intercalibration sites. Therefore the boundary setting was performed by linear division along the actual gradient of chlorophyll *a* values. The 80 %-percentile (3.4 µg chlorophyll *a* /l) marked the boundary poor-bad. The boundaries between good and moderate status as well as between moderate and poor status represent the three equal parts between high and poor state of the water type.

Poland

Reference conditions were calculated on the basis of empirical relationships obtained from contemporary monitoring data. Only data obtained in summer period (May-September) in 1999-2005 were taken into consideration – in total: 61 chlorophyll *a* concentration values with corresponding water transparency values (SD) and TN concentration values (detailed description in Annex 3.5).

Assuming that 6 m is a water transparency reference value for summer – estimated from historical data, corresponding chlorophyll *a* concentrations values were calculated by means of linear regression. Chlorophyll *a* reference values were also estimated on the basis of total nitrogen (TN) concentration reference values determined for outer Puck Bay, using the method of extrapolation of temporary trends. Results of reference value calculations obtained by different methods are shown in the Table 2.1.5.

In the calculations of the relationship between chlorophyll *a* and TN, only summer nitrogen concentrations were used because data from the winter period was incomplete. However, the relationship between chlorophyll *a* and transparency was better, and therefore the chlorophyll *a* reference concentration for Kępa Redłowska intercalibration site was estimated by using the chlorophyll *a* and water transparency regression. The obtained reference values (3.7 $mg \cdot m^{-3}$) was

Table 2.1.5: Chlorophyll *a* (chl-a) reference values for Kępa Redłowska area assessed on the basis of relationship with water transparency and total nitrogen (TN) concentrations.

Method of reference values determination	Chlorophyll *a* reference value
Linear correlation chl-a/ transparency	3.7
Exponential relationship chl-a/ transparency	3.7
Linear correlation chl-a/ TN	3.0
Exponential relationship chl-a/ TN	3.1

much higher than the reference value (1.2 mg·m^{-3}) obtained for the Swedish coastal waters type for the CWB13. However, the this value reflects the strong impact of the Vistula River – one of the biggest rivers in Europe. Therefore a significant part of the Gulf of Gdańsk (inner Gulf of Gdańsk) was assigned to be transitional waters having thus higher reference values for that region than for other coastal waters belonging to the type B13.

Discontinuities, which could indicate class boundaries, in the chlorophyll a concentration/ water transparency relationship were not found. Consequently, the ecological status classification was based on statistic methods.

Values 25 % and 50 % were agreed to be the acceptable deviation from the reference conditions (following recommendation of the HELCOM EUTRO project), that is, if the chlorophyll a concentration increases by 25 % or 50 % in relation to reference value, ecological state is still good. With regard to considerable scatter of chlorophyll a concentration values, which indicates significant natural variability of environmental conditions, the higher acceptable deviation from reference value – 50 % was adopted. In this way the boundary between good ecological status and moderate ecological status (3.7 + 50 %) was obtained and it equals chlorophyll a concentration value – 5.6 mg·m^{-3}.

The boundary between moderate and poor ecological status was established on the basis of statistical analyses on the frequency distribution of data. This value was 7.3 mg·m^{-3}, which is a median in value range 6.0<chl-a<9.0 mg·m^{-3}. Proposal of ecological status classes for Kępa Redłowska intercallibration site is presented in Table 2.1.6.

Table 2.1.6: Ecological classification for Kępa Redłowska intercallibration area.

High/ reference value	Good	Moderate	Poor	Bad
<3.7	3.8 – 5.6	5.6 – 7.3	7.3 - ?	?

Sweden

The reference values for total nitrogen (TN) were estimated using historical data on Secchi depth, and by using empirical relations between nutrients and Secchi depth based on current data. The modern relationships between nutrients and phytoplankton biovolume and chlorophyll *a* were used to estimate reference conditions for these variables. Historical secchi depth data indicate the Secchi depth has decreased in the Baltic proper since the beginning of the 20th century from ca 10 m to 7 m. We have used 10 m as reference value for summer Secchi depth in the Baltic proper.

From the empirical relationships between Secchi depth and TN, a reference value for total nitrogen of 15.3 µM (214 µg/l) was estimated for the open coastal waters of the Baltic proper (See Annex 3.6 for details). Using the TN reference value of 15.3 µM and the empirical relationships found between chlorophyll a and TN, a reference value for chlorophyll of 1.2 µg/l was estimated for the open coastal waters of the Baltic proper

Outside major freshwater outflows, salinity gradients cause conditions in the surface water bodies to vary greatly. Therefore a simple mixing model was used to calculate individual surface water body reference conditions corrected for background concentrations in freshwater total nitrogen discharges according to salinity (Annex 3.6).

The reference value indicates the average pristine condition. There is natural variation around this value. The boundary between high and good status was set at a level where this natural variation is judged to be exceeded. The 95 % confidence limit of variation was used as guidance.

Classifications for the Baltic proper coastal areas are based on dose-effect relations with total nitrogen which was used to calibrate classes to assure a similar outcome of classification exercises. In the Baltic proper the relation between phytoplankton and TN was stronger than between phytoplankton and TP and consequently only TN was used to develop classifications for phytoplankton. The empirical relationship between chlorophyll and TN was used to set corresponding boundaries for chlorophyll. The southern Gulf of Bothnia is influenced by the Baltic proper and a similar classification has been used in both areas. Further north in the Gulf of Bothnia the influence of humic substances from freshwater increases which motivate an upward adjustment of the classification limits for Secchi depth, chlorophyll *a* and phytoplankton

The classification of the general condition in open coastal areas as moderate means that the ecological status of many coastal waters is not mainly determined by the local discharges, but rather the general conditions of the Baltic Sea (Fig. 2.1.1). This calls for methods to separate the effect on coastal waters into two parts: one that is directly manageable by local measures and one part that is manageable by efforts by all countries together. An empirical model was developed to provide quantitative estimates of the possible improvements in Secchi depth and reductions in chlorophyll *a* and phytoplankton biomass resulting from local measures (Annex 3.6).

Estimated chlorophyll **a** values for reference conditions and classification boundaries, as well as the subsequent Ecological Quality Ratios for Swedish coastal waters corresponding the common intercalibration types for the Baltic Sea are presented in Table 2.1.7.

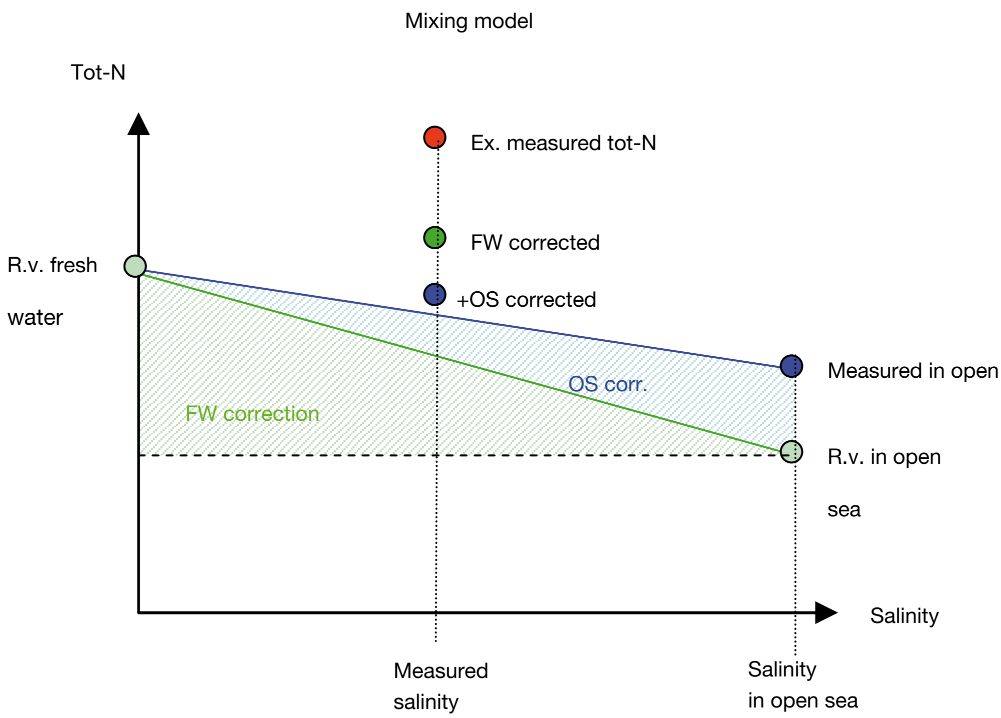


Fig. 2.1.1: Mixing model used to correct for natural background of *fresh water inputs of total nitrogen* (FW correction). The fresh water corrected value forms the basis for classification of ecological status. The model also can be used to estimate the influence of the *open sea on total nitrogen* (OS correction). The open sea correction is used to estimate the degree to which local measures will have an effect on local ecological status (R.v. = Reference values).

Table 2.1.7: Reference values (Rv), class boundaries (HG=high-good, GM= good-moderate, MP= moderate-poor, PB= poor-bad) and corresponding EQR for summer levels of chlorophyll *a* (µg/l). Grey marking means that values should be corrected according to salinity and estimated natural background load of nitrogen in respective area before comparison with class boundaries (see text). Intercalibration sites correspond to the the common intercalibration types of the Baltic GIG.

Country& period	Habitat	Area	BEQI		m-AMBI	
			EQR	status	**EQR**	status
		Netherlands				
2003-2005	Brakish Sub-Littoral	Western Wadden Sea	**0.57**	moderate	**0.79**	good
	High Littoral Mud	Balgzand				
2003-2005		Groninger Wad	**0.56**	moderate	**0.767**	good
		Balgzand				
	Middle Littoral Muddy Sand	Groninger Wad				
2003-2005		Piet Scheveplaat	**0.73**	good	**0.683**	good
2003-2005	Low Littoral Sand	Balgzand	**0.795**	good	**0.8**	good
		Germany				
1998-2002	Middle Littoral Muddy Sand	Norderney	**0.63**	good	**0.66**	good
1998-2003	High Littoral Mud	Leybucht	**0.51**	moderate	**0.85**	high

2.1.4 Results of the comparison and harmonisation

Type B0: Salinity 0.5-3, sheltered, shallow, >150 ice days

Sites	Preliminary Status[1]	Reference values	Calculated value 1995 – 2000	Calculated value 2000 – 2004	Time period for ref values	Finland Classification	Sweden Classification
Bergö (FI)	-	1.3	2.2	2.0	1925-1932	G	G
Gaviksfjarden (SE)	H/G	1.4		1.4	1900-1920	H	H
Holmöarne, N	G/M	1.1	Data missing				
Kvarken (SE)	H/G	1.2	1.6		1900-1920	H	H
The Quark – Orefjärden (SE)							

[1]Borderline in the Intercalibration network register

Classification table

Type B 0	Boundary high/ good	EQR H/G	Boundary good/ moderate	EQR G/M	Period
Sweden: Gaviksfj. Holmöarna Örefjärden	1.8 1.5 1.8	0.78 0.73 0.67	2.3 2.0 2.3	0.61 0.55 0.52	June – August
Finland	1.8	0.72	2.7	0.48	July – August

Type B2: Salinity 3-6, sheltered, shallow, >150 ice days

Sites	Prelimin. Status[1]	Reference values	Calculated value 1995 – 1999	Calculated value 2000 – 2004	Time period for ref values	Finland Classifikation Data 2000-2004	Sweden classifikation
Domarkobban (FI)	H/G	1.4	-	1.4	1925-1934	H	H
Järviluoto (FI)	G/M	1.4	3.8	3.2	1925-1934	M or lower	M or lower
Pjelaxfjarden (FI)	G/M	1.4	2.4	4.2	1925-1934	M or lower	M or lower
Rounakari (FI)	G/M	1.4	3.3	2.1	1925-1934	G	G
Harkskars and Trodjefjard (SE)	H/G	1.4	-		1900-1920		
Langvinds- and Skarsfjarden (SE)	H/G	1.4	-				
The Bay of Gavle, Outer (SE)	G/M	1.4	-				
Ljusnan and Voxnan (SE)	G/M	1.4	-				

[1]Borderline in the Intercalibration network register

Classification table

Type B 2	Boundary high/ good	EQR H/G	Boundary good/ moderate	EQR G/M	Period
Sweden	1,8	0,78	2.3	0,61	June – August
Finland	1,8	0,78	2,6	0,54	July – August

Type B3: Salinity 3-6, sheltered, shallow, 90-150 ice days

Sites	Prelimin. Status[1]	Reference values	Calculated value 1995 – 1999	Calculated value 2000 – 2004	Time period for ref values	Finland Classification	Sweden Classification
Bågaskär I (FI)	G/M	1.8	4.9	6.3		M or lower	M or lower
Långskär I (FI)	H/G	1.8	3.7	6.6	1925- 1934	M or lower	M or lower
Putsaari II (FI)	H/G	1,5	2.1	2.4		H	M or lower
Seili II (FI)	G/M	1.5	2.7	4.2	c.1930 –	M	M or lower
The Askoe area (SE)	G/M	1.2	2.7*	2.7**	1950	G	M or lower
The Yxla area (SE)	G/M	1.3		3.3***		G or M	M or lower

[1]Borderline in the Intercalibration network register
* 0 m, Jun-Aug 1995-1999, 1 station, ca 6 samplings/year during Jun-Aug
** 0 m, Jun-Aug 2000-2003, 1 station, ca 6 samplings/year during Jun-Aug
*** 0 m, Aug 2001, 2004, 2005, 6 stations, one sampling per year.

Classification table

Type B 3	Boundary high/good	EQR H/G	Boundary good/moderate	EQR G/M	Period
Sweden: Askoe area (More exposed)	1.5	0.80	1.8	0.67	June – August 0 m
Sweden: Yxla area	1.6[a]	0.81	1.9[a]	0.69	June - August 0 m
Finland I: Archipelago Sea II: Western Gulf of Finland	 2.2 2.6	 0.82 0.58	 2.9 4.0	 0.62 0.38	July – August

a: Dynamic reference values (and boundaries) estimated from salinity during sampling.

Type B 12b: Salinity 6-22, sheltered, shallow

Sites	Borderline in Intercalibration network database	Reference value	Calculated value 1995-1999	Calculated value 2000-2004	Time period for reference values	German classification	Swedish classification	Danish classification
Faxe Bay, DK	G/M	1,4		1,9	1904-1911	G/M	G/M	G/M
Hjelm Bay, DK	G/M	1,4		1,6	1903-1912	G	G	G
Darss-Zingst DE	G/M	<1,1	1,8	1,8		G	G	G
Geltinger Birk, DE	G/M	<1,1	4,6	2,7		M or lower	M or lower	M or lower
Arc. Torhamn, SE	G/M	1,2	2,7*	2.6**		M or lower	M or lower	M or lower
Koiguste laht, EE	G/M	1,8						

* June - Aug 1996-1999, discrete samples between 0-4m, 1 station, 1 sampling per year (station K19).
** June - Aug 2000-2004, discrete samples between 0-4m, 1 station, 1 sampling per year (station K19).

Classification table

Type B 12	Boundary high/good	EQR H/G	**Boundary good/moderate**	**EQR G/M**	**Period**
Denmark	1.5	0.93	1,9	0.74	May – September
Sweden	1,5	0.80	1,9	0.63	June – August
Germany, Geltinger Birk[1)]	1,1	-	1,9	< 0.58	June – September
Germany, Darss-Zingst	1,1	-	1,9	< 0.58	June – September
Estonia	2.2	0.82	2.7	0.66	June – September

[1)] Site at the mouth of Flensburger fjord.

Type B 13: Salinity 6-22, exposed, shallow

Sites	Borderline in Intercalibration network database	Reference value	Calculated value 1995-000	Calculated value 2000-2004	Time period for ref values	Estonia/Latvia classification	Danish classification
Bornholm vest (DK)	G/M	1,2		1,4	1958-59	G	G
Kudema Bay (EE)	H/G	1,1					
Open coast 6 (LT)	G/M	3.2		4,5	?		
Baltic Sea 3 (TW) (LT)	G/M	3.85		8,6	?		
LI (LV) [1]	H/G	1.1					
Rowy (PL) *	H/G	<1,5		?	?		
Kepa Redlowska (TW) (PL)	G/M	< 3.7		?	?		

Classification table

Type B 13	**Boundary high/good**	**EQR H/G**	**Boundary good/moderate**	**EQR G/M**	**Period**
Denmark	1,3	0.92	1,6	0.75	May – September
Estonia and Latvia[1]	1,3	0.85	1.65	0.66	June – September
Data below are not comparable with the information from other countries					
Lithuania coast	4		5		?
Lithuania outside lagoon (TW)	4.8		6		May – September
Poland Rowy*	1.5		3		June - September
Poland Kepa Redlowska (TW)	3.7		5.6		May – September

* Polish coastal water type B 13 – Rowy is situated too close to river mouth so it is not representative for type or water body.
[1] LV has adopted the assessment made by Estonia. The sites in Estonia and in Latvia are very similar (expert judgement). LV has not developed their own assessment system and has no data from the intercalibration site.

Type B 14: Salinity 6-22, sheltered, shallow lagoons

Sites	Borderline in Intercalibration network database	Reference value	Calculated value 1995-2000	Calculated value 2000-2004	Time period for ref values	Polish Classification	Danish Classification
Dybsø Fjord (DK) Mielizna Borzynska (PL) [1]* Piaski Dziewicze (PL) [1]*	G/M H/G G/M	0,9 5 4	2,3	1,3			G/M

Classification table

Type B 14	Boundary high/good	EQR H/G	Boundary good/moderate	EQR G/M	Period
Denmark	1,1	0.82	1,6	0.56	May – September
Data below not comparable with the information from other countries					
Mielizna Borzynska (PL)*	5		10		?
Piaski Dziewicze (PL)*	4		7,5		?

* Transitional waters. Data not comparable with the information from other countries

2.1.5 Results of the harmonization – Boundary EQR values

Summery table with the results of the phytoplankton intercalibration in the Baltic Sea area.

	Reference conditions	Boundary High/good	EQR High/Good	Boundary Good/ moderate	EQR Good/ Moderate
Type B 0					
Sweden	1.1 – 1.4	1.5 – 1.8	0.67 – 0.78	2.0 – 2.3	0.52 – 0.61
Finland	1.3	1.8	0.72	2.7	0.48
Type B 2					
Sweden	1.4	1.8	0.78	2.3	0.61
Finland	1.4	1.8	0.78	2.6	0.54
Type B 3					
Sweden: Askoe area (More exposed)	1.2	1.5	0.80	1.8	0.67
Sweden: Yxla area	1.3	1.6	0.81	1.9	0.69
Finland I: Archipelago Sea II: Western Gulf of Finland	1.8 1.5	2.2 2.6	0.82 0.58	2.9 4.0	0.62 0.38
Type B 12					
Denmark	1.4	1.5	0.93	1.9	0.74
Sweden	1.2	1.5	0.80	1.9	0.63
Germany, Geltinger Birk	< 1.1	1.1	-	1.9	< 0.58
Germany, Darss-Zingst	< 1.1	1.1	-	1.9	< 0.58
Estonia	1.8	2.2	0.82	2.7	0.67
Type B13 CW					
Denmark	1.2	1.3	0.92	1.6	0.75
Estonia and Latvia	1.1	1.3	0.85	1.65	0.67
Type B13 TW					
Poland	< 3.7	3.7	?	5.6	0.67
Lithuania	3.85	4,8	0.80	6,0	0.64
Type B 14					
Denmark	0.9	1.1	0.82	1.6	0.56

Phytoplankton: parameter indicative of biomass (Chlorophyll a)
Results: Ecological quality ratios and parameter values
The following results refer to summer mean May/June – September

Type and country	Ecological Quality Ratios for the national classification systems		Parameter values/ranges Chlorophyll a µg/l	
	High-Good boundary	***Good-Moderate boundary***	***High/-Good boundary***	***Good-Moderate boundary***
CW B0 All countries sharing the type	0.76	0.56	1.7 (1.5 – 1.8)	2.3 (2.0 -2.7)
CW B2 All countries sharing the type	0.78	0.56	1.8	2.5 (2.3 -2.6)
CW B3 a Sheltered All countries sharing the type	0.71	0.49	2.4 (2.2 - 2.6)	3.5 (2.9 – 4.0)
CW B3 b Exposed All countries sharing the type	0.81	0.68	1.6 (1.5 – 1.6)	1.9 (1.8 – 1.9)
CW B 12 a Eastern Baltic Sea Salinity 5-8 psu All countries sharing the type	0.82	0.66	2.2	2.7
CW B 12 b Western Baltic Sea Salinity 8 -22 psu All countries sharing the type	0.92	0.63	1.3 (1.1 – 1.5)	1.9
CW B 13 Denmark, Estonia and Latvia	0.92	0.75	1.3	1.6
CW B 14 Denmark	0.82	0.56	1.1	1.6
TW B 13 All countries sharing the type	0.90	0.66	4.2	5.8

Countries sharing types that have been intercalibrated:
Types CWB0, CWB2, CWB3a, CWB3b: Finland, Sweden.
Type CWB12a: Estonia
Type CWB12b: Germany, Denmark, Sweden.
Type CWB13: Denmark, Estonia, Lithuania, Latvia, Poland.
Type CWB14: Denmark, Poland
Type TWB13: Lithuania, Poland.

2.1.6 Open issue and need for further work

All member states were participating in the intercalibration of the chlorophyll a metrics and setting of reference conditions and boundaries as well as EQR values for chlorophyll *a* (Latvia agreed on the boundaries for type CWB13 developed by other member states). Further development and intercalibration is needed for other phytoplankton parameters. Assessment methods were not yet ready for phytoplankton species composition and phytoplankton blooms in any of the Baltic GIG member states.

2.2 Black Sea GIG

2.2.1 Intercalibration approach

Typology

In the Black Sea was identified only one common type, as is shown in the table below:

Tab 2.2.1: Black Sea GIG Coastal Water Types included in the intercalibration.

Type	Salinity	Tidal range	Depth	Substratum
CW – BL1	mezohaline	microtidal	shallow	mixed

The countries participating at this intercalibration exercise into the Black Sea region are Bulgaria and Romani

2.2.2 Reference conditions and class boundary setting

a. BULGARIA

Dr. Assoc. Prof. Snejana Moncheva, Junior Res. Natalia Slabakova, Marine Biology and Ecology Department, INSTITUTE OF OCEANOLOGY, BAS, Varna

Identification of type-specific biological reference conditions for the coastal waters

After the critical review of the possible phytoplankton descriptors and the outlined constrains and uncertainties for the identification of type-specific biological reference conditions for the coastal waters the following metrics have been selected:

- Phytoplankton biomass [mg/m^3]
- Chlorophyll a [mg/m^3] and
- Phytoplankton blooms (need of further development)

Approach

The approach for the identification of reference conditions is based on:

- historical data from the relatively pristine period of the Bulgarian Black Sea coast (1954-1970 – published seasonal data);
- 10^{th} percentile (lower quartile) of a long-term data set (period 1983-2005) to test applicability for definition of reference values and for the selection of "bad values" (from the period of intensive anthropogenic eutrophication – the 80-ies) (Heiskanen et al., 2005). This step was crucial for the identification of reference values for chlorophyll a. Due to lack of chlorophyll a measurements from the reference period and before 1990, the 10^{th} percentile from a long-term data set (1990-2006) was determined and its applicability tested against the results from the analysis of the phytoplankton data set .
- expert judgment
- As initially phytoplankton is an indicator of eutrophication mainly, the typology groups were considered in relation to the level of exposure and depth. In addition due to the influence of the Danube river nutrient enriched waters through the main Black Sea current along the Black Sea coast the moderately-exposed-intermediate and exposed-shallow types were combined

into one group. Thus the classification tool was developed for the following 2 typology groups: moderately exposed - shallow (CW602330; CW602310) and moderately-exposed-intermediate/exposed-shallow types (CW602210; CW602220; CW602230; CW602321).

- Due to the high seasonal variability of phytoplankton communities composition and abundance the average annual values are not adequate and the classification tool should be developed on seasonal bases

In order to illustrate the approach the Galata/Varna Bay sites were selected on the basis of the best (only) available long-term data set, over the period 1954-2006 (phytoplankton) and 1990-2006 (chlorophyll a).

The data sets of the two sites were merged into one data array based on the following assumptions:

1) the intensive hydrophysical interaction between the two sites that to a great extent determine the spatial distribution and dynamic of phytoplankton communities thus reflecting the synergy between the local land base sources and the Danube influence through the main Black Sea currents.
2) Principally the analytical procedure of phytoplankton processing (biomass estimation) are comparable for the data array 1954-1998, assuming 25 % analytical error inherent in the method itself. The difference of sampling frequency (seasonal sampling for the historical data versus monthly /bimonthly sampling after 1980) should be underlined.
3) Originally phytoplankton is the biological quality element most sensitive to external forcing (nutrient over-enrichment (eutrophication), light, currents etc.).
4) the values estimated for the period 1954-1970 correspond to High status, and those from the period 1983-1998 to Bad status, and they represent the worst High and the worst Bad along the Bulgarian Black Sea coast (Moncheva et al, 1995, 2001, Moncheva, Kamburska, 2002).

Results

Table 2.2.2: Statistical summary of phytoplankton biomass B[mg/m3] by seasons (Galata-Varna Bay).

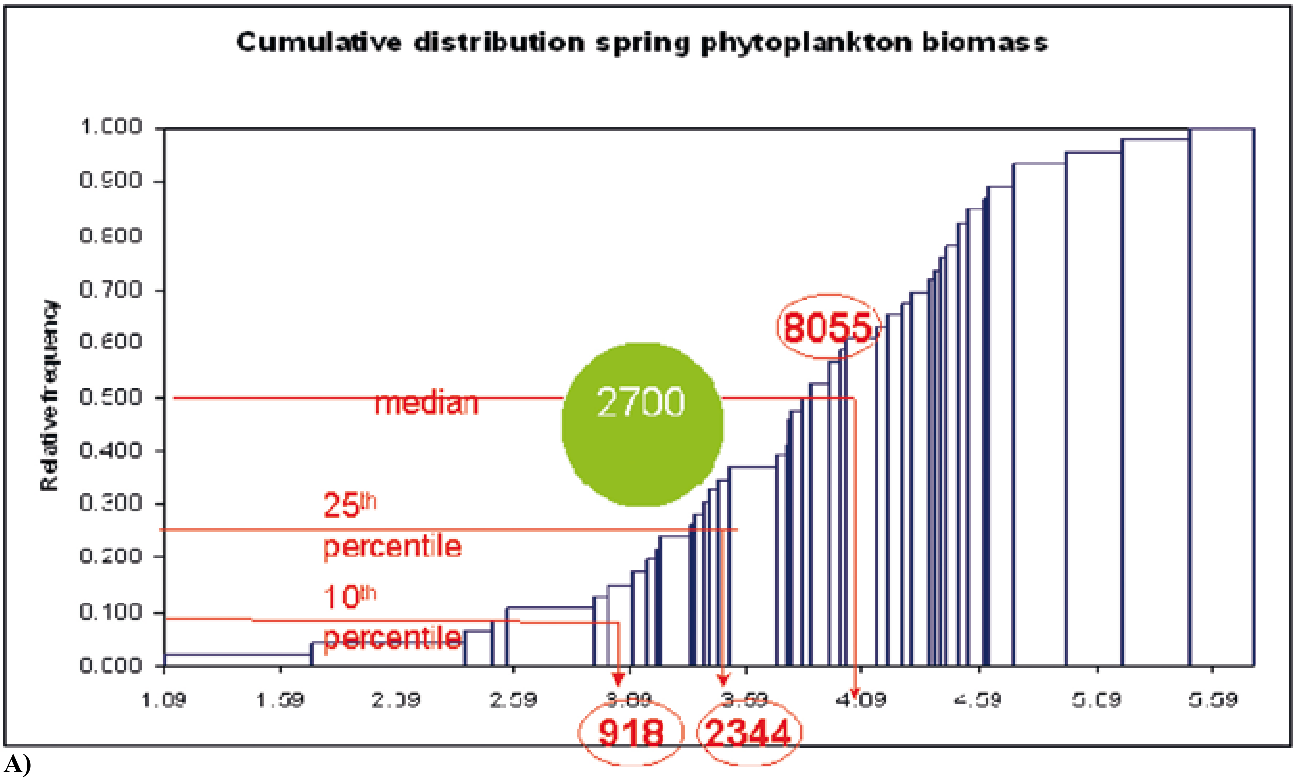
	winter	**winter**	**spring**	**spring**	**summer**	**summer**	**autumn**	**autumn**	**annual**	**annual**
years	1954-1970	1983-1997	1954-1970	1983-1997	1954-1970	1983-1997	1954-1970	1983-1997	1954-1970	1983-1997
n	15	18	14	45	11	39	15	18	55	120
Average*	2075	7763	3995	36892	526	39035	783	31639	1845	28832
Median*	1214	3088	2700	8055	534	23196	489	854	1234	8798
Max*	6800	42260	12290	583139	900	157789	2483	296675	12290	583139

*the values within the 95 % confidence interval after the statistical processing of the data array

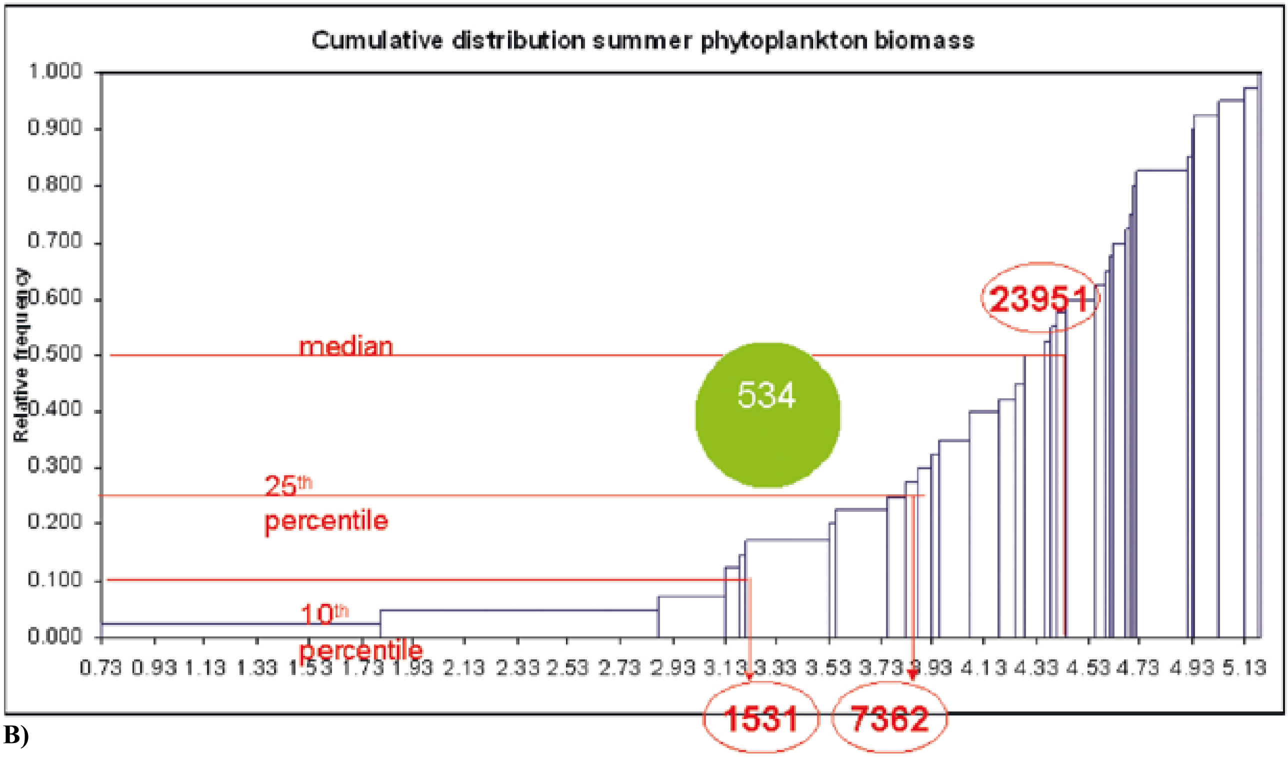
As apparent from the data presented the average annual gives no idea about the alterations in the seasonal pattern of phytoplankton dynamics, the severe increase in summer biomass in particular (related hypoxia/anoxia conditions and ecosystem deterioration as one of the most important ecological concerns) bearing implications for the proper environmental management policy and monitoring design.

Principally the historical median was selected as the seasonal reference value, and the “average” for autumn only. The threshold “bad” was based on the 90 percentile of the long-term data set and expert judgment.

As apparent from Fig. 1. the lower quartile of the long-term data set for spring (A) is very close to the historical reference, which does not hold for summer (B).



A)



B)

Fig. 2.2.1: Cumulative distribution of spring and summer phytoplankton biomass [mg/m^3].

Table 2.2.3: Inventory of chlorophyll a [mg/m3] data by sites.

Site	Winter		Spring		Summer		Autumn		Total
	period	No Samples	period	No Samples	period	No Samples	period	No Samples	No Samples
Kaliakra	1995-2005	9	1990-2006	22	1990-2006	28	1990-2006	23	82
Varna Bay	1990-2005	39	1991-2005	101	1990-2006	93	1990-2006	88	321
Galata	1990-2005	9	1990-2006	16	1990-2006	30	1990-2006	11	66
Burgas Bay	1987-2005	32	1984-2006	51	1987-2006	53	1982-2006	46	182

The chlorophyll a reference and threshold values were determined based on the 10 (or 25) percentile and the 90 percentile (bad) of the long-term data set – Fig.2., Table.3

The proposed classification and QRs based on phytoplankton biomass and chlorophyll a to be applied for the Bulgarian Black Sea coastal waters is presented in Tab.4 and 5.

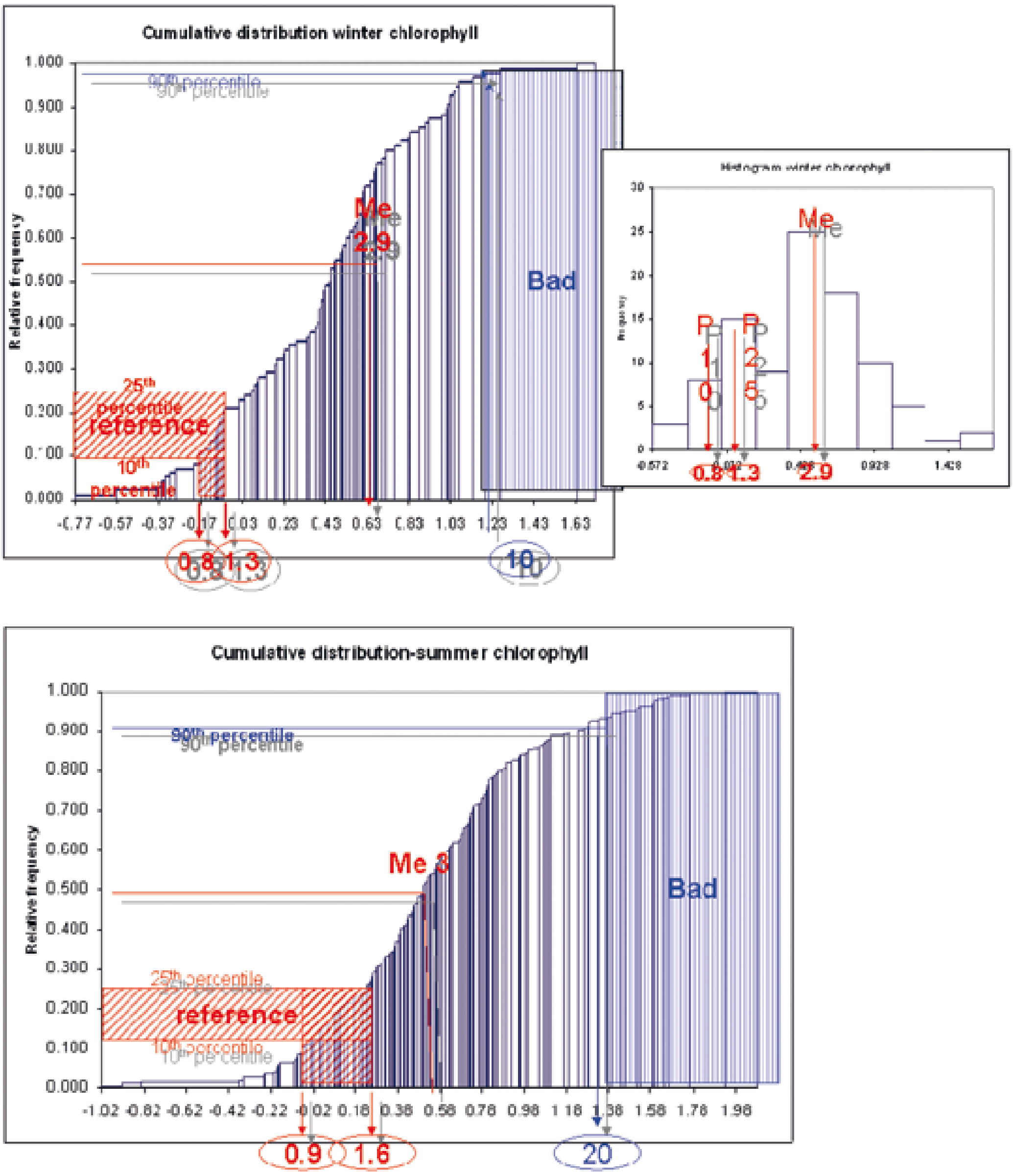


Fig. 2.2.2: Cumulative distribution of long-term chlorophyll a data for winter and summer.

Table 2.2.4: Seasonal "high" values as inferred by the cumulative distribution.

Site	High – chl. a [mg/m³]							
	Winter		Spring		Summer		Autumn	
	10th %	25th %	10th %	25th %	10th %	25th %	10th %	25th %
Kaliakra	0.77	2.30	0.79	0.97	0.65	1.12	1.32	1.82
Galata V. Bay	1.4	1.7	1.3	2.2	1.2	2.1	0.8	1.3
Burgas Bay	1.05	1.7	1.4	2.1	0.8	1.2	0.7	1.2

Table 2.2.5: Classification and EQR based on phytoplankton biomass [mg/m^3] by seasons.

Type	*Winter*					
	Index	**High**	**Good**	**Moderate**	**Poor**	**Bad**
CW602330 CW602310	PhB [mg/m3]	(1200) 2070	4150	7600	11600	>15000
CW602210 CW602220 CW602230 CW602321	PhB [mg/m3]	(1200)1700	3200	6000	9100	>12000
	EQR	0.85	0.7	0.45	0.16	
Type	*Spring*					
	Index	**High**	**Good**	**Moderate**	**Poor**	**Bad**
CW602330 CW602310	PhB [mg/m3]	(2700) 3700	5200	9500	14500	>15000
CW602210 CW602220 CW602230 CW602321	PhB [mg/m3]	(2500)2900	4100	7400	11600	>15000
	EQR	0.85	0.7	0.45	0.16	
Type	*Summer*					
	Index	**High**	**Good**	**Moderate**	**Poor**	**Bad**
CW602330 CW602310	PhB [mg/m3]	(600-900) 1160	2750	5500	7600	>10000
CW602210 CW602220 CW602230 CW602321	PhB [mg/m3]	(750) 1100	2150	4000	6100	>8000
	EQR	0.85	0.7	0.45	0.16	
Type	*Autumn*					
	Index	**High**	**Good**	**Moderate**	**Poor**	**Bad**
CW602330 CW602310	PhB [mg/m3]	(1000) 1650	3300	6100	9200	>12000
CW602210 CW602220 CW602230 CW602321	PhB [mg/m3]	(1000) 1350	2700	4950	7500	>10000
	EQR	0.85	0.7	0.45	0.16	

Table 2.2.6: Classification and EQR based on chlorophyll a data [mg/m^3] by seasons.

Type	*Winter*					
	Index	High	Good	Moderate	Poor	Bad
CW602330	Chl a [mg/m3]	(1.6) 1.9	3.9	7.3	9.4	15
CW602310	Range	Chl a<1.9	1.87<Chla<3.92	3.93<Chla<7.28	7.28<Chla <9.41	Chl a >13
CW602210	Index	High	Good	Moderate	Poor	Bad
CW602220	Chl a [mg/m3]	(1.3) 1.5	3.6	6.7	8.6	12
CW602230	Range	Chla <1.5	1.54<Chla <3.59	3.59<Chla <6.67	6.67<Chla <8.62	Chl a >12
CW602321						
	EQR	0.85	0.65	0.35	0.16	
Type	*Spring*					
	Index	High	Good	Moderate	Poor	Bad
CW602330	Chl a [mg/m3]	(2.3) 3.0	6.2	9.5	14.8	20
CW602310	Range	Chl a<3.0	3.0 <Chla<6.2	6.2<Chla<9.5	9.5 <Chla <14.8	Chl a >20
CW602210	Index	High	Good	Moderate	Poor	Bad
CW602220	Chl a [mg/m3]	1.7 (2.3)	4.6	7.3	11.2	15
CW602230	Range	Chla <2.3	2.3<Chla <4.6	4.6<Chla <7.3	7.3<Chla <11.2	Chl a >15
CW602321						
	EQR	0.85	0.65	0.35	0.16	
Type	*Summer*					
	Index	High	Good	Moderate	Poor	Bad
CW602330	Chl a [mg/m3]	(0.9) 1.35	3.2	5.1	7.6	10
CW602310	Range	Chl a <1.35	1.35<Chla <3.2	3.2<Chla <5.1	5.1<Chla <7.6	Chl a >10
CW602210	Index	High	Good	Moderate	Poor	Bad
CW602220	Chl a [mg/m3]	(0.7) 1.23	2.8	4.5	7	9
CW602230	Range	Chla <1.23	1.23<Chla <2.8	2.8<Chla <4.5	4.51<Chla <7	Chl a >9
CW602321						
	EQR	0.8	0.65	0.35	0.16	
Type	*Autumn*					
	Index	High	Good	Moderate	Poor	Bad
CW602330	Chl a [mg/m3]	(1.5) 1.7	4	7.5	9.7	13
CW602310	Range	Chl a<1.7	1.73<Chla<4.03	4.03<Chla<7.48	7.48<Chla <9.66	Chl a >13
CW602210	Index	High	Good	Moderate	Poor	Bad
CW602220	Chl a [mg/m3]	(1.2) 1.6	3.7	6.8	8.8	12
CW602230	Range	Chla <1.6	1.58<Chla <3.68	3.68<Chla <6.83	6.83<Chla <8.82	Chl a >12
CW602321						
	EQR	0.85	0.65	0.35	0.16	

In addition based on the correlation chl. α/Secchi depth as inferred from the available data for the north-western Black sea the regression equation was used (Fig.3) to suggest classification thresholds for the Secchi depth presented on – Table 6.

At this stage it is not possible to suggest classification based on phytoplankton blooms for the following reasons.
The reference data (1954-1970) are seasonal, as well as the recent data which makes quantification and the application of the 6 years monthly frequency records as suggested by some expert groups (WFD ITR, 2007) impossible. The records for the species that were the most frequent drivers of ecosystem dysfunction (Prorocentrum minimum and the related hypoxia events in summer during the eutrophication period – Moncheva et al., 1995, 2001) are of little use due to the shifts in the phytoplankton taxonomic composition after 2000 (Moncheva et al, 2006) – Table 7.

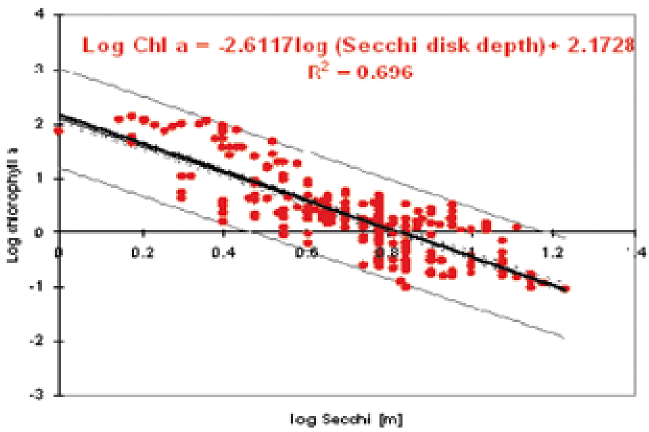


Figure 2.2.3: Correlation between chlorophyll a [mg/m3] and Secchi disk depth [m] – log scale (after V. Doncheva. 2007).

Table. 2.2.7: Classification based on TRIX and Secchi depth.

All types and seasons					
Index	**High**	**Good**	**Moderate**	**Poor**	**Bad**
TRIX	2-4.5	4.5-5.4	5.5-6.4	6.5-7.9	>8
Secchi depth[m]	>4.5	4.5-3.5	3.5-2.5	2.5-1.5	<1.5
EQR	0.9	0.7	0.5	0.3	

Table 2.2.8: Summary of phytoplankton blooms (Galata-Varna Bay).

Descriptor	**Winter**	**Winter**
	1954-1970	**1983-1998**
	Bacillariophyceae	**Bacillariophyceae**
	Skeletonema costatum	Skeletonema costatum
Most typical species	Pseudonitzschia seriata	Pseudonitzschia seriata
	Detonula confervaceae	
		Dinophyceae
		Heterocapsa triquetra
		Prorocentrum minimum
No species	3	13
No blooms	6	31
Max N [$1x10^6$ cells/l]	8.2	43.47
Descriptor	**Spring**	**Spring**
	1954-1970	**1983-1998**
	Bacillariophyceae	**Bacillariophyceae**
	Pseudonitzschia delicatissima	Skeletonema costatum
Most typical species	Pseudinitzschia seriata	P. delicatissima
	Dinophyceae	**Dinophyceae**
	Prorocentrum minimum	Heterocapsa triquetra
		Prorocentrum minimum
No species	7	24
No blooms	16	63
Max N [$1x10^6$ cells/l]	13.5	220
Descriptor	**Summer**	**Summer**
	1954-1970	**1983-1998**
	Bacillariophyceae	**Bacillariophyceae**
	C.caspia	Cerataulina pelagica
Most typical species		Pseudonitzschia delicatissima
		Nitzschia tenuirostris
		Pseudinitzschia seriata
		Rhizosolenia fragilissima
	Dinophyceae	**Dinophyceae**
	Prorocentrum minimum	Gymnodinium uberimum
		Prorocentrum minimum
		Alexandrium monilatum
		Lingulodinium polyedrum
		Haptophyceae
		Phaeocystis pouhettii
No species	2	21
No blooms	4	73
Max N [$1x10^6$ cells/l]	2.1	481
Descriptor	**Autumn**	**Autumn**
	1954-1970	**1983-1998**
	Bacillariophyceae	
	Skeletonema costatum	Skeletonema costatum
Most typical species	Detonula confervaceae	Detonula confervaceae
	Leptocylindrus minimus	Leptocylindrus minimus
	Dinophyceae	**Dinophyceae**
		Prorocentrum minimum
		Alexandrium monilatum
		Lingulodinium polyedrum
		Oxyphisis oxytoxoides
No species	2	15
No blooms	2	33
Max N [$1x10^6$ cells/l]	5.7	60.17

b. ROMANIA

The site selected was Constanta/Mamaia Bay were the phytoplankton samples have been collected twice per week. The long-term data set is available for the period 1960-1970 and 1986-1997 for this site.

Phytoplankton samples have been collected from the surface layer (enabling more then 500 ml of water per sample) and immediately treated with a formaldehyde solution of 4 % concentration, followed by further processing based on sedimentation method (Morozova-Vodyanitzkaya, 1954; Bodeanu, 1987-1988). The taxonomic determination and cells counting by species of respective samples has been carried out by means of plankton inverted microscopes, equipped with 40x magnification lens, in the case of small-sized cells (less then 15-20 µm) or with 16.3x / 20x magnification lens, in the case of larger-sized cells. Afterwards numerical densities ($cell \times l^{-1}$) and biomass (mg/m^3) were calculated for each specific component of each taxonomic group and for total phytoplankton.

In order to identify the biological reference condition for the Romanian coastal waters the phytoplankton biomass (mg/m^3) has been selected. The identification of reference condition is based on historical data from pristine period of the Romanian Black Sea coast (1960-1970), from period of intensive eutrophication ('80 years).

In order to identify the EQRs for the common body waters, we found the values estimated for the period 1960-1970 corresponding to high status and those from the period 1986-1997 to bad status. The limits between these classes, for good, moderate and poor have been decided based on expert judgment.

Table 2.2.9: Summary of phytoplankton biomass (mg / m^3) by season.

	Winter	Spring	Summer	Autumn	Annual
1960-1970					
Average	2692	2583	690	540	1627
Max	5725	19165	1035	921	19165
1986-1997					
Average	1255	5064	10664	6603	6747
Max	5465	34647	46809	61195	31195

Table 2.2.10: Classification based on phytoplankton biomass (mg/m^3).

High	Good	Moderate	Poor	Bad
		Winter		
2500	3600	5500	8000	> 10000
EQR				
		Spring		
3000	5700	9000	14000	> 15000
EQR				
		Summer		
1000	2500	6000	7500	> 12000
EQR				
		Autumn		
1500	3500	7200	9500	> 15000
EQR				

2.2.3 Results of the harmonization – Boundary EQR values

Table 2.2.11: Phytoplankton biomass.

Ecological status	High	Good	Moderate	Poor	Bad
Winter					
Range	< 2000	2000 - 3000	3001 - 5500	5501 - 12000	> 12000
EQR	> 0.85	0.7	0.45	0.16	< 0.16
Spring					
Range	< 3000	3000 - 5000	5001 - 9000	9001 - 20000	> 20000
EQR	> 0.85	0.7	0.45	0.16	< 0.16
Summer					
Range	< 1000	1000 - 2500	2501 - 5000	5001 - 10000	> 10000
EQR	> 0.85	0.7	0.45	0.16	< 0.16
Autumn					
Range	< 2000	2000 - 3500	3501 - 6500	6501 - 15000	> 15000
EQR	> 0.85	0.7	0.45	0.16	< 0.16

It was agreed that the assessment system for phytoplankton have to be applied for surface layer samples, with a monthly frequency sampling, at least between May and September; the assessment should be made based on seasonal values and for annual assessment will be made using the "one out, all out" principle; harmonization of EQR values resulted with separation of the most northern water body of the common water body type in Bulgaria (CW602230) from the rest of the bodies at the same type, and eventually separation of a new type.

The rest of the metrics have not been harmonized because Romanian part was not ready with their reference values and EQRs.

2.2.4 Open issues and need for further work

The future work plan will focus on preparation of assessment system for bloom concentrations, bloom species and chl α, according to the methods provide in the Black Sea Commission Manual. Other parameters will be considered at a later stage.

2.3 Mediterranean GIG

2.3.1 Intercalibration approach

Participation of countries in the Phytoplankton subgroup:

Cyprus
France
Greece
Italy
Slovenia
Spain (Valencia, Catalonia, Balearic Islands)
+ Croatia (Accession Country)

Data availability

The examination of the datasets provided by each MedGIG Member States highlighted a huge data heterogeneity, mainly due to different monitoring schemes. Table 1 summarized the main features of the datasets, highlighting the availability of key features (sites, parameters, vertical profile and amount of data) needed for the typological identification of water bodies.
Data used are referred to Chl *a* concentrations measured in "coastal waters", as defined in the Directive: *"within the 1 nautical mile distance from the coastline",*
A common decision is to recommend the use of nearshore data only (500-1500 m from the coast), (while for the IC all "coastal" data have been considered, as reported above), when not differently advised by MS[23].

Table 2.3.1: Data features for MED GIG MSs datasets.

MS	N° Sites	N° Records	Period	Freq (d)	Profile	Temp Sal data
France	8	1250	1997-2006	7	not available	available
Italy	11	2541	2001-2004	15	available	available
Slovenia	2	332	1997-2004	30	available	available
Cyprus	48	158	2005	60	not available	not available
Spain	117	1109	1991-2006	regional	available	available
Croatia	19	1784	2000-2004	120	not available	available
Greece	5	1225	2000-2004	30	available	available

Typology

Phytoplankton experts defined that the 4 Mediterranean Coastal IC types, (see Section 1 General Part), based primarily on the substratum composition and the depth profile, cannot be applied to the IC for the current BQE, within the Mediterranean basin: the classification criterion is based mainly on the morphological features of the bottom and therefore it is not so meaningful in a "phytoplankton perspective".
A new typology has been developed, mainly focused on hydrological parameters characterizing water bodies' dynamics and circulation. The typological approach is based on the introduction of the static stability parameter (derived from temperature and salinity values in the water column): such a parameter, having a robust numerical basis, can describe the dynamic behaviour of a coastal system. The group agreed then to adopt surface density as a proxy indicator for static stability as both Temperature and Salinity are relevant in the dynamic behavior of a coastal marine system: both are involved in circulation and mixing dynamics and all information is then nested in the surface density parameter (Russo et al., 2006, submitted).

On the basis of surface (density (σ t) values three major water types have been defined:

Table 2.3.2: density thresholds defining new coastal water types.

	Type I	Type II	Type III
σ t (density) (kg m^{-3})	<25	25<d<27	>27

[23] NOTE from Spain: From 90s in Spain there has been an important effort to sample Chl-*a* stations located at inshore (over the line coast). In the main of cases these data are the base of the methods used along the country and the base of the part of the results Spain shares in this intercalibration exercise. Although no other MSs work with this kind of data, it is important to stress that all the reference conditions and boundaries proposed in this intercalibration exercise are referring to the nearshore.

The same three water types are defined below as salinity classes, since for Spain (Catalonia) variability due to seasonal fluctuation is marginal: using relationships between density and salinity, Spain calculated the following table (at 18 °C)

Table 2.3.3: salinity thresholds defining new coastal water types.

	Type I	**Type II**	**Type III**
Annual Mean Salinity (psu)	<34.5	34.5<d<37.5	>37.5

This type subdivision based only on salinity, is perfectly comparable with the previous ones, based on density, agreed by the rest of Mediterranean MSs. For more details about the use of salinity instead of density see the Annex 3.8: Spain MS report on phytoplankton element.

The three different water types, in an ecological perspective, can be described as follows:

Type 1 coastal sites highly influenced by freshwater inputs
Type 2 coastal sites not directly affected by freshwater inputs
Type 3 coastal sites not affected by freshwater inputs

A further distinction has been suggested and approved by the MSs, regarding the splitting of the coastal water type 3 in two different sub basins, the Western and the Eastern Mediterranean one, according to the different trophic conditions[24]:

Type 3 WM
Type 3 EM

Furthermore, Spain proposed the subdivision of type II, which include marine waters with intermediate salinities) in two subtypes: type II-A and type II-B[25].

Types presence in the different MSs was reviewed, and finally defined, as shown in the following Table 2.3.4.

---

[24] The Levantine Basin of eastern Mediterranean is characterized as nutrient-deficient and therefore ultra-oligotrophic in comparison to the Atlantic Ocean (Berman et al., 1984). Furthermore, eastern Mediterranean is more P-limiting to the growth of phytoplankton, in contrast to the general dogma that N is the more limiting nutrient in marine systems (Krom et al., 1991). Recent studies made on phytoplankton biomass in the deeper waters of eastern Mediterranean reveal that prevailing oligotrophic conditions result in low chlorophyll-a concentrations ranging from 0.1 to 0.2 µg L-1 (Krom et al., 1992). It has also been shown that chlorophyll-a concentrations off the coast of Cyprus are among the lowest in the region and ranged from 10 to 90 ng L-1 (Bianchi et al., 1996). Recent studies along the coastal waters of Cyprus confirmed its oligotrophic status (Argyrou, 2005, 2006).
The coastal waters of Cyprus are classified as Type III (no freshwater input – density greater of 27), due to their hydrographical features and the prevailed physicochemical characteristics; in fact mean salinity of coastal waters of Cyprus is 39,1 psu. The annual mean of Chl a for the years 2004 to 2006 ranged from 0,07 to 0,11 µg L-1 while, the calculated 90th Percentile ranged from 0,09 to 0,2 respectively. The overall average level of Chl a for the entire period, 2004 to 2006, was 0,086 and the respective 90th Percentile was 0.188. These values were used for the assessment of the ecological status of the coastal waters of Cyprus according to the Eutrophication Scale, which was developed by Ignatiades et al. (1992) and Karydis (1999), and further modified by Siokou & Pagou, 2000; Pagou, 2000) based on nutrient and phytoplankton data collected from several coastal and marine areas from Greece.

[25] The South of Spain (the main part of Andalusian coast) is clearly affected by the influence of the Atlantic waters, so the natural salinity, nutrients and Chl-*a* concentrations do not correspond with type III. Moreover the lower salinities of before defined type II were explained by freshwater inputs, coming mainly from the continent. It should be emphasized that in the vicinities of Gibraltar Strait there are also lower salinities that come from the Atlantic, and that is why this subdivision in Type II-A (the original one) and Type II-B (affected by atlantic influence) was proposed by Spain. For more details consult the *Annex: Spain MS report on phytoplanckton element*. It should be also considered the relationship between the reference conditions and boundaries defined by the NEA GIG for the atlantic waters in the western part of the Gibraltar Strait.

Table 2.3.4: Types occurrence per MSs in Coast MED GIG.

Types description			France	Spain	Italy	Slovenia	Croatia	Greece	Cyprus
Type I	Highly influenced by freshwater input		X		X				
Type II	A	Moderately influenced by freshwater input (continent influence)	X	X	X	X			
	B	Influenced by Atlantic waters		X					
Type III	W	Continental coast , Not influenced by freshwater input	X	X	X		X		
	E	Not influenced by freshwater input						X	X

Based on this new Typology and on the available data for the different types in the Member States, Intercalibration was performed as follows:

for **Type I** France is not able at the moment to provide enough data to be compared with the Italian data; therefore no Intercalibration is performed for this typology at the present phase

for **Type II** Spain had to make distinction within the same type (as specified below (3)). As this subdivision is not found in the other Mediterranean MS, IC performed on Type II does not include the Spanish Type II B (influenced by Atlantic waters)

for **Type III** the distinction between Type III Western Mediterranean and Type III Eastern Mediterranean was already agreed for evident ecological differences within the 2 Mediterranean basins. Only Greece and Cyprus belong to the Eastern Mediterranean basin, therefore Type III E Intercalibration was performed only between these 2 countries

2.3.2 National methods that were intercalibrated

Since we did not elaborate a methodology based on a common data set, which would be then adopted at national level of each MS, we compared boundaries (Chl-*a* concentrations and EQRs) derived from national methods having different status at present (under development, finalized, officially accepted). Available methodologies descriptions are detailed or cited in the Annex 3.8: National methods included in the intercalibration.

Only one parameter of the BQE phytoplankton was considered for this phase of the Intercalibration process: Chlorophyll *a* concentration as parameter/indicator for biomass.

Common statistical analysis on Chl-*a*, nutrients and physico-chemical data, and some multivariate techniques have been performed in order to facilitate the reaching of a wide agreement for the intercalibration process. Since a methodology based on a common data set, which would be then adopted at national level of each MS, was not elaborated, different metrics of this parameter and different statistical approaches for setting the boundaries (derived from national methods, where defined) were analysed and compared. Boundaries are in terms of Chl-*a* concentrations and EQRs that have different status of implementation/finalization, at present in the Mediterranean Member State (see Table below). Different metrics of this parameter and different statistical approaches for setting the boundaries were compared.

The finally agreed approach that has been followed for the intercalibration, is an the hybrid option, as described in the Intercalibration Guidance as follows:
"Boundary values are first established with national classification assessment methods (as in Option 3). The subsequent comparison of the boundary values could then be done with the help of a common metrics method (as in Option 2)."

National methods adopted, mostly, three kinds of metrics: percentile90th, annual geometric mean (geomean) and average. Depending on the MS the metrics were calculated using only surface data or water-column integrated data, covering different period (e.g. one year in case of geomean and 5 to 6 years when using percentile 90th. The metrics used by MSs are shown in the table below.

Table 2.3.5: Metrics adopted by MSs.

MS	Percentile90th	Average (A-arithmetic, G-geometric)	Based on: Raw data (R)/ Geomean (G)
France	X		R
Spain	X	A	R
Italy	X		G
Slovenia		G	R
Greece	X	A	
Cyprus	X	A	

2.3.3 Reference conditions and class boundary setting

For the 3 intercalibrated types every MS defined reference conditions, boundaries and EQRs applying their own methodologies. These methodologies are detailed or cited in the 2.3. 1.

Reference conditions

Reference conditions will be different according to different water types.
Each MS proposed its own reference conditions based on their phytoplankton experts' knowledge. All of them based their calculations of reference conditions by selecting High status stations from their monitoring programmes. For more details of the methods see Annex 3.8: National MSs reports on phytoplankton element.
Type-specific reference conditions, as suggested by MSs, are listed in table 2.3.6, below.

Table 2.3.6: Type-specific Reference conditions, expressed as surface Chl a concentration (µg /L)

RC	Type II-A	Type III WM	Type III EM	Metric
France	<2	<1	*	Percentile90th
Spain	1.9	1.10	*	Percentile90th
Spain	0.98	0.46	*	Average
Italy	0.77	0.4	*	Percentile 90th (Geomean)
Slovenia	0.99	*	*	Annual (geomean)
Greece	*	*	0.08	Annual average
Cyprus	*	*	0.08	Annual average

* Not applicable

Setting of Boundaries

In the following table the MSs boundaries, as chl *a* concentration, (µg /L), for each water type are listed:

Table 2.3.7: Type-specific MSs boundaries.

		Reference	H/G	*EQR H/G*	G/M	*EQR G/M*	Metric	
T1	France						Percentile 90th	TRUE VALUES
	Italy	1,8	2,4	*0,75*	3,5	*0,51*	Geometric mean	
T2 - A	France	<2	2		4		Percentile 90th	TRUE VALUES
	Spain	1,9	2,3	*0,83*	3,5	*0,54*	Percentile 90th	
	Spain	0,98	1,15	*0,85*	1,72	0,57	Mean	
	Italy	0,77	1	*0,77*	1,24	*0,62*	Geométric mean	
	Slovenia	0,99	1,28	*0,78*	1,62	*0,61*	Geometric mean	
T3 – Western Med	France	<1	1		2		Percentile 90th	TRUE VALUES
	Spain	1,1	1,3	*0,85*	1,8	*0,61*	Percentile 90th	
	Spain	0,46	0,54	*0,85*	0,7	*0,66*	Mean	
	Italy	0,4	0,51	*0,78*	0,64	*0,63*	Geométric mean	
T3 – Eastern Med	Cyprus	0,08	0,1	*0,8*	0,4	*0,2*	Percentile 90th	
	Greece	0,08	0,1	*0,8*	0,4	*0,2*	Percentile 90th	

2.3.4 Results of the comparison and harmonization

Phytoplankton experts from MSs decided to adopt a final agreement based on 90th percentile on raw data and, at least, monthly sampling frequency. In order to make MSs values more comparable Slovenia and Italy decided to translate their own values in new boundaries calculated using the same metric as the other MSs (90^{th} percentile). For details on MS calculations see Annex 3.8.
Using the same metric the proposed boundaries by each country were very similar, thus the group came to the agreement reported in the following paragraph.
Harmonization of boundaries and EQR values
The boundary values are expressed for the metric 90^{th} percentile, assuming that at least 5years data are available, with monthly sampling, in the surface layer.

Type I: not intercalibrated, only Italy has enough data

Type II

Table 2.3.8: Harmonized boundaries for Type II - A.

Type	MS	REFERENCE	H/G	EQR	G/M	EQR
T2 - A	Slovenia France Spain Italy	1.9	2.4	*0.80*	3.6	*0.53*

Type III

Table 2.3.9: Harmonized boundaries for Type III.

Type	MS	REFERENCE	H/G	EQR	G/M	EQR
T3 – Western Med	France Spain Italy	0.9	1.1	*0.80*	1.8	*0.50*
T3 – Eastern Med	Cyprus Greece	0.08	0.1	*0.80*	0.40	*0.20*

2.3.5 Open issues and need for further work

A few suggestions have been pointed out by MS for an eventual further intercalibration activity:

1. Include species composition analysis or blooms frequency analysis for a better understanding of the system's behavior and efficiency/status Initially common indicator metrics will be considered (option 1 or 2), but it may be possible to move to option 3 when member states will have developed their full phytoplankton classification systems.
2. Improve dose/response analysis correlating pressures (nutrients) with trophic conditions.

2.4 NE Atlantic GIG

2.4.1 Intercalibration approach

Option 2 has been used in the intercalibration process for phytoplankton. Three metrics have been selected: chlorophyll, Indicator Taxa (Frequency of *Phaeocystis* Cell counts) and Taxa Cell Counts (Frequency of phytoplankton taxa cells counts). Boundaries have been agreed for all countries for the measurement of chlorophyll a using a 90th percentile metric. Total cell counts and *Phaeocystis* metric are not relevant in all countries' waters, but common thresholds have been agreed where applicable.

Generic typologies for NEAGIG types

In the NE Atlantic six basic intercalibration types have been agreed. These are shown in the Table 1. Types were distinguished by salinity, tidal range, depth, velocity, exposure, mixing and residence time.

The above types occur in Member State's waters as detailed in Table 2.

Table 2.4.1: Broad geographical types within the North East Atlantic GIG.

New Type ID	Name	Salinity (PSU)	Tidal range (m)	Depth (m)	Current velocity	Exposure	Mixing	Residence time
CW –NEA1/26 a,b,c,d,e	Exposed or sheltered, euhaline, shallow	Fully saline (> 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or sheltered	Fully mixed	Days
CW – NEA3/4	Polyhaline, exposed or moderately exposed (Wadden Sea type)	Polyhaline (18 - 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or moderately exposed	Fully mixed	Days
CW – NEA7	Deep, low current, sheltered	Fully saline (> 30)	Mesotidal (1 - 5)	Deep (> 30)	low (< 1 knot)	Sheltered	Fully mixed	Days
CW – NEA8	Polyhaline, microtidal, sheltered, shallow (Skagerrak inner arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Shallow (< 30)	low (< 1 knot)	Sheltered	Partially Stratified	Days-Weeks
CW – NEA9	Fjord with a shallow sill at the mouth with a very deep maximum depth in the central basin with poor deepwater exchange.	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	low (< 1 knot)	Sheltered	Permanently Stratified	Weeks
CW – NEA10	Polyhaline, microtidal exposed, deep (Skaggerak outer arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	low (< 1 knot)	Exposed	Permanently Stratified	Days
TW – NEA11 (will be split into sub-types)	Transitional waters	Oligo-Euhaline (0 - 35)	Mesotidal (1 – 5)	Shallow (< 30)	Medium	Sheltered or moderately Exposed	Partially- or Permanently Stratified	Days-Weeks

Table 2.4.2: The occurrence of the broad geographical types and sub-geographical types within each member state. **NB.** Type CW – NEA1/26 has been split down into 5 sub-types due to regional geographic differences within the North East Atlantic Geographic Intercalibration Group.

Type	BE	DK	FR	DE	IE	NL	NO	PT	ES	SE	UK
CW - NEA1/26a			X		X		X		X		X
CW - NEA1/26b	X		X			X					X
CW- NEA1/26c		X		X							
CW- NEA1/26d		X									
CW- NEA1/26e								X	X		
CW – NEA3/4				x		x					
CW – NEA7							x				x
CW – NEA8		x					x			X	
CW – NEA9							x			x	
CW – NEA10							x			x	
TW – NEA11	X		X	X	X	X		X	X		X

2.4.2 National methods that were intercalibrated

For the phytoplankton element, three metrics were chosen:

- Chlorophyll a
- Elevated cell counts
- *Phaeocystis* blooms

National methods from each country have all used a variation of these metrics within their own assessment protocols. All countries have used chlorophyll-*a* as a primary assessment tool for phytoplankton, though typically chlorophyll is used within a suite of phytoplankton tools and calculated as an EQR. All MS have agreed to use chlorophyll-*a* as a metric within the intercalibration process. Elevated cell counts and *Phaeocystis* are not all used uniformly across member states in the national methods and as such, have not been adopted as an intercalibration tool for all MS. Five countries are using the elevated count tool and four counties are using the *Phaeocystis* tool. National methods for each MS are listed below and further discussed in Table 3.

UK

Biomass	- 90th percentile of chlorophyll *a* data
Blooms/Counts	- Frequency (%) of blooms of any single phytoplankton taxa - Seasonal succession of functional groups

SPAIN

Biomass	- 90th percentile of chlorophyll *a* data
Blooms/Counts	- Frequency (%) of blooms of any single phytoplankton taxa.

REPUBLIC OF IRELAND

Biomass	- 90th percentile and median of chlorophyll-*a* data
Blooms/Counts	- Frequency (%) of blooms of any single phytoplankton taxa - Seasonal succession of functional groups (under development)

NETHERLANDS

Biomass	- 90th percentile of chlorophyll *a* data
Blooms	- *Phaeocystis* counts

FRANCE

Biomass	- 90th percentile of chlorophyll *a* data
Blooms/Counts	- Frequency (%) of blooms of any single phytoplankton taxa. Blooms of all species
Composition	- Blooms of harmful species

PORTUGAL

Biomass	- 90th percentile of chlorophyll *a* data
Blooms/Counts	- Frequency (%) of blooms of any single phytoplankton taxa. Blooms of all species

SWEDEN

Biomass	- 90th percentile and median of chlorophyll-*a* data, (original Swedish metric: mean of chlorophyll-*a* data)
Blooms/Counts	- Frequency (%) of phytoplankton, total cells of all taxa, divided into two size fractions, >20 µm and 2-20 µm (>20 µm includes common species, e.g. *Skeletonema costatum* and many *Chaetoceros* spp.)]

DENMARK

Biomass	- 90th percentile of chlorophyll a data

BELGIUM

Biomass	- 90th percentile of chlorophyll a data
Blooms/Counts	- *Phaeocystis* counts
Composition	- Blooms of harmful species (under development, thus not intercalibrated yet)

GERMANY

Biomass	- 90th percentile of chlorophyll-*a* data
Blooms/Counts	- *Phaeocystis* counts

NORWAY

Biomass	- 90th percentile of chlorophyll-*a* data - 90th percentile of biovolume/cellcarbon (under development)
Blooms/Counts	- Frequency (%) of blooms of any single phytoplankton taxa >5 µm (Need further development) - Seasonal succession of functional groups (under development)

Table 2.4.3: Description of national methods using a chlorophyll a metric.

	Description of national method/ assessment of phytoplankton biomass using the plant pigment chlorophyll	Boundary classes agreed for national assessment	Variation in methods
UK	Metric: 90th percentile of the chlorophyll-a data set ($\mu g\ L^{-1}$). • Sampling frequency: monthly (*) • Season: growth period, from March to October (*) • Period: 6-year (*)	**Atlantic Coast - Exposed West Coast of England & Northern Ireland (NEA 1/26a)** **High** -90th % <= 5 **Good** -90th % <= 10 **Mod** -90th % <=20 **Poor** -90th % <= 40 **Bad** -90th % > 40 **North Sea/Eastern Irish Sea (NEA 1/26b)** **High** -90th % <= 10 **Good** -90th % <= 15 **Mod** -90th % <=20 **Poor** -90th % <= 40 **Bad** -90th % > 40	Chlorophyll is also investigated in the elevated count tool.
Spain	Metric: 90th percentile of the chlorophyll-a data set ($\mu g\ L^{-1}$). • Sampling frequency: monthly (*) • Season: growth period, from February to October (*) • Period: 6-year (*)	**High** 90th percentile < the first threshold **Good** 90th percentile ≥ the first threshold and < the second threshold **Moderate** 90th percentile ≥ the second threshold	*Note: some difference in sampling strategy may occur along the NEA Spanish Coast* The Spanish Coast has been split into 2 NEA GIG Types 1/26a and 1/26e, and 5 regional areas, boundary values for H/G and G/M are given in the 'Results of Harmonization' section of this chapter.

ROI	Metric: 90^{th} percentile and median of the chlorophyll-a data set (µg L^{-1}). • Sampling frequency: monthly (*) • Season: growth period, from March to October (*) • Period: 5-year (*)	**High** Median and 90^{th} below H/G boundary **Good** Median and 90^{th} below G/M boundary **Moderate** Median and/or 90^{th} above G/M boundary H/G boundary = 5 (µg L^{-1})[a] G/M boundary = 10 (µg L^{-1})[a]	It differs from the proposed metric in having a second statistic based on the median value. [a]RoI use both acetone and hot methanol for chlorophyll extraction, hence the need to develop two sets of reference conditions relevant to the analysis (see Table 5). When reporting chlorophyll-a data, based on the hot methanol method, the data is not corrected by subtracting for Phaeopigment and is therefore reported as Total Pigment.
France	Metric: 90^{th} percentile of the chlorophyll-a data set (µg L^{-1}). • Sampling period: March to October. • Sampling frequency: once a fortnight or once a month. • Period: 6-year • Concern coastal waterbodies	**For the Channel and Atlantic coast except North of France (type NEA 1/26a)** **High** 90^{th} % <= 5 **Good** 90^{th} % > 5 and <= 10 **Mod.** 90^{th} % > 10 and <= 20 **Poor** 90^{th} % > 20 and <= 40 **Bad** 90^{th} % > 40 **For the French Northern coast (type NEA 1/26b)** **High** 90^{th} % <= 10 **Good** 90^{th} % > 10 and <= 15 **Mod.** 90^{th} % > 15 and <= 20 **Poor** 90^{th} % > 20 and <= 40 **Bad** 90^{th} % > 40	
Netherlands	*Metric: mean summer (March – September) chlorophyll-a concentration (µg/l) and on the maximum abundance of Phaeocystis (10^6 cells/l).* The class boundaries for the 90 %ile summer chlorophyll-a concentrations are water-type specific. For Phaeocystis the boundaries high/good and good/moderate are set to 10^6cells/l and 10^7 cells/l, respectively. These boundaries are identical for all waterbodies.	**NEA 1/26b)** **High** 90^{th} % <= 10 **Good** 90^{th} % <= 15 **Mod.** 90^{th} % <= 30 **Poor** 90^{th} % <= 60 Bad 90^{th} % > 60	

Norway	Metric: 90th percentile of the chlorophyll-a data set (µg L^{-1}). • Sampling frequency: once a fortnight or once a month. • Season: growth period, from February to October • Period: 6-year	**For the North Sea and Norwegian Sea (NEA 1/26a) and Skagerrak (NEA 9)** **High** 90th % <= 2.5 **Good** 90th % > 2.5-5.0 **Mod**. 90th % > 5.0 **For Skagerrak (NEA 8)** **High** 90th % <= 1.5 **Good** 90th % > 1.5-3.0 **Mod**. 90th % > 3.0 **For Skagerrak (NEA 10)** **High** 90th % <= 3.0 **Good** 90th % > 3.0-6.0 **Mod**. 90th % > 6.0	Chlorophyll-*a*: Norway is using methanol for chlorophyll a extraction according to Norwegian Standard 4767.
Denmark	Metric: 90th percentile of the chlorophyll-a data set (µg L^{-1}).	NEA 1/26c – North Sea – German Bight & Jutland High/Good: 5 µg/L Good/Moderate: 7.5 µg/L NEA 1/26d – Denmark Skagerrak High/Good: 3 µg/L Good/Moderate: 4 µg/L NEA 8 – (Kattegat + small area of Skagerrak) High/Good: 1.5 µg/L Good/Moderate: 3 µg/L	
Sweden	Metric: 90th percentile of the chlorophyll-a data set (µg L^{-1}). • Sampling frequency: monthly • Season: growth period, March - September • Period: 6-year	**High** Median and 90th below H/G boundary **Good** Median and 90th below G/M boundary **Moderate** Median and/or 90th above G/M boundary H/G boundary NEA8 (Kattegat + small area of Skagerrak): 1.5 µg/L NEA9 (Skagerrak): 2.5 µg/L NEA10 (Skagerrak): 3 µg/L G/M boundary NEA8 (Kattegat + small area of Skagerrak): 3 µg/L NEA9 (Skagerrak): 5 µg/L NEA10 (Skagerrak): 6 µg/L	Chlorophyll-*a* The Swedish classification tool differs by using mean values during June-August, resulting in different reference values and borders between the classes.
Portugal	Metric: 90th percentile of the chlorophyll-*a* data set (µg L^{-1}). • Sampling frequency: all available data • Season: all available data • Period: all available data Sa	Type NEA 1/26e **High** 90th percentile <8 µg/L **Good** 90th percentile ≥ 8 - <12 **Moderate** 90th percentile ≥12	*Note: some difference in sampling strategy may occur along the NEA Portuguese Coast. Data was sparse so all available data was used*

Belgium	Metric: 90th percentile of the chlorophyll "a" data set (µg L^{-1}). • Sampling frequency: monthly or once a fortnight from 2008 onwards • Season: growth period, from March to October • Period: 5-year, in 2007 with lower frequency	**NEA 1/26b** **High** 90th % <= 10 **Good** 90th % > 10 and <= 15 **Mod**. 90th % > 15 and <= 30 **Poor** 90th % > 30 and <= 45 **Bad** 90th % > 45	Chlorophyll is measured with the same frequency as the *Phaeocystis* counts measurements (once a month or once a fortnight, from 2008 onwards).
Germany	Metric: 90th percentile of the chlorophyll "a" data set (µg L^{-1}).	**NEA GIG Type 1/26c – North Sea - German Bight & Jutland –** High/Good: 5 µg L^{-1} Good/Moderate:7.5 µg L^{-1}	

Table 2.4.4: Description of national methods using phytoplankton counts and/or composition.

	Description of national method 1 Frequency (%) of blooms of any single phytoplankton taxa. 2 Frequency (%) Phaeocystis above 10^6 Cells/l)	Boundary classes agreed for national assessment	Variation in methods/ Comments on tools
UK	Elevated counts of phytoplankton groups are recorded and compared against thresholds, and calculated as a percentage of the total number of sampling occasions. There are 4 sub--metrics of phytoplankton, including • Total cell count, • individual species count, • *Phaeocystis* count and • Chlorophyll concentrations. Final assessment is on the mean of the four phytoplankton metrics	**Single (individual) taxa cell counts:** Threshold: 250,000 cells per liter (large phytoplankton) Percentage exceeding threshold: High – Count (%) < 20 % Good – Count (%) < 20 %-39 % Mod – Count (%) < 40 %-69 % Poor – Count (%) < 70 %-90 % Bad – Count (%) > 90 % ***Phaeocystis* cell count metric:** Threshold: 1,000,000 cells per liter Percentage exceeding threshold: High – Count (%) < 10 % Good – Count (%) 10 %-17 % Mod – Count (%) > 17 % Poor – Count (%) > 35 % Bad – Count (%) > 80 %	Elevated counts of single species are taken as a sub-metric within a tool, and final assessment is calculated as a combination of the 4 sub-metrics Other tools are used in the national assessment including • Seasonal succession of functional groups • Community indicator species (under development) Boundaries apply to UK NEA Type 1/26a & NEA Type 1/26b
Spain	The phytoplankton taxa included in the analysis are the species routinely monitored in coastal waters. • Three different approaches are followed depending on the degree of expertise in the identification and counting step ➢ Only diatoms, dinoflagellates and euglenophyceans are considered. Small flagellates and coccoids are not included. ➢ Small flagellates and coccoids are included and the phytoplankton is classified by two size categories: small (< 20 μm) and large phytoplankton (> 20 μm). ➢ Small flagellates and coccoids are included, but size categories are not established. • Sampling frequency: monthly (*) • Season: whole year, from January to December (*) • Period: 6-year (*)	**All geographical areas except Iberian upwelling coast and Western Cantabrian coast NEA 1/26a** **High -**Frequency of samples exceeding the threshold < 20 % **Good-**Frequency of samples exceeding the threshold ≥ 20 % and < 40 % **Moderate-**Frequency of samples exceeding the threshold ≥ 40 %. **Iberian Coast and Western Cantabrian Coast NEA 1/26e** **High -**Frequency of samples exceeding the threshold < 30 % **Good-**Frequency of samples exceeding the threshold ≥ 30 % and < 50 % **Moderate-**Frequency of samples exceeding the threshold ≥ 50 %.	The use of two thresholds (large and small phytoplankton) is not convenient for the national methodology. But, for intercalibration purposes in the coast of the País Vasco (Eastern Cantabrian coast) thresholds have been calculated for • large (75,000 cells/L) and • Small phytoplankton (750,000 cells/L). *Note: some difference in sampling strategy may occur along the NEA Spanish Coast* NB: Cells/litre thresholds for the other Spanish regions and types are documented, in the reference conditions and 'Results of Harmonisation' sections of this chapter.
ROI	Percentage of samples where counts of individual phytoplankton taxa (> 20 μm) are above a threshold of 250,000 cells/l. Sampling frequency: monthly between January and December. Assessment period: 5-6 years	**NEA Type 1/26a** **High -**Frequency of samples exceeding the threshold < 20 % **Good -**Frequency of samples exceeding the threshold ≥ 20 % and < 40 % **Moderate -**Frequency of samples exceeding the threshold ≥ 40 %.	For Irish coastal waters it will be necessary to distinguish between phytoplankton populations that are known to originate offshore and show no response to local nutrient conditions and those resident populations that do. Blooms that are considered to have originated offshore (based on existing knowledge of the region's oceanography) rather than in response to in situ growth conditions will be omitted from the analysis.

France	Metric: percentage of samples where a single taxa count is above the exceeding threshold. • Definition of the exceeding threshold: 100 000 cells per liter for large cells, and 250 000 cells per liter for small cells. • Sampling period : all the year • Sampling frequency: once a fortnight or once a month. • Concern coastal waterbodies • Period: 6-year	**Blooms of all species** **Types NEA 1/26a & 1/26b** **High** - Count (%) < 20 **Good** - Count (%) >= 20 and < 40 **Mod –** Count(%) >= 40 and < 70 **Poor –** Count(%) >= 70 and < 90 **Bad –** Count (%) >= 90	
Portugal	Phytoplankton counts are carried out. When elevated chlorophyll concentrations are detected (>12μg L^{-1} G/M threshold). Elevated counts of phytoplankton single taxon groups are recorded and compared against the cell count threshold, (microphytoplankton 100,000 cells /L, micro+nanophytoplankton 1,000,000 cells /ml). The occurrences of single taxon blooms are calculated as a percentage of the total number of sampling occasions. The metrics are • total cell count, • single taxon counts	**Iberian Upwelling Coast** **NEA 1/26e** **High -**Frequency of samples exceeding the threshold < 30 % **Good-**Frequency of samples exceeding the threshold ≥ 30 % and < 50 % **Moderate-**Frequency of samples exceeding the threshold ≥ 50 %.	The ***Phaeocystis* metric** is not applicable for the Iberian Upwelling Coast
Denmark	N/A	**N/A**	N/A
Norway	Percentage of samples where a single taxa count is above the exceeding threshold. • Definition of the exceeding threshold: 500 000 cells per liter. • Sampling period : all the year • Sampling frequency: once a fortnight or once a month. • Period: 6-year	**Blooms of all species, frequency of samples – TBC** **High** - Count (%) < 20 **Good** - Count (%) >= 20 and < 40 **Mod. –** Count(%) >= 40 and < 70 **Poor –** Count(%) >= 70 and < 90 **Bad –** Count (%) >= 90	The metric need further development because among other factors the cell size is not taken into consideration. Postponed to phase II intercalibration.
Sweden	Percentage of samples with Total cells of phytoplankton above a set threshold. Microplankton (>20 μm): **Tentative – no threshold (cells/L) at present** Nanoplankton (2-20 μm): **Tentative – no threshold (cells/L) at present** • Sampling frequency: monthly • Season: growth period, March - September • Period: 6-year	**Total cell counts, frequency of samples - TBC** **High** – < 20 % **Good** – 20 % - 39 % **Moderate** – 40 % - 69 % **Poor** – 70 % - 90 % **Bad** – > 90 %	This metric is not fully developed and evaluated for the Swedish NEA GIG 8, 9, 10, but needs further work, if to be used at all. In Sweden phytoplankton biovolume mean values during June-August have been used, resulting in different reference values and borders between the classes. Postponed to phase II intercalibration.

Belgium	Metric *Phaeocystis* counts • Sampling frequency: monthly or once a fortnight from 2008 onwards • Period: 5-year • Cell Threshold: 1,000,000 Cells/l	% Phaeocystis samples exceeding threshold (NEA Type 1/26b) HIGH - <10 % GOOD - 10 – 17 % MODERATE - >17 % POOR- >35 % BAD- >80 %	A metric evaluating elevated counts of harmful phytoplankton taxa is under development.
Netherlands	Metric *Phaeocystis* counts • Sampling frequency: monthly during growing season (March-Sept (incl.)) • Period: 6-year • Cell Threshold: 1,000,000 Cells/l	% Phaeocystis samples exceeding threshold (NEA Type 1/26b) HIGH - <10 % GOOD - 10 – 17 % MODERATE - >17 % POOR- >35 % BAD- >80 %	Sampling takes place in the growing season only. The frequency is calculated under the assumption that there are no *Phaeocystis* blooms outside the growing season.
Germany	Metric *Phaeocystis* counts • Cell Threshold: 1,000,000 Cells/l	**NEA GIG Type 1/26c – North Sea - German Bight & Jutland & NEA Type 3/4 – North Se - Ems, Weser, Elb Coast** HIGH - <10 % GOOD - 10 – 17 % MODERATE - >17 % POOR- >35 % BAD- >80 %	

2.4.3 Reference conditions and class boundary setting

Initially reference conditions for the phytoplankton quality element were put forward by each MS, using established background criteria from unimpacted sites (assumed to be in reference condition), modelling and regression from impacted site data, marine conventions, scientific literature and through expert agreement in the GIG. The diversity of measures required for setting reference conditions reflects the extremely diverse and localised ecohydrodynamics of the NEA GIG causing gross and subtle modification to phytoplankton responses to nutrients and climate.

The following outlines the reference setting protocols established by each Member state, and the reference thresholds that were derived for each of the different types.

United Kingdom (NEA GIG TYPE 1/26A and 1/26B)

• Atlantic Coast – Exposed West Coast of England and Northern Ireland (NEA 1/26a)

• North Sea, Eastern Irish Sea and Channel (NEA 1/26B)

Chlorophyll-a metric

An appropriate standard for assessing chlorophyll *a* concentration can be derived from the background nutrient concentrations by making some reasonable assumptions about nutrient conversion to plant biomass. From practical experience the UK has adopted 10 µg L^{-1} chlorophyll *a* as a guide for assessment in North Sea, Channel and Eastern Irish Sea Waters. It is therefore proposed that for UK Type 1/26b offshore waters that 10 µg L^{-1} chlorophyll *a* is adopted as the 50 % elevated value (implying a background value of 6.7 µg L^{-1} and a reasonable C : Chl factor of 0.012). For further information on conversion of C : Chl see Malcolm *et al* (2002).

For Atlantic Coastal Waters which include the Exposed West Coast of England and Northern Ireland, the UK has adopted 5 µg L^{-1} chlorophyll *a* as a guide for assessment. It is therefore proposed that for Atlantic Coast offshore waters 5 µg L^{-1} chlorophyll *a* is adopted as the 50 % elevated value (implying a background value of 3.33 µg L^{-1}).

Proposed Chl-a reference values for UK waters
• NEA GIG Type 1/26A – Atlantic Coast – 3.33 µg L^{-1} • NEA GIG Type 1/26B – North Sea, Channel, Eastern Irish Sea – 6.7 µg L^{-1}

Taxa count metric

Proposed reference values for the taxa count metric – UK
• NEA GIG Type 1/26a – Atlantic Coast & NEA 1/26b – North Sea, Channel, Eastern Irish Sea Cell Threshold: 250,000 cells/L (large phytoplankton) • Reference Percentage exceedent threshold: 16.7 % (2 samples, associated with natural spring and autum blooms. Assumes monthly sampling) • Percentage exceedent thresholds: H/G 20 %, G/M 40 %

Phaeocystis metric

Proposed reference values for the *Phaeocystis* metric – UK
• NEA GIG Type 1/26a – Atlantic Coast & NEA 1/26b – North Sea, Channel, Eastern Irish Sea Cell Threshold: 1,000,000 cells/L. • Reference percentage exceedent threshold: 8.30 % (1 sample in 12 months as part of natural spring bloom. Assumes monthly sampling) • Percentage exceedent thresholds: H/G 10 %, G/M 17 %

Spain (NEA GIG 1/26a and 1/26e)

The NEA coast of Spain has been divided into five different sub-areas that reflect the natural variation in abiotic factors driving the phytoplankton communities: Eastern Cantabrian Coast, Western Cantabrian Coast, Western Iberian Coast (upwelling), Canary Islands and Atlantic Coast (Southern Spain). A summary of the boundary setting protocol in each of these areas is provided below.

Eastern Cantabrian Coast (NEA 1/26a)

At the North of Spain, it extends between the border with France in País Vasco (43º 22´ N, 1º 46´ W) and Cape Peñas in Asturias (43º 39´ N, 5º 50´ W).

Chlorophyll-a metric

The 90^{th} percentile of the chlorophyll-a in reference stations ranged between 0.8 µg L^{-1} (País Vasco) and 2.8 µg L^{-1} (Asturias). If these values are compared to those observed in other Atlantic coastal waters (e. g. France, Portugal) the phytoplankton biomass in the Eastern Cantabrian coast is considerably lower. However the specific hydrographical conditions of the Eastern Cantabrian coast in relation to other Atlantic areas can explain the differences in chlorophyll “a” concentration. The Cantabrian shelf is narrower compared to the French shelf. Also, nutrient fluxes to the Cantabrian shelf are distributed among several small rivers along the coast and no large plumes are formed (Diez et al. 2000). The intensity of the Iberian upwelling system decreases eastward along the Cantabrian shelf and it only slightly affects the Eastern Cantabrian Coast (Valencia et al. 2004; Mason et al. 2005; Lavin et al. 2006).

Taxa count metric

Phytoplankton counts were conducted since 2002 in the coastal waters of País Vasco. The anthropogenic eutrophication impact in the studied stations is considered negligible due to the strong hydrodynamic forces that act on this coast and, therefore, the phytoplankton is assumed to be in High or Good status. Low chlorophyll concentrations (see above) support this assumption. Water samples were collected at surface every 6 months. Sampling seasons corresponded to spring (May) and summer (August). Standard methods were used for field and laboratory: Van-Dorn bottle sampling, inverted microscopy and Uthermöhl. Based on the work of Borja et al. (2004), a bloom frequency of 20 % was considered the boundary between High and Good status and 40 % the boundary between Good and Moderate status. In order to calculate the abundance threshold that defines a bloom**,** three different approaches were followed:

1. Only diatoms, dinoflagellates and euglenophyceans were included in the data analysis. Small flagellates and coccoids were not included. By this method, **500,000 cells L^{-1}** was the most suitable threshold to define a bloom.
2. Small flagellates and coccoids were included in the data analysis and the phytoplankton community was split into two size categories: small (< 20 μm) and large phytoplankton (> 20 μm). By using a criteria based on the Equivalent Spherical Diameter (ESD), 102 taxa were classify as large phytoplankton and 126 taxa as small phytoplankton. The thresholds that best fitted with this method were: **75,000 cells L^{-1} for large phytoplankton and 750,000 cells L^{-1} for small phytoplankton**.
3. Small flagellates and coccoids were included in the data analysis, but size categories were not established. The resulting threshold to be applied to any single phytoplankton taxa (regardless of its cellular size) was **750,000 cells L^{-1}**.

The most accurate method was based on the use of two different thresholds, for large and small phytoplankton respectively (approach 2). However, the size-fractionation method was very time-consuming as it required obtaining and processing the information of the ESD for the phytoplankton taxa. Therefore, it was found not suitable as a routine phytoplankton monitoring method. Other alternative methods were based on the use of a single threshold (approach 1 and 3) and the results obtained by them in the Basque coast (País Vasco) were acceptable and very similar. At present, in the Eastern Cantabrian Coast the approach 3 and the 750,000 cells L^{-1} threshold have been chosen because the monitoring programs provide accurate counts of the small phytoplankton forms.
However, it is important to note that the abundance threshold to define a phytoplankton bloom depends on the methodology used (the counting expertise and the type of data analysis employed). This is evidenced by the different thresholds obtained with similar samples when using the approach 1 (only some taxonomic groups), the approach 2 (size-fractionation) or the approach 3 (all phytoplankton taxa).

Western Cantrabrian Coast (NEA 1/26e)

At the North of Spain, it extends between Cape Peñas in Asturias (43º 39' N, 5º 50' W) and Cape Estaca de Bares in Galicia (43º 47′ N, 7º 41′ W).

Chlorophyll -a metric

The chlorophyll "a" data were obtained in the coast of Asturias (43º 36′ N, 6º 08′ W). The 90th percentile value in the reference station was 2.2 μg L^{-1}. In addition to the time series of field data from Asturias, the influence and regional variation of the upwelling process over the Western Cantabrian coast needs to be taken into consideration to establish sensible limits for the whole region. The review of published data by Bode et al. (1996) showed geographic differences in the upwelling regime, so that the mean chlorophyll "a" concentration increases westward along the

N-NW Spanish coast in most oceanographic stages. These geographic differences are lower during unproductive phases (summer stratification and winter) and the highest during the upwelling stage, when the mean integrated chlorophyll “a” concentration increases twofold from the Western Cantabrian Coast to the Western Iberian Coast (Southern Galicia). In turn, this geographic gradient in the mean chlorophyll “a” concentration should reflect on a gradient for the limits of the 90th percentile chlorophyll “a” along the N-NW coast of the Iberian Peninsula.

Taxa count metric

By expert judgement, in accordance with the westward gradient of increasing upwelling influence along the N-NW Spanish coast, 750,000 cells L^{-1} is proposed as the most suitable threshold for defining a phytoplankton bloom in the Western Cantabrian Coast and, similarly to the Iberian upwelling coast (see below), 30 % and 50 % as the boundaries between classes (High/Good, Good/Moderate). As with phytoplankton biomass, it is important to stress that the current paucity of data available for intercalibration, together with the high spatial and temporal variability associated to the wind-driven upwelling processes, may require a thorough revision of the proposed thresholds, once suitable databases become available.

Western Iberian Coast (upwelling) (NEA 1/26e)

The Iberian upwelling coast in Spain extends along the coast of Galicia, from the Cape Estaca de Bares (43º 47’ N, 7º 41’ W) to the border with Portugal.

Hydrographic changes associated with upwelling and their influence on nutrient regimes and phytoplankton cycles in the Galician coast are well-documented. Many studies have focused on the hydrographical and biological features of the Iberian upwelling system with particular emphasis on the enhancement of biological productivity (Fraga 1976; 1981; Álvarez-Salgado et al. 1996; Moncoiffé et al. 2000; Cermeño et al 2006). The Galician coast is also characterised by the presence of wide coastal embayments or rías. These are deep estuaries (ca. 40 m depth near the mouth), whose intense exchange of water, and hence materials, with the ocean leads to their consideration as coastal intrusions of the shelf. The fertilizing process in the rías leads to higher primary production rates than in other estuarine or coastal systems (Boynton et al. 1982). For example in the Ría de Vigo mean annual primary production rates are about 800 mg C m^{-2} d^{-1} (Prego 1994) and the maximum values from spring to autumn range between 700 and 1800 mg C m^{-2} d^{-1} (Vives and Fraga 1961, in Nogueira et al. 1997). In terms of biomass and primary production there are not significant differences between the upwelling season and the spring bloom. Although seasonally variable in frequency and intensity, the upwelling is an episodic process driven by the wind. The close relationship between wind regime, nutrient inputs and chlorophyll concentration has been reported in several papers (Moncoiffé et al. 2000, Cermeño et al. 2006). This close relationship with the wind regime reflects on a high frequency of phytoplankton blooms and very high short-scale variability in sea surface chlorophyll “a” concentration.

Chlorophyll-a metric

The data were obtained at the Ría de A Coruña (43° 21.8’ N, 8° 22.2’ W) and in the middle zone of the Ría de Vigo (42º 14.5’ N, 8º 45.8’ W). The calculated values of the 90th percentile were 4.73 µg L^{-1} at the A Coruña station (monthly sampled, from 1992 to 2006) and 10.55 µg L^{-1} at the Vigo station (two samples per week, from 1987 to 1996). A previous analysis by the INTECMAR Centre in Galicia (*Phytoplankton tools for NEA type 1 Galician waters*), calculated the 90th percentile from 37 stations distributed in the southern part of the Spanish upwelling coast (between the border with Portugal and Cape Finisterre) and 13 stations in the northern part (between Cape Finisterre and Cape Estaca de Bares). All the stations were weekly sampled from 1992 to 2004 and the values obtained for the 90th percentile were 4 and 5 µg L^{-1} in the southern and northern part of the Spanish upwelling coast, respectively.

Taxa count metric

Phytoplankton cell counts in the Ría de A Coruña (43° 21.8’ N, 8° 22.2’ W) were carried out from 1992 to 1998. Samples were collected monthly, preserved with Lugol’s solution and counted under an inverted microscope following the technique described by Uthermöl (1958). The Ría de A Coruña can be considered a bay with important oceanic influence, whose status is considered High or Good. According to the data from the Ría de A Coruña, a value of 10^6 cells L^{-1} is proposed as the threshold for a phytoplankton bloom. This coincides with the value proposed by the INTECMAR Centre (*Phytoplankton tools for NEA type 1 Galician waters*) after a study of data from 40 stations weekly sampled along the Galician coast from 1999 to 2004. According to the data analysis and the knowledge of the high variability in sea surface phytoplankton in the area (see above), the High/Good and Good/Moderate boundaries are set at 30 % and 50 % bloom frequencies, respectively.

Canary Islands (NEA 1/26a)

Chlorophyll-a metric

The Canary Islands (~28° N) are located in a transition zone between the oligotrophic waters of the open ocean and the NW Africa upwelling system (Barton et al. 1998). Although there is a slight increasing gradient in chlorophyll-a towards the Eastern islands, the mean values are low during most of the year all around the Archipelago and maxima are typically < 1 µg L^{-1} (Aristegui 1990).

Taxa count metric

Taking into account the oligotrophic character of the Canary Islands in comparison to other Spanish NEA coastal waters, 500,000 cells L^{-1} is proposed as the threshold for defining a phytoplankton bloom and 20 % and 40 % as the boundaries between classes (High/Good, Good/Moderate) in this coastal area.

Atlantic Coast (NEA 1/26a)

The Atlantic coast in the South of Spain extends from the mouth of the Guadiana River at the Portuguese border to the mouth of the Guadalmesí River at the Straight of Gibraltar. This coastline belong to the area of the Gulf of Cadiz in Andalucía and it can be distinguished from the coastline open to the ocean (from Cape San Vicente to the North of Spain) as it represents an area of transition between the Mediterranean Sea and the Atlantic Ocean (OSPAR 2000).

Chlorophyll-a metric

The Atlantic coast in the South of Spain presents a large continental platform and it receives the runoff from two important rivers (Guadalquivir and Guadiana Rivers).

In addition, some upwelling influence has been described in the Gulf of Cadiz (Garcia et al. 2002). Also, the continuity with the Portuguese coast, westward of the Guadiana mouth, must be considered and the thresholds for phytoplankton biomass should not differ importantly between them. Extensive data series for chlorophyll in reference monitoring stations are not available. Therefore, on the basis of expert judgment, the thresholds proposed for the 90^{th} percentile of chlorophyll “a” in the Atlantic coast (S Spain) are 5 µg L^{-1} (between high and good status) and 10 µg L^{-1} (between good and moderated status). Nevertheless, in the future, chlorophyll data that are being acquired now could lead to consider a new definition of these thresholds for the Atlantic coast in the South of Spain.

Taxa count metric

For the determination of the cell abundance that indicates a bloom event in the Gulf of Cadiz, data series corresponding to 6-year periods have been studied. The minimum sampling frequency was monthly and, in some cases, weekly or fortnightly. Small phytoplankton forms (mainly flagellates) were not recorded. Different abundance values to define a phytoplankton bloom were studied both, in reference stations and, in other areas considered with a slightly higher level of eutrophication. Attending to the results, the threshold of 500,000 cells L^{-1} was set to define a bloom event, with 20 % and 40 % as the boundaries between classes (High/Good, Good/Moderate).

Proposed reference values for the chlorophyll “a” (90th percentile) metric – Spain
• Eastern Cantabrian Coast (NEA Type 1/26a) 1 - 3 µg L^{-1} • Western Cantabrian Coast (NEA Type 1/26e) 2 – 5 µg L^{-1} • Western Iberian Coast upwelling (NEA Type 1/26e) 4 -11 µg L^{-1} • Canary Islands (NEA Type 1/26a) < 1 µg L^{-1} • Atlantic Coast (NEA Type 1/26a) < 5 µg L^{-1}

Proposed reference values for the taxa count metric – Spain
• Eastern Cantabrian Coast (NEA1/26a)-Threshold 750,000 cells/L- % Frequency Exceeding cell threshold: Reference 16.70 %, H/G 20 %- G/M 40 % • Western Cantabrian Coast (NEA1/26e)- Threshold 750,000 cells/L- % Frequency Exceeding cell threshold: Reference 25 %, H/G 30 %- G/M 50 % • Western Iberian Coast (upwelling, NEA1/26e)- Threshold 1,000,000 cells/L- % Frequency Exceeding cell threshold: Reference 25 %, H/G 30 %- G/M 50 % • Canary Islands (NEA1/26a)- Threshold 500,000 cells/L- % Frequency Exceeding cell threshold: Reference 16.70 %, H/G 20 %- G /M 40 % • Atlantic Coast (NEA1/26a)- Threshold 500,000 cells/L- % Frequency Exceeding cell threshold: Reference 16.70 %, H/G 20 %- G/M 40 %

Portugal

NEA GIG TYPE 1/26e Western Iberian Coast (upwelling)

Chlorophyll-a metric

The coast of Portugal extends north from Cape St Vincent to the border with northern Spain at the Minho. This coastline has a narrow continental shelf and is subject high hydrodynamics and upwelling.

The south coast of Portugal extends west from Cape St Vincent to the border with southern Spain at the Guadiana. Although less exposed than the west coast it also has a narrow continental shelf and is subjected to upwelling, (Garcia *et al.* 2002).

The continuity with the Andalucian coast, east of the Guadiana mouth, and the northern border with Spain must be considered, and the thresholds for phytoplankton biomass should not differ significantly between Portugal and Spain. The 90th percentile of chlorophyll “a” for Portugal is 5 µg L^{-1} (boundary 8 µg L^{-1} between high and good status) and 10 µg L^{-1} (boundary 12 µg L^{-1} between good and moderate status). Nevertheless, these may be revised in the future, as more chlorophyll data becomes available.

Proposed Reference chl-a values for Portuguese waters
• NEA GIG TYPE 1/26e Western Iberian Coast (upwelling) - • Reference condition <4 µg L^{-1}, High/ Good- 8 µg L^{-1}, Good/Moderate - 12 µg L^{-1}

Taxa count metric

Proposed Reference taxa counts values for Portuguese waters
• NEA GIG TYPE 1/26e Western Iberian Coast (upwelling) Cell Threshold: 100,000 cells/L (large phytoplankton threshold), 1,000,000 cells/L (small phytoplankton threshold) • Percentage exceedent thresholds: Reference = 25 % (3 blooms expected per year, spring, summer and autumn, with monthly sampling), H/G <30 %, G/M <50 %

Republic of Ireland

NEA GIG TYPE 1/26a (Atlantic Coast and Western Irish Sea)

Chlorophyll-a metric

Reference criteria were based on contemporaneous data collected from undisturbed waterbodies that were considered to be close to or at reference as determined by expert judgment, the Article 5 Characterisation report and supporting environmental data. The proposed class boundaries for excessive cell counts were based on the analysis of phytoplankton data collected from coastal waterbodies that are considered to be close to or at reference. Reference criteria were provided for 2 of the 3 intercalibration common metrics (i.e. chlorophyll biomass and excessive cell counts).
The metric based on *Phaeocystis* was not considered to be relevant for Irish coastal waters and was therefore not included in Ireland's intercalibration exercise.

A 100 % agreement was obtained in a comparison of the intercalibration common metric against the national assessment method (see Appendix x). It should be noted that the chlorophyll data used by the Republic of Ireland for the intercalibration exercise was derived using 2 different extraction methods that are known to have different extractant efficiencies. Intercomparisons of the two extractants have shown that chlorophyll levels extracted using the hot methanol method can be, on average 25 - 40 % higher than cold acetone. The difference can vary considerably depending on phytoplankton species composition at time of sampling. As a consequence of these differences it was deemed necessary to develop separate class boundary criteria for both extraction methods.
The boundary setting criteria for chlorophyll in Irish coastal waters, for both extraction techniques, is summarised in Table 5**.** The difference in magnitude between both sets of criteria, with the hot methanol criteria being twice that of the cold acetone, appears to be consistent with observations, first, that methanol is the more efficient extractant and second, that the hot methanol technique is likely to overestimate chlorophyll values because it does not correct for degradation products.

Table 2.4.5: Median and 90^{th} percentile-a (μg L^{-1}) boundary criteria for different pigment extraction techniques.

Extractant	Cold Acetone		Hot Methanol	
	Median	90 %ile	Median	90 %ile
Boundary				
high/good	2.5	5.0	5.0	10.0
good/moderate	5.0	10.0	10.0	20.0

Proposed reference chl-a values for ROI waters
• NEA GIG Type 1/26A – Irish Sea (Acetone Method) – 3.7 μg L^{-1} • NEA GIG Type 1/26A – Atlantic Sea (Acetone Method) – 3.4 μg L^{-1}

Taxa count metric

Proposed reference taxa count values for ROI waters
• NEA GIG Type 1/26A – Irish Sea & Atlantic Sea Cell Threshold: 250,000 cells/L (Large phytoplankton) • Reference Percentage exceedent threshold: 16.7 % (2 samples, associated with natural spring and autumn blooms. Assumes monthly sampling) • Percentage exceedent thresholds: H/G 20 %, G/M 40 %

Netherlands

NEA Type 3, 4 & NEA Type 1/26b

• Holland and North Delta Coast (NEA Type 3)
• Dutch Wadden Sea (NEA Type 4)
• Zeeland Coast and Wadden Coast (NEA Type 1, 1/26b)

Chlorophyll-a metric

Originally all coastal waters of UK (east) and NL were classified as one type (NEA1) and therefore had the same reference values. However, with regard to the mean salinity values the Holland Coast more properly fell in the NEA3 type. The difference between the UK discharges and the Dutch discharges is a factor 1.7, and between the German discharges and the Dutch discharges is a factor 2.3. Assuming that the concentrations of nutrients in the pristine situation were identical in the British and the continental rivers, the nutrient load on the Holland Coast can be expected to be 1.7 times higher than that in the British waters and 2.1 times higher than in the German waters. New class boundaries of NEA3 have been calculated based on freshwater discharges. Therefore the elevated values for the 90-percentile of the chlorophyll-*a* concentrations in the Holland Coast will be in the range of 11 to 17 μg L^{-1} i.e. at least a factor 2.1 higher than the values of the German Coast and at most 1.7 higher than the values for the English coast due to the higher run off of the Netherlands/Holland Coast typology.
According to Cadée and Hegeman (2002) the annual average chlorophyll-*a* concentrations in the 70's of the last century have been between 2 and 5 μg L^{-1}. With the relationships between annual mean and summer mean, the related range of the summer mean could be calculated and by multiplying the numbers of this range with the factor 2 (relation mean – 90-percentile) the range of the summer 90-percentile has been derived, as being 5 – 13 μg L^{-1} [1]. Assuming that the chlorophyll concentrations in the 70's were elevated in relation to the undisturbed background concentrations, but still below 1.5 times the background, the value 14 μg/l deduced from the freshwater discharges agrees very well with the value deduced from Cadée & Hegeman (2002). In the framework of the "Watersysteemverkenning" (Water System Exploration) in the Netherlands, so called reference values (which were in fact the boundaries between High and Good) for a number of functional groups and individual species have been calculated (Baptist & Jagtman, 1997). For chlorophyll the "reference" value has been expressed as the 90-percentile value of the concentration. The calculation of this value has been based on model simulations and expert judgement and is calculated as 14.3 μg/l for NEA3 in Dutch coastal waters. Taking 14 μg/l as boundary between High and Good gives a reference value of 9.3 μg L^{-1} (2/3 * 14) and a boundary between Good and Moderate of 21 μg L^{-1} (1.5 *14).

The waterbodies Zeeland Coast and Wadden Coast are belonging to the euhaline water type, NEA1. Although the influence of freshwater in the Zeeland Coast is slightly higher than in UK coastal waters the class boundaries for the 90-percentile summer chlorophyll-*a* concentrations for both areas could be set on 10 and 15 µg L^{-1}, with a reference values of 6.7 µg L^{-1}
Class boundaries for NEA4 for the Dutch Wadden Sea are set equal to NEA3 (14 and 21 µg L^{-1}), based on comparable influence of Rhine discharges.

Proposed reference chl-a values for Netherlands Waters
• NEA GIG Type 4 – Dutch Wadden Sea – 9.3 µg L^{-1} • NEA GIG Type 3 – Holland and North Delta Coast – 9.3 µg L^{-1} • NEA GIG Type 1 (1/26b) – Zeeland Coast and Wadden Coast – 6.7µg L^{-1}

Phaeocystis metric

Proposed reference *Phaeocystis* values for Netherlands waters
• NEA GIG Type 1 (1/26b) - Zeeland Coast and Wadden Coast, NEA Type 3 - Holland and NEA Type 4 - North Delta Coast, Dutch Wadden Sea - Cell threshold: 1,000,000 cells/L • Reference percentage exceedent threshold: 8.30 % (1 sample in 12 months as part of natural spring bloom. Assumes monthly sampling) • Percentage exceedent thresholds: H/G 10 %, G/M 17 %

France

Chlorophyll-a metric

French Channel and Atlantic coast (except Northern part) / type NEA 1/26a
Reference values are based on data collected from undisturbed sites that are considered to be close to or at reference as determined by expert judgment. The 90th percentile calculated on these reference sites shows that the reference values may be different between sites:

site	90th percentile
Cherbourg Port / 2004-2005	4.40
Donville / 2002-2006	2.58
Chausey / 2001-2006	2.29
Bréhat / 1992-2006	1.71
Ouessant - cale de Porz Arlan / 2006	1.30
Groix nord / 1996-2006	3.99
Port Joinville / 2006	2.20
Bouée 7 / 2003-2006	2.85

This is considered as reflecting the diversity of the French coast, since the values are consistent between large geographical regions: the lowest values concern the Northern and Western parts of Brittany, while the highest ones concern primarily Normandy. The reference value is therefore proposed as a range between the lowest (1.30) and the highest value (4.4) of these reference sites.

Northern part of the French coast (North Sea) / type NEA 1/26b

No reference site could be found in this region. However, data collected in two offshore sites (site 4 SRN Dunkerque and site 3 SRN Boulogne) show that the values of 90th percentile chlorophyll-*a* on the period 1992-2006 are respectively 9.9 and 6.2 µg/L. It may be considered by expert judgment that these values are consistent with the reference value calculated for the type NEA 1/26b. So it is decided that, in the absence of reference site for this part of French coast, the calculated value of 6.7 µg/L should be the reference value.

Proposed reference chl-a values for French Waters
• NEA GIG Type 1/26a – French Channel and Atlantic Sea – 1.3 – 4.4 µg L^{-1} • NEA GIG Type 1/26b – French North Sea – 6.7 µg L^{-1}

Taxa count metric

Proposed reference taxa count values for French waters
• NEA GIG Type 1/26a – French Channel and Atlantic Sea – 100,000 cells/L (Large phytoplankton threshold, 250,000 cells/L (Small phytoplankton threshold) • Reference Percentage exceedent threshold: 16.7 % (2 samples, associated with natural spring and autumn blooms. Assumes monthly sampling) • Percentage exceedent thresholds: H/G 20 %, G/M 40 %

Sweden

NEA GIG TYPE 8, 9 and 10
• Öresund and Kattegat (8)
• Skagerrak (9)
• Skagerrak (10)

Chlorophyll-a metric

Sweden is using the method outlined in the Combine manual of HELCOM (www.helcom.fi).
In short this means; fluorometric measurement, calibrated against spectrophotometric method, ethanol as solvent. Sweden reports Chlorophyll-*a*, without subtraction of Phaeopigment.

Along the Swedish west coast (Skagerrak, Kattegat and Öresund) reference values of chlorophyll are based on off shore data. Temporal changes of Secchi depth and Total Nitrogen were used to establish “unaffected” (background) values. The relations between Secchi depth, Total Nitrogen, salinity and chlorophyll-*a* were then used to establish the Chlorophyll a reference value for each Type Area. Boundary classes were calculated using the relationships between Secchi depth, Total Nitrogen and chlorophyll-*a*. The boundary between GOOD and MODERATE was set with the assumption that the Skagerrak and Kattegat are not unaffected by man. Other class boundaries were set by a 50-75 % increase between the classes.

Proposed reference chl-a values for Swedish waters
• NEA GIG Type 8 – Öresund and Kattegat – 1.0 µg L^{-1} • NEA GIG Type 9 – Skagerrak – 1.7 µg L^{-1} • NEA GIG Type 10 – Skagerrak – 2.0 µg L^{-1}

Abundance of phytoplankton as a metric has not been used in Sweden and reference values and boundaries can therefore not be presented at present. However Sweden is developing phytoplankton abundance as a metric, which can be used as a parallel to the Swedish phytoplankton metric BIOVOLUME of phytoplankton.

Norway

Chlorophyll-a metric

The method for measuring chlorophyll-*a* in Norway is in accordance to Norwegian Standard 4767 using methanol for pigment extraction. There is no correction for pheopigments.
For establishing reference values and boundary criteria "Classification of environmental quality in fjord and coastal waters. A guide" (Norwegian Pollution Control Authority 1997) and expert judgment has been used. Lack of long term series from monitoring programs from relevant areas have prevented use of historical data.

Proposed reference chl-a values for Norwegian waters
• NEA GIG Type 1/26a – North Sea, Norwegian Sea – 1.7 µg L^{-1} • NEA GIG Type 8 – Skagerrak (see map) – 1.0 µg L^{-1} • NEA GIG Type 9 – Skagerrak (see map) – 1.7 µg L^{-1} • NEA GIG Type 10 – Skagerrak (see map) – 2.0 µg L^{-1}

Norway has not used abundance of phytoplankton as a metric, as reference values and boundaries can not be presented at this stage. However, phytoplankton as a metric is under development as well as cell carbon and biovolume.

Belgium

NEA GIG TYPE 1/26b

Chlorophyll-a metric

For Chl-*a*, a background Chl-*a* concentration was determined for the application of phytoplankton assessment criteria for the Belgian Continental Shelf. Eutrophication in the BCS occurs in the form of massive, ephemeral *Phaeocystis* colony blooms during spring (April-May). *Phaeocystis* colonies generally occur simultaneously with diatom (mainly *Guinardia* spp.) blooms that considerably affect the Chl-a concentrations. Analysis of historical data (ASMO 98/3/Info.1-F) suggests that foam accumulation would occur from a *Phaeocystis* cell concentration of 10^7 l^{-1}. This cell concentration can be converted into a Chl-a concentration of 5 µg Chl-a l^{-1} (C : Chla = 29) using the experimentally determined factor of 0.5 pg Chl-a /*Phaeocystis* cell (Rousseau *et al.*, 1990).
The contribution of diatoms to the spring bloom has been established from the analysis of a 13 year time series at a monitoring station (330) located in the centre of the Belgian Continental Shelf. Seasonal evolution of Chl-a concentrations measured on a weekly basis indicates that the average Chl-*a* concentration of 9.2 µg l^{-1} corresponds to the *Phaeocystis* pre-bloom situation, itself determined as a *Phaeocystis* concentration higher than $1x10^6$ cells l^{-1} (Cadée & Hegeman, 1991).
This allows us to consider a threshold of 15 µg Chl-*a* l^{-1} from which nutrient over-enrichment would result in ecosystem disturbance.

Proposed reference chl-a values for Belgium waters **(Calculated reference condition, based on 50 % deviation of reference from the high/good boundary)**
NEA GIG Type 1/26b – North Sea – 6.7 µg L^{-1}

Phaeocystis metric

Proposed reference *Phaeocystis* values for Belgium waters
NEA GIG Type 1/26b – North Sea – Cell threshold: 1,000,000 cell/L
Reference percentage exceedent threshold: 8.30 % (1 sample in 12 months as part of natural spring bloom. Assumes monthly sampling)
Percentage exceedent thresholds: H/G 10 %, G/M 17 %

Denmark

- NEA GIG Type 1/26c – North Sea (German Bright & Jutland)
- NEA GIG Type 1/26d – Denmark Skagerrak
- NEA GIG Type 8 – Kattegat + Small area of Skagerrak

Chlorophyll-a metric

Proposed reference chl-a values for Denmark waters (Calculated reference condition, based on 50 % deviation of reference from the high/good boundary)
• NEA GIG Type 1/26c – North Sea (German Bright & Jutland) – 3.3 μg L^{-1} • NEA GIG Type 1/26d – Denmark Skagerrak – 2.0 μg L^{-1} • NEA GIG Type 8 – Kattegat + Small area of Skagerrak – 1.0 μg L^{-1}

Germany

Chlorophyll-a metric

Proposed reference chl-a values for German waters
• NEA GIG Type 1/26c – North Sea - German Bright & Jutland – 3.3 μg L^{-1} • NEA GIG Type 3/4 – North Se - Ems, Weser, Elb Coast – 3.3 μg L^{-1}

Phaeocystis metric

Proposed reference *Phaeocystis* values for German waters
• NEA GIG Type 1/26c – North Sea - German Bright & Jutland – Cell threshold: 1,000,000 cell/L • NEA GIG Type 3 4 – North Sea - Ems, Weser, Elb Coast – Cell threshold: 1,000,000 cell/L • Reference percentage exceedent threshold: 8.30 % (1 sample in 12 months as part of natural spring bloom. Assumes monthly sampling) • Percentage exceedent thresholds: H/G 10 %, G/M 17 %

Class Boundary Setting Procedures

The reference values put forward by each MS for the typologies were specific to each type, and vary geographically. The boundary setting protocols investigated conditions that influenced the geographical difference between the NEA GIG types. Examination of the conditions influencing the variation between NEA types supported the delineation of the geographical areas, and the reasoning behind the differences in reference conditions.

The NEA GIG covers a very large geographic area from Southwest Spain (including the Canary Islands, 28ºN) to Northern Norway (71ºN). These areas will have different non-anthropogenic pressures, which would affect the onset, rate of growth and overall productivity of phytoplankton populations in identical typologies, consequently different thresholds need to be set. The main non-anthropogenic drivers are:

- Isolation
- Freshwater discharge & catchment geology
- Upwellings
- Continental shelf width / break
- Coastal geomorphology & Sediment loads
- Tidal height & coastal currents
- Basic salinity regime

The OSPAR Quality Status Review (2000) recognizes some of these hydromorphic features in its approach to regional seas (Figure 2.4.1).
However for the purposes of setting chlorophyll boundaries and thresholds the local nature of all the factors above also needs to be taken into account.

Major influences on sub-types

The first layer of types was determined primarily on freshwater inflow. Details of freshwater flow characteristics are found in Table 6. On the basis of freshwater flow, an initial boundary setting procedure differentiates 9 broad coastal types as shown in Table 7
Higher freshwater flows within the Dutch coastal zone compared to other NEA-GIG coasts, change the typology of the Holland Coast from North Sea euhaline (NEA1) into a polyhaline (NEA3).
However, freshwater flow does not account for all the variability between GIG types and further delineation is explained by both upwelling and localised influences.

Within each of the broad typologies, further delineation was required to account for upwelling and localized and regional variations within the typologies.

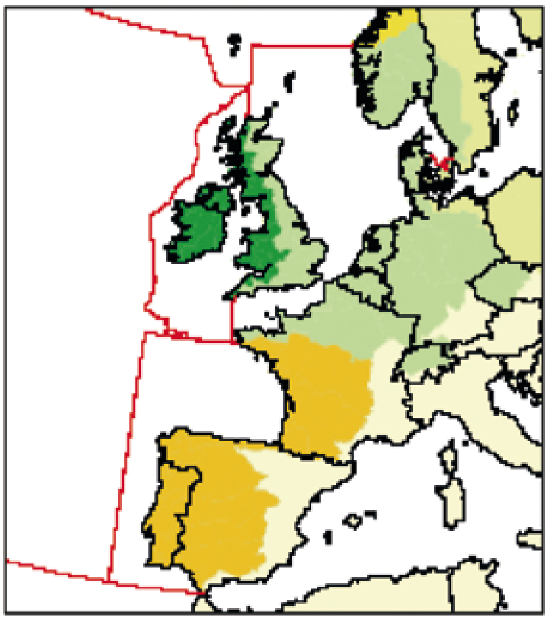


Figure 2.4.1: Hydromorphic areas of the NEA GIG Atlantic area.

Table 2.4.6: Freshwater flow characteristics of each region associated with reference conditions of chlorophyll.

Zone	Member States	Freshwater Discharge/ Runoff (km^3/year)
Western Iberian coast upwelling	Portugal / Spain West coast	63
Cantabrian coast	North Spanish Coast	16
Atlantic Coast	French Atlantic coast	46.5
Atlantic Coast	Atlantic Coast (UK and IE)	40
North Sea	North Sea (UK, NL, Belgium)	122
Irish Sea	Irish Sea (UK & IE)	80
German Bight	Germany & Denmark	22
Scandinavian Coast	Denmark, Sweden & Norway	21.5

For example, because of the extreme variation in latitude within the type NEA1/26 it was not possible to agree a single range of chlorophyll thresholds across the entire area as there are significant differences in how phytoplankton reacts to natural and anthropogenic nutrient enrichment. There are clear differences between the open clearer Atlantic waters and the more enclosed sea areas and special cases e.g. where natural upwelling occurs, particularly on the Western Iberian coast.

It was therefore agreed by the GIG that the only way to produce meaningful results was to split type NEA1/26 into five sub-types, NEA1/26a, b, c, d, e. The sea areas grouped under this new typology are described in Table 7.

Type 1/26a generally reflects open clearer Atlantic Waters and Type1/26b generally reflects more enclosed sea areas. Spain and Portugal are both affected by the Iberian Upwelling, which reacts very differently from other regions in the NEA GIG, with naturally nutrient enriched coastal waters and more natural phytoplankton blooms expected through out the year. This upwelling area has been designated Type 1/26e.

Kattegat and Skagerrak also behave differently from the other areas. It is a complex system with low salinity (surface values from salinity 10-12 in south Kattegat increasing up 25-30 in the Skagerrak) and the areas are greatly influenced by inflow of water from the Baltic Sea, the German Bight, the North sea and the Atlantic. Stratification, sometimes three layers, occurs in most of the area in the growing season due to the different salinity in the different inflowing water types and the relatively shallow water. Due to these complex factors, the Scandinavian coast is divided into type 8, 9, 10 with additional separation of small areas into 1/26a, 1/26c and 1/26d.

There is also a change of chlorophyll thresholds across the Spain/ France border. The differences in morphological and hydrographical features between the Spanish and French shelves have been taken into account to justify lower thresholds of the Eastern Cantabrian coast (Spain'NEA1/26a). The Eastern Cantabrian coast has almost no coastal plain and the rivers are small, whereas the French shelf presents a wide coastal plain and much higher river runoff.

Table 2.4.7: Description of NEA GIG types and associated areas.

New Type	Areas
NEA1/26a	Eastern Cantabrian Coast, Canary Islands. Atlantic Coast, Western Irish Sea, Scandinavian Coast: North Sea Norway + Norwegian Sea
NEA1/26b	Eastern Irish Sea, North Sea (UK East Coast, Channel, NL Wadden Coast, Zeeland Coast, Belgium Coast)
NEA1/26c	German Bight & Jutland
NEA1/26d	Scandinavian Coast: Denmark Skagerrak
NEA 1/26e	Western Iberian Coast upwelling, Western Cantabrian Coast
NEA8	Denmark, Norway, Sweden: Kattegat + small area of Skagerrak
NEA9	Sweden, Norway: Skagerrak
NEA10	Sweden, Norway: Skagerrak

Thus if we take into account, the variation between freshwater flow, upwelling and localised regional differences, the NEA GIG can be separated into 9 distinct coastal areas. The nine coastal types will be intercalibrated in Phase I. Intercalibration on NEA Type 11 (Transitional waters) will be taken up in Phase 2 Intercalibration. The final delineation of NEA GIG Coastal Types for Phase I intercalibration is illustrated in Figure 2.

NB. The map in Figure 2 shows the regions and areas and the coastal types that apply, Germany & Netherlands appears to have two types overlying each other, this is due to off shore coastal islands that respond to North Sea influences defined by type NEA 1/26b and an inshore area (Wadden Sea Coast) which response to influences defined by type NEA3/4.

2.4.4 Results of the comparison

As option 2 has been used, the boundaries have been agreed by experts representing all countries in the GIG phytoplankton sub-group. Therefore the final agreed results presented below are the results of comparing expert views on what the boundaries should be related to type differences and reference levels.

2.4.5 Results of the harmonization – Boundary thresholds and EQR values

Metric-Chlorophyll-a **concentration (90th Percentile) (Boundary Setting Procedure and Results (All Coastal Water Types: NEA 1/26a,b,c,d,e, NEA3/4, NEA7, NEA8, NEA9, NEA10))**

This tool is based on the location of the 90th percentile of chlorophyll concentration against boundary thresholds set for the High/Good and Good/Moderate boundaries. The tool assumes that 6 years of chlorophyll data are available with monthly sampling during the growing season. The assessment is made against the phytoplankton agreed growing season which varies with latitude. Recommendations from Quasimene on chlorophyll analysis show an accepted 12 % error in sampling and analysis. The inherent sampling error can then account for the small differences in reference values within the same type and same boundary conditions (i.e. Portugal and Spain – Iberian Upwelling coast).

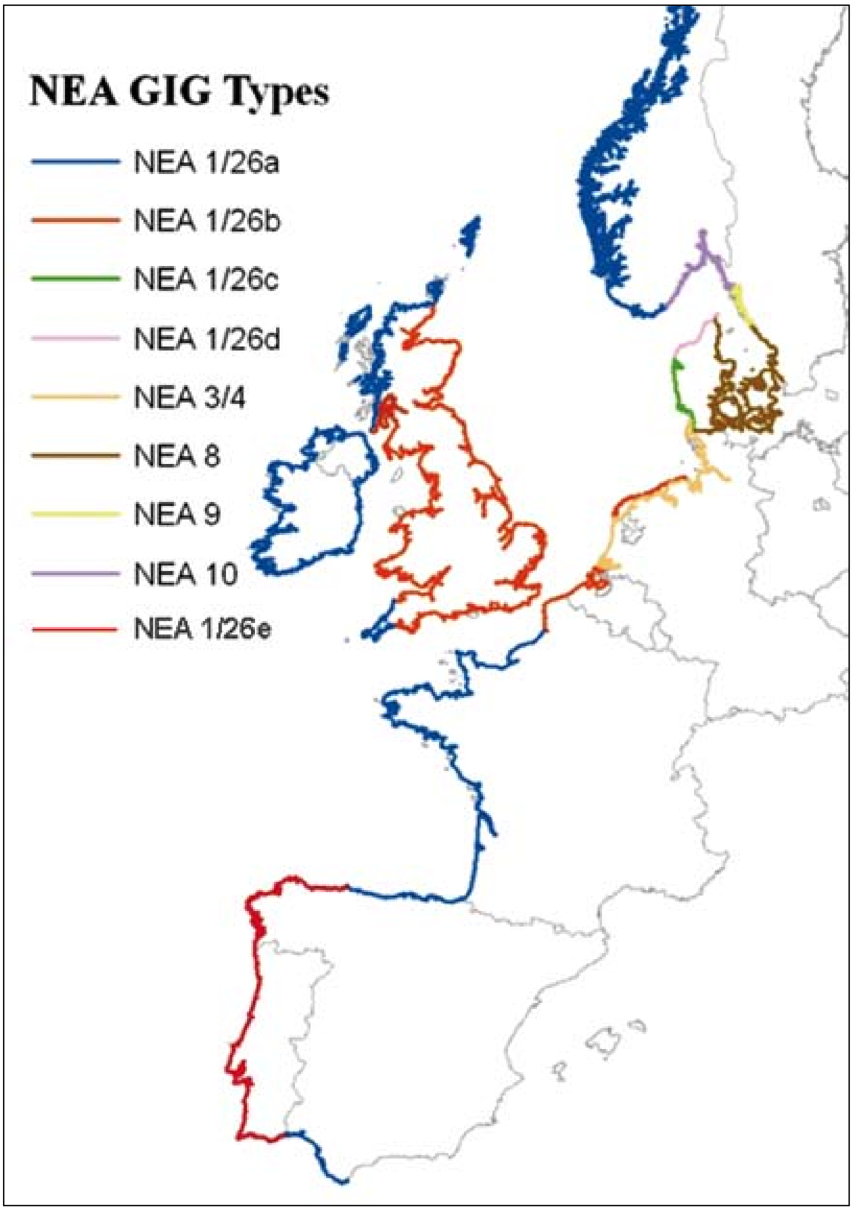


Figure 2.4.2: NEA Coastal Typology Delineation, Phase I Intercalibration.

The same level of deviation from reference values to the High/Good and Good/Moderate boundaries have been agreed within these new (and existing) types and numeric 90th percentile thresholds set for each of the geographic areas described above. There have been three different levels of deviations within types set for chlorophyll, which translates into type specific EQRs for the three different deviations (see Table 8). The three levels of difference between high/good and good/ moderate thresholds are set at 100 % for type 1/26a, 8, 9, 10, 50 % for type 1/26b, 1/26e, 3 and 4 and 33 % for type 1/26c and 1/26d. These typologies reflect the different way the phytoplankton reacts in these waterbodies. In the clear open Atlantic waters, the phytoplankton respond quickly to small changes in nutrients while those areas of more enclosed seas and upwelling's tend to have higher turbidity and nutrients, so may respond more slowly initially but bigger natural blooms can be maintained.

NEA Types 1/26a, 8, 9, 10 are all relatively clear waters with relatively low natural inputs of nutrients (therefore fast initial reaction but may not be sustained). For these types with low reference thresholds, it was decided that in the absence of good empirical data, 100 % deviation was considered to give a reasonable indication of a slight to moderate deviation from the high/good boundary. ROI, France, Spain and Scandinavian countries all have the same percentage of change between boundaries in areas where reference values are lower than 5 µg/L.

NEA Type 1/26b, 1/26e, 3, 4 are a combination of relatively high amounts of natural nutrients (either from run off or upwelling) and / or relatively high turbidity (slower reaction which can sustain bigger and longer blooms). In these areas where the reference values are higher, a 50 % change has been agreed on in keeping with the OSPAR recommendation that 50 % is adequate to describe a slight to moderate deviation from the reference level.

In NEA Types 1/26c and 1/26d, the inflow of deep water, relatively rich in nutrients but initially unavailable to algae enters the Kattegat bottom waters and eventually mixing to the surface water and re-exports to the Skagerrak frontal area.

Table 8 shows both the numeric chlorophyll thresholds and the associated EQRs. Table 9 shows a summary of the type specific EQRs only. Extent of boundary conditions is illustrated in Figure 3.

Table 2.4.8: Final boundary thresholds grouped by NEA GIG geographic zones and types for chlorophyll a metric.

Geographical Zone	Member States	New Type	Reference (calculated from High/Good)	90 %ile High / Good	90 %ile Good / Mod	H/G EQR	G/M EQR
Canary Islands	ES	NEA1/26a	0.67	1	2	0.67	0.33
Western Iberian Coast upwelling	PT, ES	NEA1/26e	5.33	8	12	0.67	0.44
Western Cantabrian Coast	ES	NEA1/26e	4.00	6	9	0.67	0.44
Eastern Cantabrian Coast	ES	NEA1/26a	2.33	3.5	7	0.67	0.33
Atlantic Coast	FR , UK (exposed West Coast & NI.), ROI, ES (Gulf of Cadiz, Sweden	NEA1/26a	3.33	5	10	0.67	0.33
Western Irish Sea	UK (NI), ROI	NEA1/26a	3.33	5	10	0.67	0.33
Eastern Irish Sea	UK (England, Wales, Scotland)	NEA1/26b	6.67	10	15	0.67	0.44
North Sea	NL (Wadden coast), BE, UK (East Coast, Channel), FR	NEA1/26b	6.67	10	15	0.67	0.44
North Sea - , "German Bight", & Jutland	DE, DK	NEA1/26c	3.33	5	7.5	0.67	0.44
Scandinavian Coast	DK	NEA 1/26d	2.00	3	4	0.67	0.50
Scandinavian Coast	DK,NO,SE	NEA8	1.00	1.5	3	0.67	0.33
Scandinavian Coast	NO,SE	NEA9 NEA1/26a	1.67	2.5	5	0.67	0.33
Scandinavian Coast	SE,NO	NEA10	2.00	3	6	0.67	0.33

Table 2.4.9: Summary of EQR ratios of Chlorophyll-a for the NEA GIG types and areas.

Type	Areas	H/G EQR	G/M EQR
NEA1/26a	Eastern Cantabrian Coast, Canary Islands. Atlantic Coast, Western Irish Sea, Scandinavian Coast: North Sea Norway + Norwegian Sea	0.67	0.33
NEA1/26b	Eastern Irish Sea, North Sea (UK East Coast, Channel, NL Wadden Coast, Zeeland Coast, Belgium Coast)	0.67	0.44
NEA1/26c	German Bight and Jutland	0.67	0.44
NEA1/26d	Scandinavian Coast: Denmark Skagerrak	0.67	0.50
NEA1/26e	Western Iberian Coast upwelling, Western Cantabrian Coast	0.67	0.44
NEA8	Denmark, Norway, Sweden: Kattegat + small area of Skagerrak	0.67	0.33
NEA9	Sweden, Norway: Skagerrak (see map)	0.67	0.33
NEA10	Sweden, Norway: Skagerrak (see map)	0.67	0.33

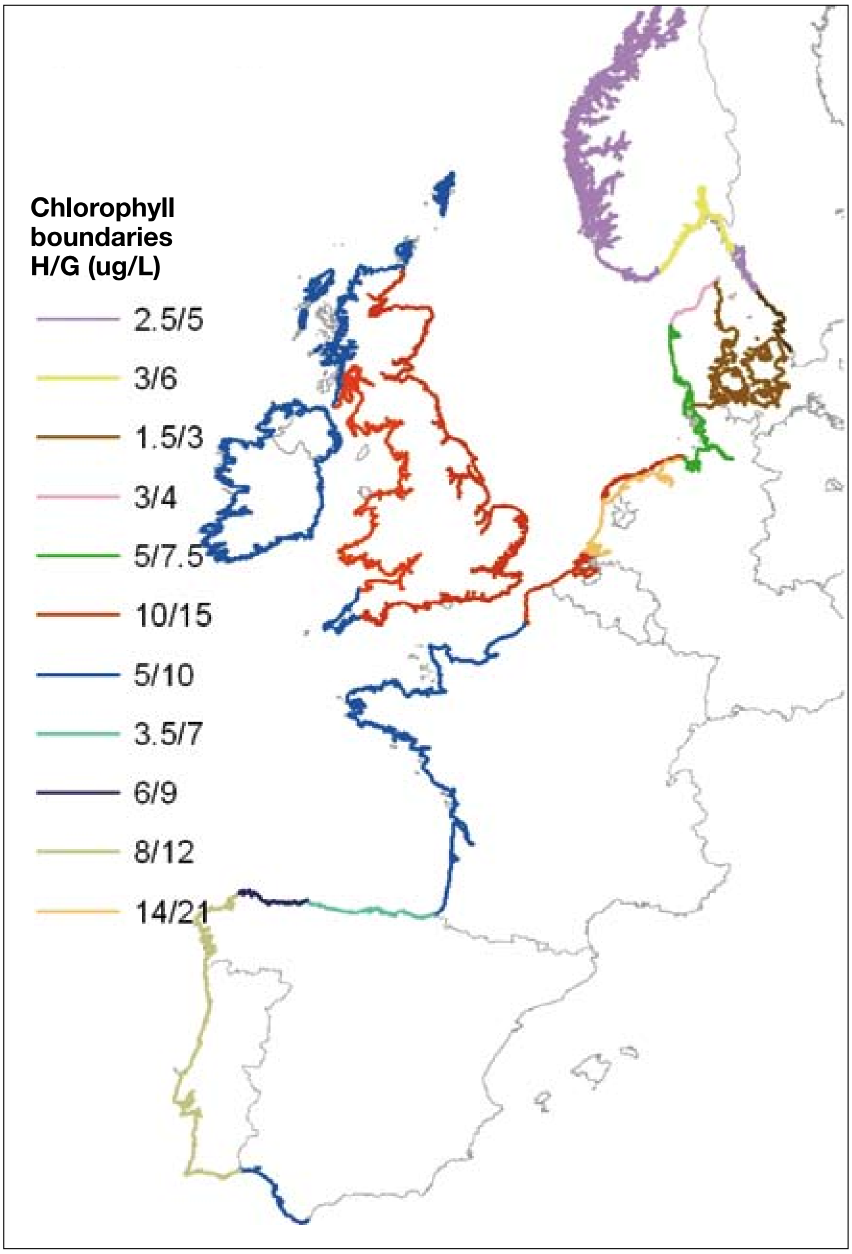


Figure 2.4.3: Chlorophyll boundary conditions for NEA GIG types. (Needs updating in early Phase II).

Metric - Taxa Cell Counts ("frequency of phytoplankton cell counts above a predefined threshold, ranging approximately from 10^5 to 10^6 cells/l") (Boundary Setting Procedure and Results (All Coastal Water Types: NEA 1/26a,b,c,d,e, NEA3/4, NEA7, NEA8, NEA9, NEA10))

Frequent occurrences of phytoplankton taxa above bloom levels over the growing season are an indication of ecosystem disturbance. Natural blooms in the spring and autumn are to be expected but more frequent occurrences throughout the rest of the year are considered indicative of a degraded system.
The sub metric is the frequency of blooms of any routinely identifiable phytoplankton taxa over a defined threshold from monthly sampling is calculated over a 6 year period.

The definition of the index is based on:

- Number of samples where a single taxa count is over a predefined threshold
- Six years of routine monitoring data should be used from the whole year
- A minimum of 12 sampling occasions (monthly) per year is recommended (but not essential).
- CLASSIFICATION is based on the number of sampling times where the single taxa counts exceed a threshold and is calculated as a percentage of the total number of samples collected within one water type for the six year period.

As the chlorophyll thresholds are region-specific, the cell-count threshold has been adapted for regions that present different trophic richness due to their natural (hydromorphological) features. Therefore, different cell-count thresholds will be used within the different types and regions. For example, the upwelling coast of NW Spain will have a higher threshold, as blooms there are of high magnitude due to natural processes of nutrient enrichment. As the upwelling intensity decreases gradually eastward, the Cantabrian Coast would present lower thresholds. The Canary Archipelago, the most oligotrophic area in Spain, would present the lowest threshold. As for the frequency of blooms, it should be noted that the Iberian upwelling zone is subject to a higher frequency of blooms than expected in other coastal waters due to the occurrence of blooms during the summer, which is a season of high upwelling intensity in that area. Therefore, the boundary classification should be adapted for this special region where blooms are not always related to anthropogenic eutrophication.
It was accepted at GIG meeting in Mieres that member states will use different phytoplankton analytical levels. Two groups of routinely identified phytoplankton were determined. These are loosely termed "large" (mainly microphytoplankton, chain formers and other readily identified groups) and "small" (which includes, nanoplankton, microflagellates and the more difficult taxa). There tend to be fewer large taxa then small, so two thresholds of cell count are required. Where a member state identifies and counts phytoplankton from both groups the assessment requires a primary assessment against the "large" threshold, if the sample passes then it is assessed against the small threshold. The reference level is the frequency these local numbers are exceeded. These are presented in the table below:

Table 2.4.10: Summary of final intercalibrated boundary thresholds grouped by NEA GIG geographic zones and types for taxa count metric. N/P = not participating.

Member state	Typology	Area	Reference (% Single Taxa counts exceeding thresholds)	Large Phytoplankton Threshold	Small Phytoplankton Threshold
			Reference = number of times a single taxa count exceeds the threshold. If we expect a spring and autumn bloom and only sample monthly then 2/12 = 16.7 %. High <20 % *Upwelling Area Reference = 3 blooms per year spring, summer and autumn expected, if sample monthly then 3/12 = 25 %, High <30 % in Upwelling regions, †This symbol indicates the assessment is made on all phytoplankton regardless of size		
Spain	NEA1/26e	*Western Iberian Coast	25	N/P	1,000,000†
Spain	NEA1/26e	*W. Cantabrian Coast	25	N/P	750,000†
Spain	NEA1/26a	E. Cantabrian Coast	16.70	N/P	750,000†
Spain	NEA1/26a	Canary Islands	16.70	N/P	500,000†
Spain	NEA1/26a	Atlantic Coast	16.70	N/P	500,000†
Portugal	NEA1/26e	*Western Iberian Coast	25	100,000	1000,000
France	NEA1/26a		16.70	100,000	250,000
France	NEA1/26b		16.70	100,000	250,000
Belgium	NEA1/26b		N/P	N/P	N/P
Netherlands	NEA1/26b		N/P	N/P	N/P
Netherlands	NEA3		N/P	N/P	N/P
Netherlands	NEA4		N/P	N/P	N/P
Germany	NEA1/26c		N/P	N/P	N/P
Germany	NEA3		N/P	N/P	N/P
Germany	NEA4		N/P	N/P	N/P
Denmark	NEA1/26c		N/P	N/P	N/P
Denmark	NEA1/26d		N/P	N/P	N/P
Denmark	NEA8		N/P	N/P	N/P
Sweden	NEA8		N/P	N/P	N/P
Sweden	NEA9		N/P	N/P	N/P
Sweden	NEA10		N/P	N/P	N/P
Norway	NEA1/26a		N/P	N/P	N/P
Norway	NEA8		N/P	N/P	N/P
Norway	NEA9		N/P	N/P	N/P
Norway	NEA10		N/P	N/P	N/P
RoI	NEA1/26a		16.70	250,000	N/P
UK	NEA1/26a		16.70	250,000	N/P
UK	NEA1/26b		16.70	250,000	N/P

Not all member states are participating in this metric. Participating countries include Portugal, Spain, France, Republic of Ireland, and UK. The associated typologies are: NEA 1/26a, 1/26b, 1/26e, 3, and 4.

All single taxa counts above the threshold are calculated as a percentage of the overall sampling count (over the six-year period). Percentages are calculated and scored as per Table 11(Borja et al, 2004). The value of the percentage decides the boundary classification.

Table 2.4.11: Agreed Percentage Boundaries NEA1/26a, b, NEA3/4.

Phytoplankton Boundary Percentages	Boundary classification
<20 % single taxa counts exceeding threshold	HIGH
20 - 39 % single taxa counts exceeding threshold	GOOD
40-69 % single taxa counts exceeding threshold	MODERATE
70-90 % single taxa counts exceeding threshold	POOR
>90 % single taxa counts exceeding threshold	BAD

These boundaries equate to H/G and G/M EQRs of 0.84 and 0.43 calculated by dividing e.g. reference %, 16.7 by boundary % e.g. 20 = 0.84 at the High/Good boundary etc.

Table 2.4.12: Agreed Percentage Boundaries NEA1/26e (Spain & Portugal) for coastal waters affected by upwelling.

Phytoplankton Boundary Percentages	Boundary classification
< 30 % single taxa counts exceeding the threshold	HIGH
30 - 49 % single taxa counts exceeding the threshold	GOOD
50 - 69 % single taxa counts exceeding the threshold	MODERATE
70 - 90 % single taxa counts exceeding the threshold	POOR
>90 % single taxa counts exceeding the threshold	BAD

These boundaries equate to H/G and G/M EQRs of 0.83 and 0.51 calculated by dividing e.g. reference %, 25 by boundary % e.g. 30 = 0.83 at the High/Good boundary etc.

Metric - Indicator Taxa (Frequency of *Phaeocystis* Cell counts above 10^6 Cells/l) (Boundary Setting Procedure and Results (All Coastal Water Types: NEA 1/26a,b,c,d,e, NEA3/4, NEA7, NEA8, NEA9, NEA10))

Classification is based upon percentage of months with one or more occasions where the counts of *Phaeocystis* exceed the predetermined threshold. The definition of the metric is:

- Number of months with samples where the *Phaeocystis* cell count is over a predefined threshold
- Six years of routine monitoring data should be used from the whole year
- A minimum of 12 sampling occasions (monthly) per year is recommended (but not essential).
- CLASSIFICATION is based on the number of months with one or more sampling times where the *Phaeocystis* counts exceed a threshold and is calculated as a percentage of the total number of months collected within one water type for the six year period.

Table 2.4.13: Agreed Thresholds and References for Phaeocystis threshold.

Member state	**Typology**	**Reference (% *Phaeocystis* counts exceeding thresholds).** N/P = not participating. **Reference** = number of times a single taxa count exceeds the threshold. If we expect only a spring bloom and only sample monthly then 1/12 = 8.33 %. High /Good=10 %,G/M=17 %	**Phaeocystis Threshold Cells/l**
Spain	NEA1/26a	N/P	N/P
Spain	NEA1/26e	N/P	N/P
Portugal	NEA1/26e	N/P	N/P
France	NEA1/26a	N/P	N/P
France	NEA1/26b	N/P	N/P
Belgium	NEA1/26b	8.30	1000000
Netherlands	NEA1/26b	8.30	1000000
Netherlands	NEA3	8.30	1000000
Netherlands	NEA4	8.30	1000000
Germany	NEA1/26c	8.30	1000000
Germany	NEA3	8.30	1000000
Germany	NEA4	8.30	1000000
Denmark	NEA1/26c	N/P	N/P
Denmark	NEA1/26d	N/P	N/P
Denmark	NEA8	N/P	N/P
Sweden	NEA8	N/P	N/P
Sweden	NEA9	N/P	N/P
Sweden	NEA10	N/P	N/P
Norway	NEA1/26a	N/P	N/P
Norway	NEA8	N/P	N/P
Norway	NEA9	N/P	N/P
Norway	NEA10	N/P	N/P
RoI	NEA1/26a	N/P	N/P
UK	NEA1/26a	8.30	1000000
UK	NEA1/26b	8.30	1000000

Only the Netherlands, Germany, UK and Belgium, are participating in this tool. Classification is taken from the final percentage count of exceedances over the total sampling number. The present threshold percentages related to boundary classifications are presented in Table 14. There is still some discussion on the boundaries for moderate, poor and bad and further work is required on these boundaries. As all participating countries have agreed the same method in terms of reference conditions, boundary thresholds and EQRs within types then this intercalibration fulfils the criteria set out under Option 1 in the intercalibration process.

Table 2.4.14: Phaeocystis boundary thresholds.

Phaeocystis **Percentage exceedance boundaries**	**Boundary classification**
<10 % *Phaeocystis* exceeding threshold	HIGH
10 – 17 % *Phaeocystis* exceeding threshold	GOOD
>17 % *Phaeocystis* exceeding threshold	MODERATE
>35 % *Phaeocystis* exceeding threshold	Poor
>80 % *Phaeocystis* exceeding threshold	Bad

These boundaries equate to H/G and G/M EQRs of 0.92 and 0.49 calculated by dividing e.g. reference %, 8.3 by boundary % e.g. 10 = 0.83 at the High/Good boundary etc.

2.4.6 Open issues and need for further work

- Transitional Waters – all metrics need agreement. Need to normalize data to salinity values. At present there is only one ECO TW type, this will be a problem that we need to resolve. Flow regime also affects diversity; this is another issue that would require normalization for all countries in NEA GIG.
- Sampling frequency – how can this affect the boundary thresholds.
- Phytoplankton composition/community – requirement for further investigation.
- Toxic species?
- Cell carbon and biovolume methods for intercalibration
- UK and ROI are possibly looking to modify chl-a 90 %ile tool, cell counts and functional groups metrics to be applicable in transitional waters. NL and other countries are looking at chl-a in transitional waters as well. Turbidity/light will be another normalizing issue.
- Risk of misclassification – share ideas.
- Primary production – develop new, cheaper and more reliable methods.
- Standardize size classes that phytoplankton taxa belong to. Start by country and construct a standard size class list at MS level.
- A literature search needs to be carried out to find minimum and maximum size ranges for phytoplankton.
- Indicator species for eutrophication – OSPAR list exists but the relation between eutrophication is not for all the species on the list. It is suggested we refine and develop a better list.
- Diversity and Composition metric needs to be linked to nutrient enrichment and other pressures.
- Rules for combining elements for classification need to be agreed (Possible output from CIS June Workshop in Paris).
- Methods of chl a measurement need to be agreed/ intercalibrated

3 Discussion

3.1 Comparability between GIGs

Table 3.1.1: Summary of intercalibration topics per GIGs.

	NEA GIG	MED GIG	BAL GIG	BLA GIG
METRICS	Chl α, Indicator Taxa, Taxa Cell Count	Chl α	Chl α	Biomass
N°. TYPES	8	3	8	1
OPTION	2	2/3	2/3	2
BOUNDARY SETTING PROCEDURE	Experts, 90iles, H/G, G/M	Percentiles, H/G, G/M	Percentiles, H/G, G/M	Quantiles, All boundaries
REFERENCE CONDITION	MS-specific, 90ile	High status sites	Different approaches, empirical relationships between chl α and Secchi, mixed models, …	Historical data 10th percentile Expert judgement
OPEN ISSUES	Improve dose-response relationships	Missing metrics, species composition	…	Missing metrics

Metrics

Intercalibration for the phytoplankton quality element has been successfully performed by all of the four Coastal Waters GIGs, using Chlorophyll-α as metric. Other metrics have been considered and tested by some GIGs (Indicator Taxa, Biomass).

Types

Not all types for each GIG have been covered, mainly because there were not shared by all MSs.

Option

Option 2 (MS agreed on the common metrics, created data sets relating MSs assessment methods to the common metrics, agreed on High/Good and Good/Moderate class boundaries and established relationships between common and national metrics) and a hybrid Option 2 and 3 (preliminary national boundaries were compared and harmonized with those obtained by using a common dataset for setting the final boundaries) approaches have been adopted for the intercalibration.

Boundary Setting Procedure

All Coastal Waters GIGs are providing results for the H/G and G/M boundaries (at least), applying similar approaches based on the expert supervised selection of appropriate percentiles as boundaries best fitting with evident changes in ecological status (90^{th} percentile is the favorite choice).

Reference Condition

There's no clear common view among GIGs (but also among MSs) about how to fix Reference Conditions: in some cases the expert judgment has been the selected option, in some others a mixture of modeling, statistics on historical data and empirical relationships with other metrics (e.g. Secchi depth, see BALTIC GIG) has been applied.

3.2 Open issues and need for further work

The main task for the next intercalibration round is almost unanimously to include other metrics (blooms, species composition, toxic species, indicator species) in order to complete the analysis. It would be useful to focus on (strengthen) dose-response relationships in order to correlate pressures and ecological status.

4 References

Agència Catalana de l'Aigua (ACA), 2005. Caracterisation of surface water bodies and analysis of the risk of failing the objectives of the water framework directive (2000/60/CE) in the catalonian basins of the Ebro, the Garona and the Senia, and revision of the internal basins of Catalonia. IMPRESS document. Departament de Medi Ambient i Habitatge. (http://mediambient.gencat.net/aca/ca//planificacio/directiva/impress.jsp).

Álvarez–Salgado X. A., Rosón G., Pérez F. F. and Y. Pazos, 1993. Hydrographic variability off the Rías Baixas (NW Spain) during the upwelling season. Journal of Geophysical Research 98(C8): 14447-14455.

Álvarez–Salgado X. A., Rosón G., Pérez F. F., Figueiras F. G. and Y. Pazos, 1996. Nitrogen cycling in an estuarine upwelling system, the Ría de Arousa (NW Spain). I. Short–time–scale patterns of hydrodynamic and biogeochemical circulation. Marine Ecology Progress Series 135: 259-273.

Argyrou, M. 2005. Report of the National Monitoring Programme of Cyprus for the Year 2004. Programme for the Assessment and Control of Pollution in the Mediterranean Region (MEDPOL /UNEP). Department of Fisheries and Marine Research, pp. 32.

Argyrou, M. 2006. Report of the National Monitoring Programme of Cyprus for the Year 2005. Programme for the Assessment and Control of Pollution in the Mediterranean Region (MEDPOL /UNEP). Department of Fisheries and Marine Research, pp. 41.

Aristegui J. 1990. Distribution of chlorophyll "a" in the Canary Island waters. Boletin del Instituto Español de Oceanografia 6(2): 61-72.

Arístegui J., Hernández–León S., Montero M.F. and M. Gómez, 2001. The seasonal planktonic cycle in coastal waters of the Canary Islands. Sci. Mar., 65 (Suppl. 1): 51-58.

Artigas L. F. 1998. Seasonal variability in microplanktonic biomasses in the Gironde dilution plume (Bay of Biscay): Relative importance of bacteria. Oceanologica Acta 21: 563-580.

Bald J. 2005. Proposal for the physico-chemical quality status assessment within the estuarine and coastal waters of the Basque Country (Northern Spain). PhD Thesis. University of Navarra (Spain) 262 p.

Bald J. A., Borja I., Muxika J., Franco and V. Valencia 2005. Assessing reference conditions and physico-chemical status according to the European Water Framework Directive: a case-study from the Basque Country (Northern Spain). Marine Pollution Bulletin, 50: 1508-1522.

Barton E. D., Aristegui J., Tett P, Canton M., Garcia-Braun J., Hernandez-Leon S., Nykjaer L., Almeida C., Almunia J., Ballesteros S., Basterretxea G., Escanez J., Garcia-Weill L., Hernandez-Guerra A., et al. 1998. The transition zone of the Canary Current upwelling region. Progress in Oceanography 41: 455-504.

Berman, T., D.W. Townsend, S.Z. El-Sayed, G.C. Trees and Y. Azov. 1984. Optical transparency, chlorophyll and primary productivity in the Eastern Mediterranean near the Israeli Coast. Oceanol. Acta, 7: 367-372.

Bianchi, T.S., A. Demetropoulos, M. Hadjichristophorou, M. Argyrou, M. Baskaran and C. Lambert. 1996. Plant Pigments as Biomarkers of Organic Matter Sources in Sediments and Coastal Waters of Cyprus (eastern Mediterranean). *Estuarine, Coastal and Shelf Science*, 42: 103-115.

Bode A. and M. Varela, 1998. Primary production and phytoplankton in three Rias Altas (NW Spain): seasonal and spatial variability. Scientia Marina 62: 319-330.

Bode A., Casas B,. Fernández E., Marañon E., Serret P. and M. Varela, 1996. Phytoplankton biomass and production in shelf waters off NW Spain: spatial and seasonal variability in relation to upwelling. Hydrobiologia 341: 225-234.

Borja A., Franco J., Valencia V., Bald J., Muxika I., Belzunce M. J., and O. Solaun. 2004. Implementation of the European water framework directive from the Basque country (northern Spain): a methodological approach. Marine Pollution Bulletin 48: 209-218.

Botas J. A., Bode A., Fernández E. y R. Anadón. 1988. Descripción de una intrusión de agua de elevada salinidades el Cantábrico Central: distribución de los nutrientes inorgánicos y su relación con el fitoplancton. Investigación Pesquera 52: 561-573.

Botas J. A., Fernández E. and R. Anadón. 1990. A persistent upwelling off the central Cantabrian coast (Bay of Biscay). Estuarine, Coastal & Shelf Science 30: 185-189.

Boynton W.R., Kemp W. M. and C.W. Keefe, 1982. A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production. In: Estuarine Comparisons. V.S. Kennedy (ed). Academic Press, New York, 69-90.

Cadée, G.C. and J. Hegeman. 1991. Historical phytoplankton data of the Marsdiep. Hydrobiological Bulletin 24: 111-188.

Cermeño P., Marañón E., Pérez V., Serret P., Fernández E., and C. G. Castro, 2006. Phytoplankton size structure and primary production in a highly dynamic coastal ecosystem (Ría de Vigo NW-Spain): seasonal and short-time scale variability. Estuarine Coastal and Shelf Science 67: 251-266.

Diez I., Secilla A., Santolaria A., and Gorostiaga J. M. 2000. The north coast of Spain. In: Seas at the Millennium. An environmental evaluation. C. Sheppard (ed.). Pergamon, Amsterdam, Volume I, 135-150.

European Environmental Agency. 2005. Source apportionment of nitrogen and phosphorus inputs into the aquatic environment. EEA Report No 7/2005.

Figueiras F. G,. Niel F. X. and C. Mouriño, 1986. Nutrientes y oxígeno en la Ría de Pontevedra (NO de España). Investigación Pesquera 50: 97-115.

Fraga F. 1976. Fotosíntesis en la Ría de Vigo. Investigación Pesquera 40 (1):151-167.

Fraga F. 1981. Upwelling of the Galician coast. Northwest Spain. In: Coastal Upwelling. F. A. Richards (ed). American Geophysical Union, 176-182.

Garcia C. M., Prieto L., Vargas M., Echevarria F., García-Lafuente J., Ruiz J., and J.P. Rubín. 2002. Hydrodynamics and the spatial distribution of plankton and TEP in the Gulf of Cadiz (SW Iberian Peninsula). Journal of Plankton Research 24: 817-833.

Gil J. 2003. Changes in the pattern of water masses resulting from a poleward slope current in the Cantabrian Sea (Bay of Biscay). Estuarine, Coastal & Shelf Science 57: 1139-1149.

Harding Jr., L. W. & E. S. Perry.1997. Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950-1994. Mar. Ecol. Prog. Ser., 157: 39-52.

Harris G. P. 1986. Phytoplankton Ecology: structure function and fluctuation. Chapman and Hall, London, 384 pp.

Ignatiades, l., Vounatsou, P., Karydis, M. (1992). A possible method for evaluating oligotrophy and eutrophication based on nutrient concentration scales. *Mar. Poll. Bull*., 24: 238-243.

Karydis, M. (1999). *Evaluation report on the eutrophication level in coastal greek areas*. Univ. of Aegean, Mytilini, February 1999 (in greek).

Krom, M.D., Kress, N., Brenner, S. Gordon, L.I. (1991). Phosphorus limitation of primary production in the eastern Mediterranean Sea. *Limnol. Oceanogr*., 36: 424-432.

Krom, M.D., S. Brenner, N. Kress, A. Neori and L.I. Gordon. 1992. Nutrient dynamics and new production in a warm-core eddy from the Eastern Mediterranean Sea. Deep-Sea Research, 39: 467-480.

Krom,D. M., N. Kress & S. Brenner. 1991. Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. Limnol. Oceanogr., 36(3): 424-432.

Lavin A., Valdes L., Sanchez F., Abaunza P., Forest A., Boucher J., Lazure P. and A. M. Jegou. 2006. The Bay of Biscay: The encountering of the ocean and the shelf (18 b, E). In: The Sea, Volume 14. A.R. Robinson & K.H. Brink (ed.). Harvard University Press, 933-1001.

Lipej L., P. Mozetič, M. Orlando Bonaca, B. Mavrič, M. Šiško & N. Bettoso. 2006. Evaluation of the Ecological Status of Coastal Waters in accordance with the European Water Framework Directive (Water Framework Directive, 2000/60/EC). Final national report. Marine Biology Station Piran, National Institute of Biology, October 2006, 180 pp. (in Slov.)

Llope M. 2005. Variabilidad hidrográfica en un área costera del Mar Cantábrico. Modelado de plancton y efectos de cambio climático. Tesis Doctoral. Universidad de Oviedo. Ph D. dissertation.

Lunven M., Guillaud J.F., Youénou A., Crassous M. P., Berric R., Le Gall E., Kérouel R., Labry C., and A. Aminot. 2005. Nutrient and phytoplankton distribution in the Loire River plume (Bay of Biscay, France) resolved by a new Fine Scale Sampler. Estuarine, Coastal & Shelf Science 65: 94-108.

Margalef R. 1974. Ecología. Ediciones Omega, Barcelona, 951 pp.

Margalef R., Durán M. and F. Sáiz. 1955. El fitoplancton en la Ría de Vigo de Enero de 1953 a Marzo de 1954. Investigación Pesquera 2: 85-129.

Mason E., Coombs S., and P. B. Oliveira. 2005. An overview of the literature concerning the oceanography of the Eastern North Atlantic region. Relat. Cient. Téc. IPIMAR, Série digital (http://ipimar-iniap.ipimar.pt) nº 33, 58 pp.

Moncoiffe G., Álvarez–Salgado X. A., Figueiras F. G. and G. Savidge. 2000. Seasonal and short–time–scale dynamics of microplankton community production and respiration in an inshore upwelling system. Marine Ecology Progress Series 196: 111-126.

Mozetič, P., J. Francé, M. Šiško & O. Bajt. 2005. Spatial and temporal patterns of phytoplankton assemblages in a shallow coastal sea (Gulf of Trieste). In: Wassmann, P. & B. Ćosović (eds.): Eutrophication in the coastal zone of the eastern Adriatic Sea. South-eastern Europe Programme Symposium. April 27-May 1, 2005, Hvar, Croatia.

Nogueira E., Pérez F. F. and A. F. Ríos. 1997. Seasonal patterns and long–term trends in an estuarine upwelling ecosystem (Ría de Vigo NW Spain). Estuarine Coastal and Shelf Science 44: 285-300.

OJEC (Official Journal of the European Communities). 2000. Directive 2000/60/EC of the European Parliament and of the Council. L327/1 - L327/72.

Ojeda A. 2004. Biomasa fitoplanctónica y clorofila–a en las Islas Canarias occidentales (Mayo 1986). Universidad de Las Palmas de Gran Canaria. Biblioteca digital.

Orive E., Franco J., Madariaga I., and M. Revilla. 2004. Bacterioplankton and phytoplankton communities. In: Oceanography and Marine Environment of the Basque Country. A. Borja and M. Collins (ed.). Elsevier Oceanography Series nº 70, Elsevier, Amsterdam, 367-393.

OSPAR Commission. 2000. Quality Status Report 2000. Region IV- Bay of Biscay and Iberian coast. OSPAR Commission, London. 134 + xiii pp.

OSPAR Commission. 2003. OSPAR Integrated Report 2003 on the Eutrophication Status of the OSPAR Maritime Area Based Upon the First Application of the Comprehensive Procedure.

Pagou, K. (2000). *Assessment of the trophic conditions in the Inner Thermaikos Gulf.* Technical Report for the Ministry of Environment, Planning and Public Works, NCMR, Athens, December 2000, 11pp.

Prego R. 1994. Nitrogen interchanges generated by biogeochemical processes in a Galician Ría. Marine Chemistry 45: 167-176.

Prego R. and F. Fraga, 1992. A simple model to calculate the residual flows in a Spanish Ria. Hydrographic consequences in the Ría of Vigo. Estuarine Coastal and Shelf Science 34: 603-615.

Prego R., Barciela M. C. and M. Varela, 1999. Nutrient dynamics in the Galician coastal area (Northwestern Iberian Peninsula): do the Rias Bajas receive more nutrient salts than the Rias Altas? Continental Self Researc 19: 317-334.

Rousseau, V., Mathot, S. and C. Lancelot. 1990. Calculating carbon biomass of Phaeocystis sp. from microscopic observations. Marine Biology 107: 305-314.

Siokou-Frangou, I. & Pagou, K. (2000). *Assessment of the trophic conditions and ecological status in the Inner Saronikos Gulf.* Technical Report for the Ministry of Environment, Planning and Public Works, NCMR, Athens, March 2000,43pp. (in greek and english edition).

Smayda T. J., 1980. Phytoplankton species succession. In: The physiological ecology of phytoplankton. I. Morris (ed.). Blackwell Science Publications, Oxford, 489 pp.

Uthermöhl H., 1958. Zur vervollkommnung der quantitative phytoplankton methodik. Mitteilungen Internationale Vereinigung fuer theoretische und angewandtelimnologie. 91-38.

Valencia V., Franco J., Borja A., and A. Fontán. 2004. Hydrography of the Southeastern Bay of Biscay. In: Oceanography and Marine Environment of the Basque Country. A. Borja and M. Collins (ed.). Elsevier Oceanography Series nº 70, Elsevier, Amsterdam, 159-194.

Varela M., Prego R., Belzunce M. J. and F. M. Salas. 2001. Inshore–offshore differences in seasonal variations of phytoplankton assemblages: the case of a Galician Ría Alta (Ría de A Coruña) and its adjacent shelf (NW of Spain). Continental Shelf Research 21: 1815-1838.

Vergara et al. 2005. (Eds. Vergara Martín, J.M., Haroun Tabraue, R. y González Henríquez, N.) Evaluación de Impacto Ambiental de Acuicultura en Jaulas en Canarias. Oceanográfica, Telde. ISBN: 84-609-4073-X. Depósito Legal: GC 422-2005.

Vives F. and F. Fraga. 1961. Producción básica en la Ría de Vigo. Investigación Pesquera 19: 129-137.

Vollenweider,R. A., A. Rinaldi & G. Montanari. 1992. Eutrophication, structure and dynamics of a marine coastal system: results of ten-year monitoring along the Emilia-Romagna coast (Northwest Adriatic Sea). Sci. Tot. Environ. [Vollenweider, R. A., R. Marchetti & R. Viviani (eds.)],. Suppl.: 63-106.

WFD CIS Guidance Document No. 14: Guidance on the intercalibration process 2004-2006

Wiltshire, K. H. & C. D. Durselen. 2004. Revision and quality analyses of the Helgoland Reede long-term phytoplankton data archive. Helogol. Mar. Res., 58(4): 252-268.

5 Annexes

Annexes can be downloaded from the following address:

http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library?l=/intercalibration_2&vm=detailed&sb=Title

Section 3 – Phytoplankton – Overview of Annexes

A – Baltic GIG

Annex 3.1: Development of chlorophyll a reference conditions in Danish coastal waters

Annex 3.2: Boundary setting for chlorophyll a in Danish coastal waters

Annex 3.3: Chlorophyll a reference values and boundary setting in Estonian coastal waters

Annex 3.4: Chlorophyll a reference values and boundary setting in Finnish coastal waters

Annex 3.5: Chlorophyll a reference values and boundary setting in Polish coastal waters

Annex 3.6: Chlorophyll a reference values and boundary setting in Swedish coastal waters

Annex 3.7: Chlorophyll a reference values and boundary setting in German coastal waters

B – Mediterranean GIG

Annex 3.8 – National methods included in the intercalibration

Section 4 – Macroalgae

1 Introduction

Two of the four coastal water GIGs have been able to produce results for the macroalgae quality element (Mediterranean and North-East Atlantic). Each GIG has several typologies that are found in these waters. Not all countries within each GIG have all the types within its borders. Information about the types and countries with each type in each GIG is described in the sections below.

Both GIGs have chosen Option 2 in this phase of the intercalibration process. Member States have developed metrics as part of their own classification schemes. Selected metrics have been chosen by each GIG for assessment and agreement of boundaries in this phase of the intercalibration process. At this stage not all the metrics that make up Member States' schemes can be intercalibrated, so it is not possible to produce EQRs for the whole quality element. Boundaries have been agreed for the selected metrics.

2 Methodology and results

2.1 Mediterranean GIG

2.1.1 Intercalibration Approach

Two classification methods were developed by two countries: EEI by Greece and CARLIT by Spain. Intercalibration Option2 (ECOSTAT WG Guidance) was adopted. The InterCalibration Common Metric (ICCM) used was the method "BENTHOS" developed by Spain.

Six different Mediterranean countries participated in the subgroup of macroalgae:

- Cyprus
- France
- Greece
- Italy
- Slovenia
- Spain

Macroalgae intercalibration has been carried out between Spain, Greece, Slovenia. Other countries that could not provide data for the IC exercise or do not have data yet (Italy, France and Malta) or provided data but shortly before the finalization of the report (Cyprus), approved the results.

Typology

During the early stages of the CIS the Mediterranean working group agreed in using only 2 parameters to distinguish water types, namely substrate composition and depth. Most of other geomorphological parameters, described in Directive Annex II (1.2.4), were not relevant (i.e. tidal regime) to distinguish different water types in relation to their ecological "significance" for the

Mediterranean Sea. Four main Types were then defined (Table 2.1.1). However, throughout the CIS, following data analyses for the different BQEs, these types did not actually proved to be relevant for the IC exercise, for some BQEs, as Mediterranean ecosystem is quite homogeneous in comparison to Northern Seas (some ecological differences do exist but within the Mediterranean scale).
For Macroalgae Intercalibration the methods used are applied to macroalgal communities (species composition and abundance) of the upper infralittoral zone (3.5 to 0.2 m depth) in rocky coasts, with no types distinction.

Table 2.1.1: Main water body types of coastal waters within the Mediterranean Sea.

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

1) Since in many cases different seabed substrata will occur within one water body type, the dominant substratum should be taken under consideration.
2) Depth division is based on 40 m. depth, at 1 mile distance from the coastline.

2.1.2 National methods that were intercalibrated

The methods intercalibrated were: EEI and CARLIT. EEI was elaborated by Greece, while CARLIT by Spain. Another method, BENTHOS, that was adopted as Intercalibration Common Metric (ICCM), has also been developed by Spain in parallel with CARLIT. All these methods share a common view for reference conditions.
The other Mediterranean countries that participated in the Macroalgae subgroup have decided which methods they already applied or are on the way to apply on their country data on macroalgae (Table 2.1.2).

Table 2.1.2: Countries decisions for macroalgal methodologies.

QE 3: Macroalgae	Assessment Method	Status
Cyprus	EEI	Officially accepted
France	CARLIT	Officially accepted
Greece	EEI	Officially accepted
Italy	CARLIT	Under consideration
Slovenia	EEI	Officially accepted
Spain	CARLIT, BENTHOS	Officially accepted

Metric: Ecological Evaluation Index (EEI)

Ecological Evaluation Index (EEI) was designed to estimate the ecological status classes (ESC) of transitional and coastal waters using benthic macrophyte communities as bioindicator[26] (Orfanidis et al., 2001, 2003; Panayotidis et al., 2004; Orfanidis, 2007). It is based on the well-known pattern that anthropogenic stress, e.g. eutrophication, heavy metal pollution, shifts the ecosystem from pristine state, where late-successional species is dominant, to degraded state, where opportunistic species through rapid growth and recruitment is dominant. This pattern can be explained from the species competition abilities under abundant and limiting nutrient conditions and is in accordance to r- and K-selection theory.

In order to evaluate the spatial scale-dependent ESC of the studied coast (EEI_{EGR}), the area-weighted value was calculated. For this purpose, the score of each site was multiplied by the percentage of the coast area for which is considered to be representative and the products were summed. EEI values higher than 0.5 indicate sustainable ecosystems of good or high ESC, whereas EEI values lower than 0.5 indicate that the ecosystems should be restored to a higher ESC (Table 2.1.3). These values should be regarded as provisional that have to be further verified in the future.

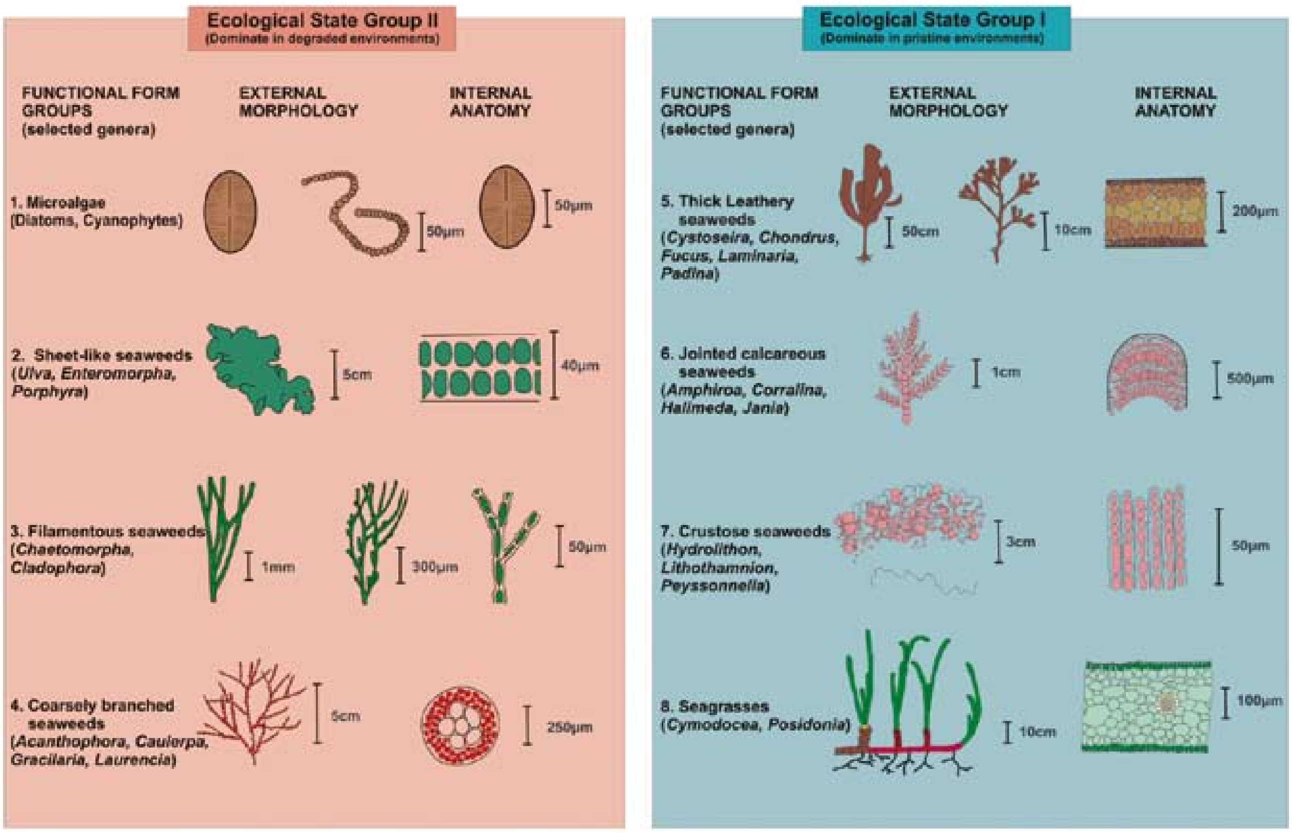


Figure 2.1.1: Ecological State Groups (ESG) I, II of marine benthic macrophytes (macrophyte graphs are based on diverse sources).

26 A readily measured component or metric of the biota that are used to provide long-term ecologically relevant information about the state and trends of ecosystem. Such an approach effectively distinguishes responses of human impact from natural variability, when supported by predictive modelling and sound ecological theory.

Table 2.1.3: Classification scheme of transitional and coastal waters based on the Ecological Evaluation Index (EEI).

Classification of antropogenic stress	Ecological Status Classes	EEI range	$EEI_{EQR\ range}$	Management target
Normal/Pristine	High	10 ≥ EEI > 8	1 ≥ EQR > 0.75	Sustainable
Slightly stresses, transitional	Good	8 ≥ EEI > 6	0.75 ≥ EQR > 0.5	Sustainable
Moderately stressed	Moderate	6 ≥ EEI > 4	0.5 ≥ EQR > 0.25	Restoration
Heavily stressed	Low	4 ≥ EEI > 2	0.25 ≥ EQR > 0	Restoration
Before azoic	Bad	2	0	Restoration

Metrics: BENTHOS, CARLIT

Both methodologies have been published recently and described in detail. The references of the papers where methodologies have been described are the following:

BENTHOS: Pinedo, S. García, M., Satta, M.P., de Torres, M. and Ballesteros, E., 2007. Rocky-shore communities as indicators of water quality: A case study in the Northwestern Mediterranean. Marine Pollution Bulletin. 55, 126-135.

CARLIT: Ballesteros, E., Torras, X., Pinedo, S., García, M., Mangialajo and L., de Torres, M., 2007. A new methodology based on littoral community cartography dominated by macroalgae for the implementation of the European Water Framework Directive. Marine Pollution Bulletin. 55, 172-180.

2.1.3 Reference conditions and class boundary setting

Reference condition is the expression of high quality structure and function of aquatic ecosystems, that should have not suffered any impact on their natural state because of human activities and there is none or only very minor evidence of disturbance on each of the general physico-chemical, hydromorphological and biological quality elements. Reference sites have been identified according to the low pressures and impacts they receive in accordance with Annex V of WFD. In all methods (EEI, BENTHOS, CARLIT) the reference sites are real sites (existing) and this allows the application of the tested methodologies in these places.

Ecological Evaluation Index (EEI)

The reference conditions have to be presented by values of relevant biological quality elements indicating high ecological status. For the description of macroalgal community of the rocky upper infralittoral zone reference conditions in Greek coastal waters 62 samples from 26 putatively pristine Aegean sites (Figure 2.1.2) dominated by *Cystoseira* cf. *crinita* community as part of the Hellenic “NATURA 2000” data-base (see Panayotidis *et al*., 2001) in combination with the biotic index Ecological Evaluation-EEI Index (Orfanidis et al., 2001; 2003) were used. The aim was (1) to develop an objective and statistically valid “virtual” list of the most common algal species in the Aegean under undisturbed conditions, and (2) to test the conceptual model and the EEI recently developed by Orfanidis et al. (2001, 2003) for the implementation of Water Framework Directive (2000/60/EC) in Greek coasts.

In total 113 taxa (73 Rhodophyceae, 25 Phaeophyceae, 15 Chlorophyceae) were identified in *Cystoseira* cf. *crinita* community of the Aegean Sea (Panayotidis et al., 2007). Nine (9) major taxa (except *C.* cf. *crinita*) contributed cumulatively by 90 % in the community: *Haliptilon virgatum*, *Cystoseira compressa*, *Jania rubens*, *Padina pavonica*, *Herposiphonia secunda*, *Corallina elongata*, *Cladophora* spp., *Sphacelaria cirrosa* and *Titanoderma cystoseirae*. Moreover, 34 taxa contributed cumulatively by 99 %. Under-storey layer considerably dominated to the community with most common representatives the red coralligenous algae *Haliptilon virgatum*, *Corallina elongata* and *Jania rubens*, and the brown alga *Padina pavonica*. It was followed by *C. crinita* epiphytes distinguished in: 1) filamentous green (*Cladophora* spp.), brown (S*phacelaria cirrosa*) and red (*Herposiphonia secunda*) algae, and 2) in encrusting red algae (*Titanoderma cystoseirae* and *Hydrolithon* spp.). *Cystoseira compressa* contributed significantly (23.08 %) to *C. crinita* community indicating that these species share common habitat resources in the Aegean Sea.

Within the common *Cystoseira* cf. *crinita* taxa 21 (62 %) belong to opportunistic ESG II, whereas 13 (38 %) taxa belong to late-successional ESG I (Table 2.1.1). By contrast the ESG I taxa dominated quantitatively (111 %; including *C.* cf. *crinita*) the ESG II (21.9 %) taxa in the *C.* cf. *crinita* community (Figure 2.1.5). This result did not change when naturally eutrophic North Aegean sites (ESG I=128 %, ESG II=21 %) were differentiated form naturally oligotrophic sites of South Aegean (ESG I=101 %, ESG II=22 %). This result is in accordance both: a) to the conceptual model of Orfanidis et al. (2001, 2003) that “in less anthropogenic stressed coastal areas (pristine) the late-successional species dominate” and b) to the basic assumption of the EEI that ESG I, II average contribution in undisturbed areas is higher than 60 % and less than 30 %, respectively. Data from tentatively pristine sites of Slovenian (Orlando-Bonaca et al., 2008) and Cyprus (pers. comm. M. Argyrou) coasts as well as from less anthropogenic impacted sites of Catalan coasts (Arévalo et al., 2007) have also verified the above hypotheses.

Since macroalgae and especially long lived genera like Fucales also follow long-term periodicity, it is important to notice that their absence from a site should be regarded as indicative of environmental degradation only if it is correlated with key abiotic parameters, e.g. water and sediment nutrient concentrations, light attenuation.

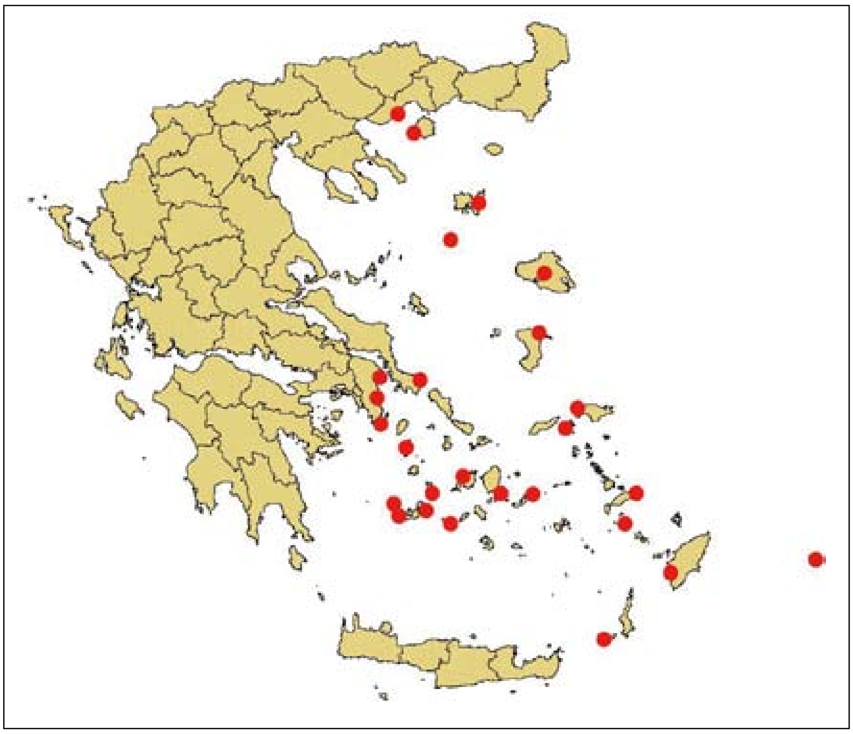


Figure 2.1.2: *Map of putatively pristine sites in the Aegean Sea, Greece.*

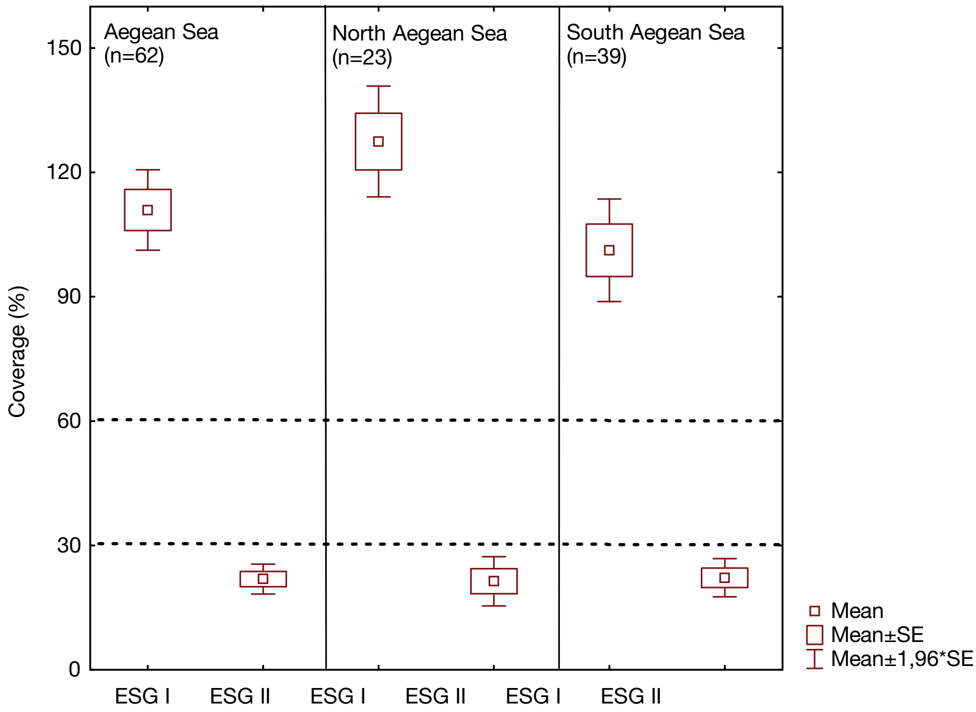


Figure 2.1.3: Average coverage (%) of ESG I, II in undisturbed Aegean Sites.

BENTHOS, CARLIT

Based on expert judgment and on historical data and information we used as RC for BENTHOS and CARLIT existing undisturbed sites or with only very minor disturbance. A problem in deriving reference conditions in the Catalan coast sites is the absence of unimpacted areas. Therefore, we have chosen three reference zones outside Catalonia: Façade maritime du Parc Naturel Régional de Corse (France), Parc Natural de Ses Salines (Balearic Islands, Spain) and Reserva Marina del Nord de Menorca (Balearic Islands, Spain) (Figure 2.1.3). All these places have in common a very low human influence with physico-chemical and hydrogeomorphological conditions similar to the Catalan coast. We have also historical data for benthic communities in the Catalan coast before 1980's (Gibert, 1918; Seoane-Camba, 1975; Polo, 1978) and in the adjacent Albères coast (Sauvageau, 1912; Feldmann, 1937; Gros, 1978; Thibaut et al., 2005) showing that previous littoral vegetation in the Catalan coast was very similar to that currently observed in the selected reference areas.

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

In order to obtain reference conditions' data for Benthos and Carlit methods we have sampled using both methodologies at the same period of time, from May to June (2001). For Benthos, reference conditions were assessed by sampling 11 stations situated within reference sites (4 in Corsica, 4 in Menorca and 3 in Freus) and to obtain CARLIT's reference conditions the whole coastline in these three reference sites was cartographied.

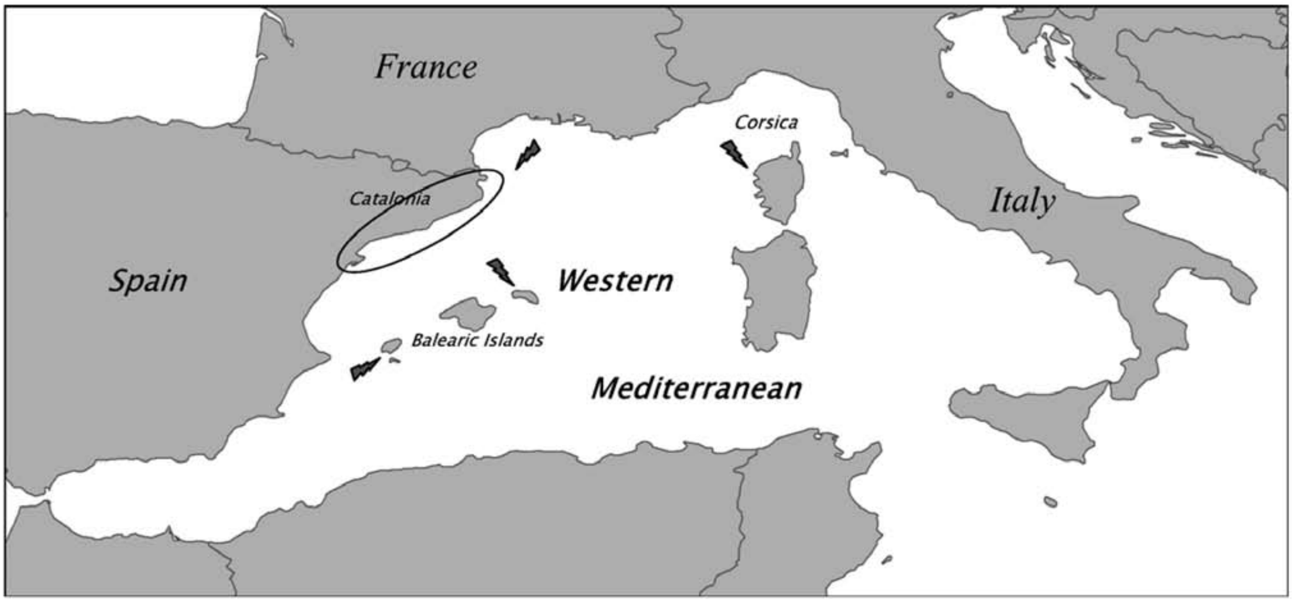


Figure 2.1.3: Location of study area and sites (Northwestern Mediterranean Sea). Reference sites are included in the map.

Common biological criteria of EEI, BENTHOS, CARLIT approaches for reference conditions

EEI, BENTHOS, CARLIT approaches share common views for reference conditions within Mediterranean Sea summarized as follows.

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

Boundary setting (national level)

Boundaries are set according to biotic index and/or combined with the results of or multivariate analysis. No statistical analysis exclusively to set boundaries. No discontinuities. Continuum of possibilities with gradual disappearance/appearance of different indicator species.

Ecological Evaluation Index (EEI)

The conceptual model proposed by Orfanidis et al. (2001, 2003) used the functional differences of benthic macrophytes related to their life-cycle strategy (r-, *K*-selected species) as a tool to evaluate the ecological status of transitional and coastal waters (Figure 2.1.4). This scheme evidences the existence of a gradient between two states, which represent the pristine (high-good) and the degraded (low-bad) conditions. The dominance of the late-successional species of the genera *Cystoseira* form communities indicative of pristine state, which is characterized, for example, by low nutrient and clear water conditions, whilst the dominance of opportunistic seaweeds as *Ulva* and *Gracilaria*, and Cyanobacteria form communities indicative of degraded state, which is characterized by high nutrients, heavy metals and turbid conditions. The coexistence of the late-successional like *Cystoseira*, *Sargassum*, *Corallina* species with opportunistic like *Ulva*, *Cladophora*, *Gracilaria*, Cyanobacteria species form communities that are indicative intermediate (moderate) conditions. In this successional model the ecological role of *Corallina elongata* and its community should be discussed in more details. *Corallina* is a slow growing calcified perennial red alga (ESG I) can be found in the infralittoral zone of most rocky Mediterranean coasts subjected to different kinds of natural, e.g. grazing, low irradiance, hydrodynamism or anthropogenic disturbance, e.g. pollution. Therefore, *C. elongata* alone could not be relevant metric of ecosystem state and trends. For EEI, which investigates anthropogenic stress at community level, *C. elongata* community is regarded as indicative of both high to good or of moderate to bad conditions depending on high quantitative contribution of ESG I or ESG II species, respectively. To estimate accurately community's contribution of small sized and delicately constructed ESG II species being very sensitive to adverse hydrological conditions which predominate in open coasts, especially during the winter season, seasonal and destructive sampling protocols are needed.

High status ($10 \geq$ EEI > 8). The composition of macroalgal taxa is consistent with undisturbed conditions. There are no detectable changes in macroalgal cover due to anthropogenic activities. This condition corresponds with unpolluted sites, where the late successionals taxa, especially species of *Cystoseira* genus, represented by the ecological group ESG I account for more than 60 % of the mean macroalgae abundance-coverage and the early successionals taxa represented by the groups ESG II account for 0-30 % of the macroalgae coverage.

Good status ($8 \geq$ EEI > 6). There are slight changes in the composition and abundance of macroalgal taxa compared to the type-specific communities. Such changes do not indicate any accelerated growth of phytobenthos or higher forms of plant life resulting in undesirable disturbance to the balance of organisms present in the water body or to the physicochemical quality of the water. This condition corresponds with slightly polluted sites (unbalanced). At the good status as is indicated by the EEI, the ESG I group may range from 30 to 60 % while the ESG II from 0 to 30 % of the macroalgae coverage, or the combination may thus that ESG I accounts for over 60 % and ESG II between 30 and 60 % of the total macroalgae coverage.

Moderate status ($6 \geq$ EEI > 4). The composition of macroalgae taxa differs moderately from type-specific conditions and is significantly more distorted from a good quality. Moderate changes in the coverage macroalgal disturbance are evident and may be such as to result in an undesirable disturbance to the balance of organisms present in the water body. This condition corresponds with moderate polluted sites. At the moderate status as is indicated by the EEI, the two groups may equally share the macroalgae coverage accounting for equally low, moderate or high percentages.

Poor status ($4 \geq$ EEI > 2). At the poor status as is indicated by the EEI, the late successional group ESG I may account for 0-30 % and the early successional group ESG II for 30-60 % or the late successional group may represent a coverage among 30 % and 60 % while the early successional group may account for over 60 %.

Bad status (EEI=2): At the bad status the sensitive group ESG I accounts for 0-30 % and the early successional group ESG II represents over 60 % of the total macroalgae coverage.

EEI application was also tested across nutrient gradients of different Mediterranean coasts of Greece (Orfanidis et al., 2001; Panayotidis et al., 2004; Orfanidis and Panayotidis, 2005) and Spain (Arévalo et al., 2007; see also Orfanidis, 2007) with available consistent seasonal data (Figure 2.1.5). The result seems to be very promising indicating clear gradients between degraded

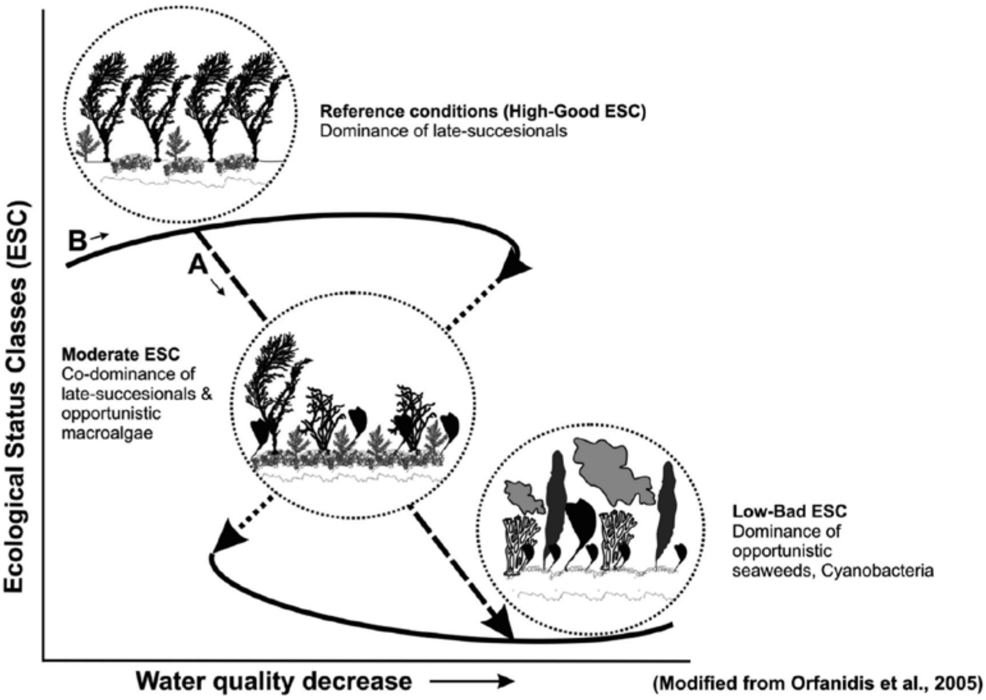


Figure 2.1.4: Conceptual model of two alternative stable states of macroalgal communities across an ecological status gradient in coastal waters. A conventional (A) and dynamic (B) view of successional changes.

and pristine sites. A disagreement appeared in Site 2 of Tossa de Mar coasts located very close to sewage outfall (Arévalo et al., 2007). In that site ESG I species, mainly *Corallina elongata*, dominated (ESG I=251 % mean annual coverage) to ESG II (12.2 %) species indicating a high ESC. The very low coverage of ESG II species of green and red algae, as well as the absence of Cyanobacteria (X. Torras correspondence) seems to be an interesting case that needs further consideration. Figure 2.1.6 shows an exponential relationship between total dissolved inorganic nitrogen (TDIN) and phosphorus mean concentrations and EEI values.

CARLIT/BENTHOS

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

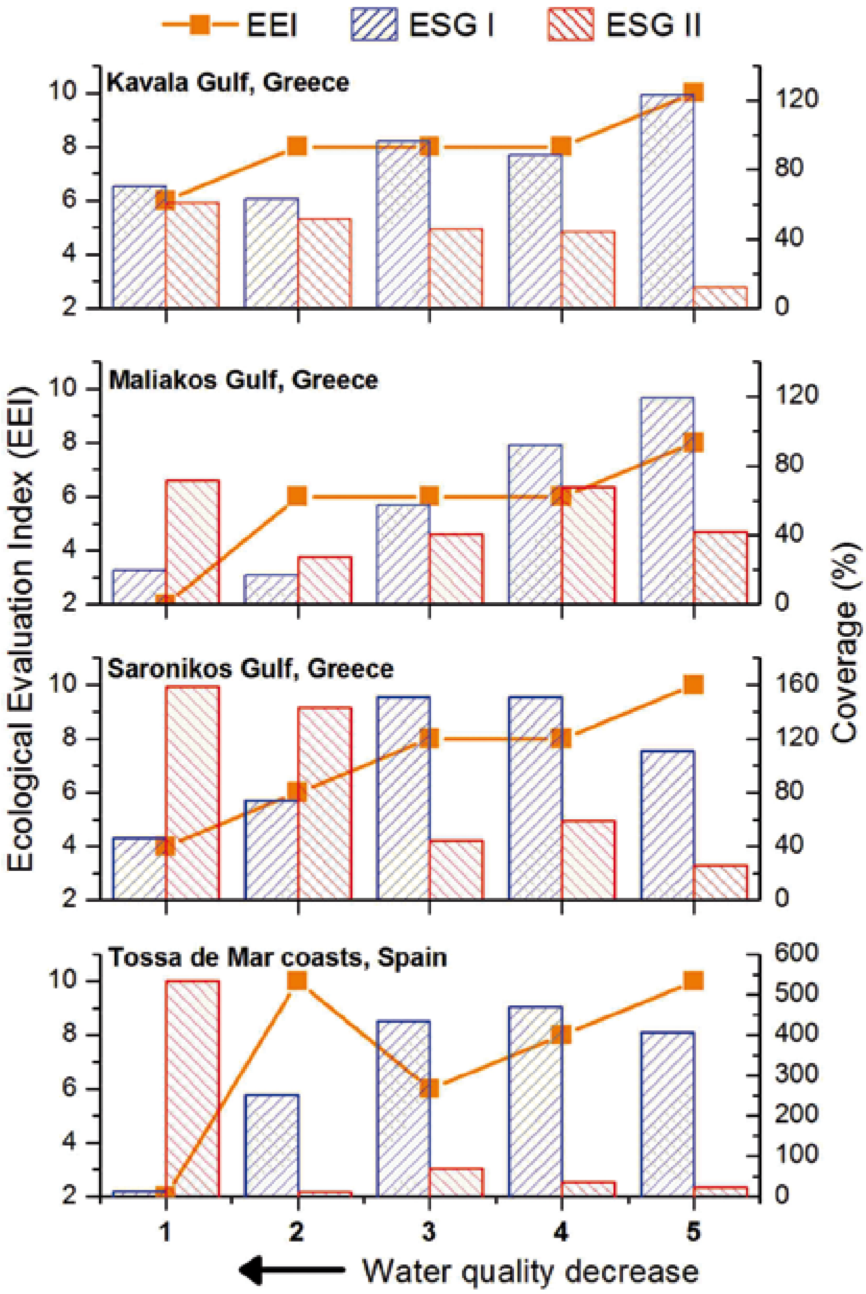


Figure 2.1.5: Implementation of EEI across water quality gradients in different Mediterranean coastal waters: Kavala and Maliakos Gulfs (Orfanidis and Panayotidis 2005), Saronikos Gulf (Panayotidis et al., 2004), Tossa de Mar coasts (Arévalo et al., 2007).

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

In Italy, Carlit method has been tested at Regional scale, in the Ligurian Sea, applying it in a moderate urban gradient (figure 2.1.7) and in four Marine Protected Areas (MPAs), proposed as hypothetical reference sites at a regional scale (Mangialajo et al., 2007). This study shows that Carlit index is suitable to detect different kinds of anthropogenic pressures obtaining a good correlation with different water column variables.

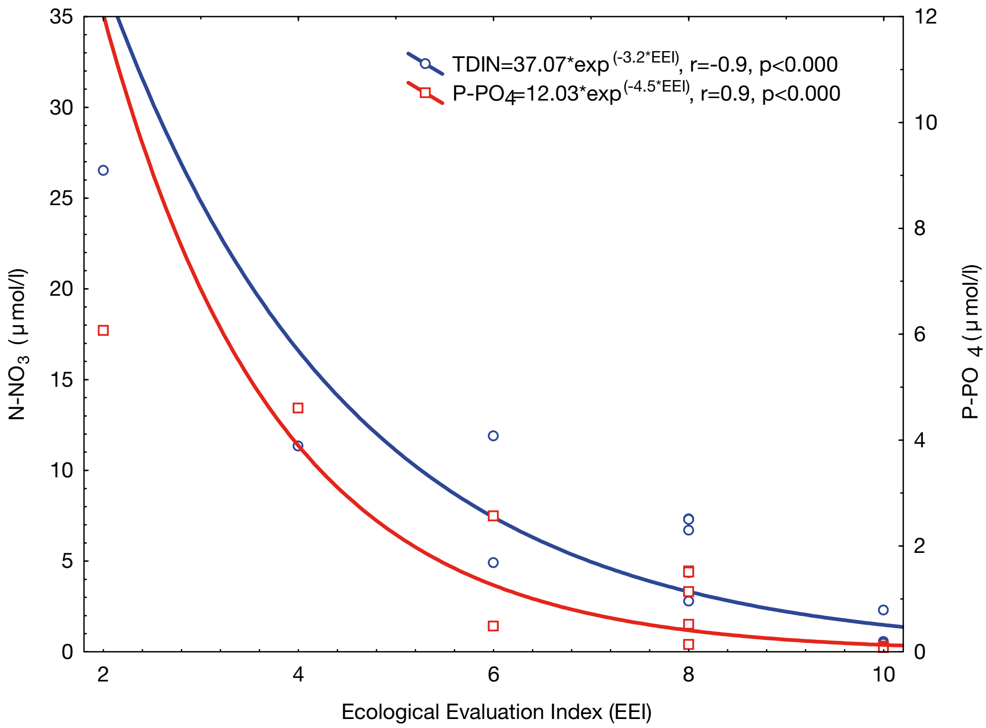


Figure 2.1.6: Total dissolved inorganic nitrogen (TDIN) and Phosphorus mean concentrations plotted against EEI values. Data from different Mediterranean coastal waters.

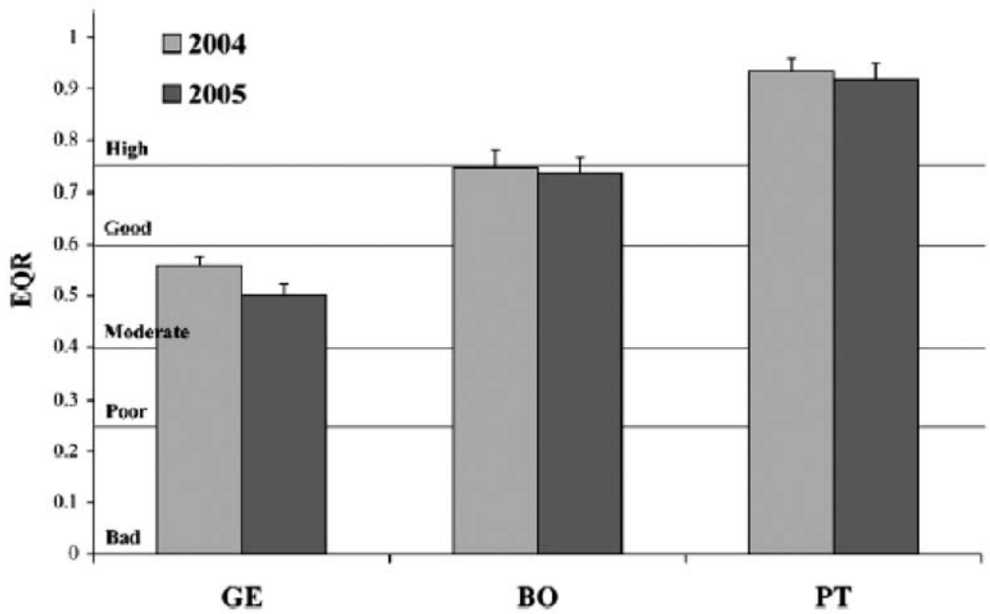


Figure 2.1.7: Carlit EQR values along a moderate urban gradient in the Ligurian sea.

2.1.4 Intercalibration

Material & Methods

Data sampled along Greek (Figure 2.1.8), Catalan (Figure 2.1.9), and Slovenian locations were exchanged for intercalibration.

Data from Greek coasts were sampled from 11 sites (96 samples in total): Saronikos (6 sites, 6 sampling periods, 36 samples in total) and Maliakos (5 sites, 4 sampling periods, 60 samples in total) Gulfs. Fifty one sites (1 sampling period, 151 samples in total) were sampled along the Slovenian coasts (Lipej et al., 2006). All samples were destructive (1 to 3 random sample from a permanent site-square 5 x 5 m per sampling period) on a quadrate 20cm x 20cm, which is considered to be the minimal sampling area in the case of the Mediterranean infralittoral communities. In the laboratory each sample was sorted carefully and the surface covered by each species in vertical projection was quantified as % of cover (4 cm^2 = 1 % sampling surface). The total coverage usually exceeded 100 % due to the presence of different layers in the vegetation (canopy, under storey layer, crusts and epiphytes). A nominal coverage value (0.1 %) was allocated to species showing insignificant abundance. Taxonomically difficult taxa were consistently sub summarized to genus level as spp.

Data from Spain coasts were sampled from 48 sites (49 samples in total) Tossa-St. Feliu coast (6 sites, High-Good Water Body) and Hospitalet-Ametlla coast (7 sites, Good-Moderate WB). Furthermore, 36 extra sites from different Catalan places with Moderate to Bad ES were included in the intercalibration exercise (Figure 2.1.9). The samples were collected in springtime in the upper

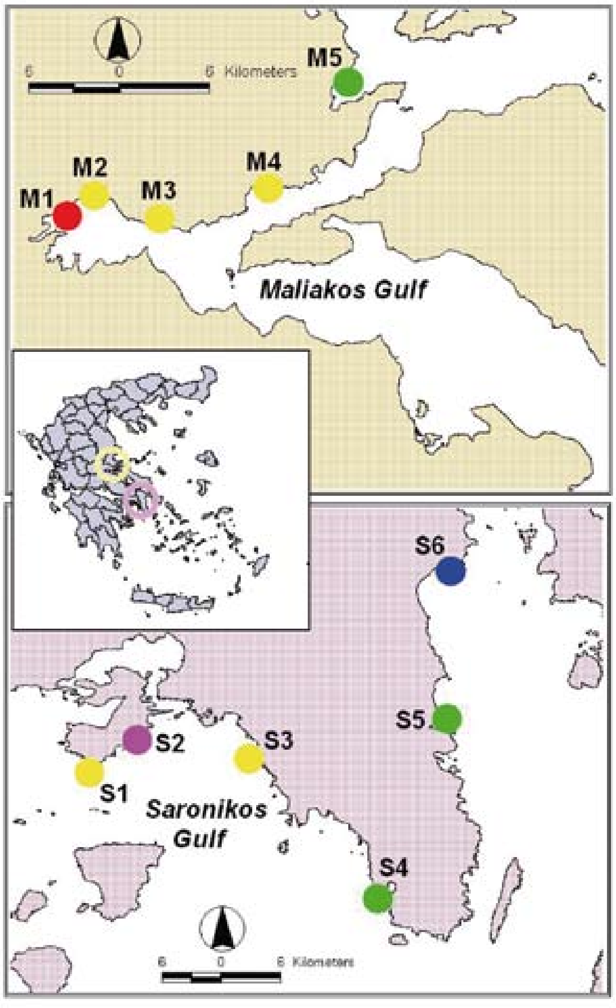


Figure 2.1.8: Map of sampling sites of Maliakos and Saronikos Gulfs. Colours correspond to Ecological Status Classes in accordance to EEI.

Figure 2.1.9: Map of Catalonia sampling sites. Colours correspond to Ecological Status Classes in accordance to Benthos methodology.

sublittoral zone on sub-horizontal rocky shores facing east, south or west. For each sample, the whole community was collected from a 15 x 15 cm surface, using a hammer and a chisel. This surface is large enough to quantitatively represent the littoral communities in the Northwestern Mediterranean (Coppejans, 1980; Verlaque, 1987; Ballesteros, 1992). Samples were preserved in formalin: sea-water at 4 % and were subsequently sorted in the laboratory. Algae and invertebrates were identified to species level and quantified in terms of coverage (horizontal surface; Ballesteros, 1992).

2.1.5 Results of the comparison

EEI and BENTHOS were compared on 62 sites in Greece (11 sites) and Slovenia (51 sites), while BENTHOS and CARLIT on 48 sites in Spain.

The relation between variable EQR-EEI and variable EQR-ICM (Benthos) is described by a linear equation:

(1) EQR-ICM = 0.594 EQR-EEI + 0.3425, coefficient of determination $R^2 = 0.85$ (Figure 2.1.10).

The relation between variable EQR-ICM and variable EQR-CARLIT is also described by a linear equation.

(2) EQR-ICM = 0.8604 EQR-CARLIT + 0.0709, coefficient of determination $R^2 = 0.77$ (Figure 2.1.11).

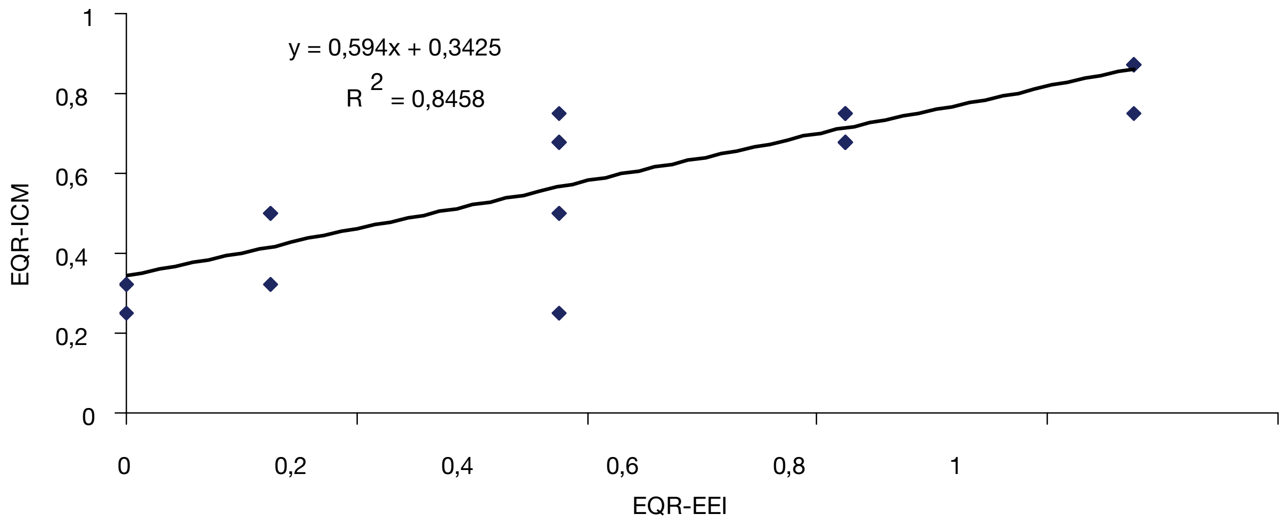


Figure 2.1.10: Relation between EQR-ICM and EQR-EEI.

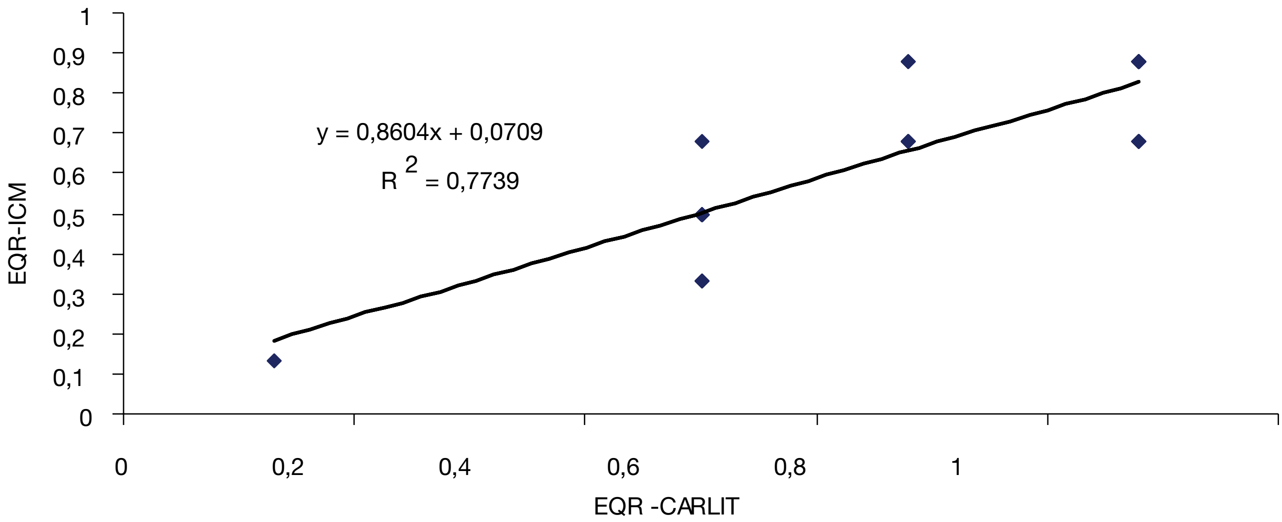


Figure 2.1.11: Relation between EQR-ICM and EQR-CARLIT.

From the equations (1) and (2) we calculated new values of class boundaries while replacing in the equation (1) EQR-EEI by the boundary values of EQR defined for method EEI (ICM-boundaries EEI) and the same for the boundary values of EQR defined for CARLIT (ICM-boundaries CARLIT) by using the equation (2) (Tables 4 and 5). CARLIT and BENTHOS (ICM) have the same values of class boundaries.

Table 2.1.4: EQR of the class boundaries used for methods EEI and CARLIT.

Boundaries	EEI-Boundaries EQR	CARLIT-Boundaries EQR
H/G	0.75	0.75
G/M	0.50	0.60

For each class boundary the new mean values of class boundaries between ICM-boundaries CARLIT and ICM-boundaries EEI were calculated as well as their acceptable range (average ± 0.05; Table 5), defined as comparability acceptable range by the ECOSTAT group.

Table 2.1.5: New EQR of the class boundaries calculated as mean values and their acceptable range (average + 0,05).

Boundaries	ICM-boundaries (CARLIT)	ICM-boundaries (EEI)	Average	Acceptable range (average ± 0,05)
H/G	0.72	0.79	0.75	0.70 - 0.80
G/M	0.59	0.64	0.61	0.56 - 0.66

ICM-boundaries (CARLIT) and ICM-boundaries (EEI) are in the acceptable range of variability, for their comparison, defined during the intercalibration process, in the ECOSTAT Working Group (Figure 2.1.12).

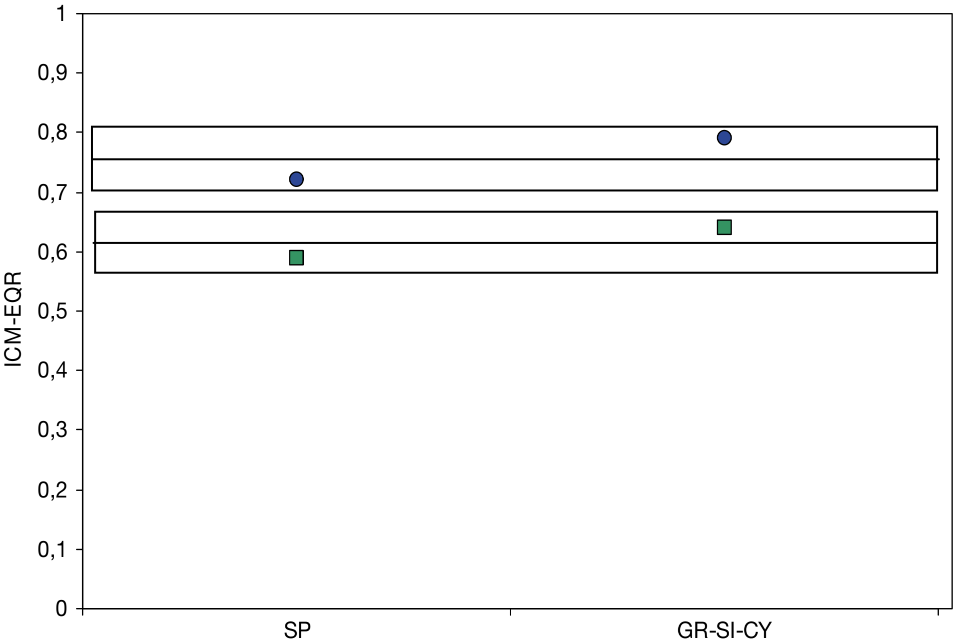


Figure 2.1.12: Intercalibrated EQR within the confidence interval.

2.1.6 Results of the harmonisation – Boundary EQR values

The harmonization process between the methods indicated that some differences, in respect to the ecological meaning of certain species and communities, exist in the methods and, as a result, each method has its own EQR values; nevertheless values are very close in all countries in which methods have been applied.

2.1.7 Open issues and need for further work

Based on above described similarities and differences between the methods following topics were emerged that needs to be searched in future:

1. To minimize differences between methods deeper knowledge in different aspects is needed: (a) clarification of scale based ecological role of species like *Corallina elongata*, *Cystoseira compressa* and their communities within the Mediterranean Sea, (b) method sensitivity to environmental stress.
2. Study of species-specific sensitivity and tolerance of dominant macroalgal species to different disturbances.
3. Development of type-specific reference conditions within rocky coasts, e.g. rocky shallow, rocky deep, using new or existing literature data.
4. Application of common actions to describe different ecological status classes and test different method effectiveness. Such an action is planned for spring/summer 2008 in Slovenian coasts, where Slovenian and French experts will intercalibrate on a similar spatio-temporal scale the CARLIT and EEI indices. The campaign will promote, if needed, the development of local scales of sensitivity levels of species (CARLIT) or possible adjustment of ecological state groups (EEI). It will also provide information/data on the ecological value of the taxa under debate.
5. Italy, after having tested the CARLIT method at Regional scale, has presently introduced its use, at the national level, in the new National Monitoring Programme (2008-2011) for coastal waters. A first training course for the Regional Agencies, that operate in the fields, has already been carried out.

2.2 NE Atlantic GIG

2.2.1 Intercalibration approach

In the NE Atlantic seven basic intercalibration types have been agreed. These are shown in table 2.2.1 below:

Table 2.2.1: NEA GIG Intercalibration types.

New Type ID	Name	Salinity (PSU)	Tidal range (m)	Depth (m)	Current velocity	Exposure	Mixing	Residence time
CW –NEA1/26a,b,c,d,e	Exposed or sheltered, euhaline, shallow	Fully saline (> 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or sheltered	Fully mixed	Days
CW – NEA3/4	Polyhaline, exposed or moderately exposed (Wadden Sea type)	Polyhaline (18 - 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or moderately exposed	Fully mixed	Days
CW – NEA7	Deep, low current, sheltered	Fully saline (> 30)	Mesotidal (1 - 5)	Deep (> 30)	low (< 1 knot)	Sheltered	Fully mixed	Days
CW – NEA8	Polyhaline, microtidal, sheltered, shallow (Skagerrak inner arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Shallow (< 30)	low (< 1 knot)	Sheltered	Partially Stratified	Days-Weeks
CW – NEA9	Fjord with a shallow sill at the mouth with a very deep maximum depth in the central basin with poor deepwater exchange.	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	low (< 1 knot)	Sheltered	Permanently Stratified	Weeks
CW – NEA10	Polyhaline, microtidal exposed, deep (Skaggerak outer arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	low (< 1 knot)	Exposed	Permanently Stratified	Days
TW – NEA11 (will be split into sub-types)	Transitional waters	Oligo-Euhaline (0 - 35)	Mesotidal (1 – 5)	Shallow (< 30)	Medium	Sheltered or moderately Exposed	Partially- or Permanently Stratified	Days-Weeks

The above types occur in Member State's waters as detailed below in table 2.2.2:

Table 2.2.2: Member States sharing the types.

Type	BE	DK	FR	DE	IE	NL	NO	PT	ES	SE	UK
CW - NEA1/26a			X		X		X		X		X
CW - NEA1/26b	X		X			X	X				X
CW- NEA1/26c		X		X							
CW- NEA1/26d		X									
CW- NEA1/26e								X	X		
CW – NEA3/4				x		x					
CW – NEA7							x				**X**
CW – NEA8		x					x			X	
CW – NEA9							x			x	
CW – NEA10							x			x	
TW – NEA11	**X**		X	X	X	X		X	X		X

Option 2 has been used in this phase of the intercalibration process. Three metrics have been selected, Perennial Intertidal Algae (in type NEA1/26a,b,e), Opportunistic Macroalgae (in types NEA1/26a,b,c/11) and Perennial Subtidal Algae (in types NEA8/9/10). Boundaries have been agreed for some countries for each metric where applicable. This is described below.

2.2.2 National methods that were intercalibrated

National methods are summarised in table 2.2.3 below. Further details can be found in the following sections and appendices

Table 2.2.3: National Methods.

Member State	RSL	CFR	P-MarMAT	MAB	Subtidal algae
BE					
DK					Yes
FR					
DE				Yes	
IE	Yes			Yes	
NL					
NO	Yes				Yes
PT			Yes	Only in TW's	
ES		Yes			
SE					Yes
UK	Yes			Yes	

RSL (Reduced Species list) – Rocky shore tool

(Perennial Intertidal Algae)

A tool for ecological quality assessment of intertidal rocky shore macroalgae based on the species richness from a reduced species list (RSL tool) of between 68 and 70 species had been developed over the last 4 years by UK and has been adopted with slight local variation by Republic of Ireland and Norway. The metric is based on five components:

- Numerical species richness which incorporates a deshoring factor to correct for the overall nature of the shore and its suitability for algal attachment.
- The proportion of Rhodophyta species within the overall sample
- The proportion of Chlorophyta species within the overall sample
- The proportion of opportunistic species within the overall sample which includes the species Blidingia, Chaetomorpha, Enteromorpha, Ulva, Ectocarpus, Pilayella and Porphyra.
- The ratio of perennial forms (ESG I) to annual or ephemeral forms (ESG II)

CFR – Quality of Rocky Bottoms

(Perennial Intertidal Algae & Opportunistic macroalgae)

Composed of three indicators: percentage cover of characteristic macroalgae (Cover), characteristic macroalgae population richness (Richness) and percentage cover of opportunistic species (Opportunists) relative to the total vegetated surface.

P-MarMAT – Portuguese Marine Macroalgae Assessment Tool

(Perennial Intertidal Algae & Opportunistic macroalgae)

This includes all components from the RSL (species richness, proportion of greens, proportion of reds, ESG ratio, proportion of opportunists and shore description) but in addition also includes coverage of opportunists (%).

MAB – Macroalgae Blooming

(Opportunistic macroalgae)

This tool is based on 5 components:

- % cover of algae over the available intertidal area (or the whole intertidal area for Germany) calculated as an average
- The total extent of the algal blooms measured in hectares
- The biomass of the algae taken as an average per squared meter over the available intertidal area
- The Biomass of the algae taken as an average per squared meter over the affected bloom area only
- Presence of entrained algae calculated as a % of the total quadrats in which it is recorded.

Subtidal Algae

(Perennial Subtidal Algae)

This tool incorporates three metrics

- Metric 1 describes the depth extension of selected perennial macroalgal species in Scandinavian coastal waters. The species selected for each type are all 1) perennial, 2) commonly occurring in the type and 3) easy to determine.

- Metric 2 describes the cover of the macroalgal community along depth gradients. This is still under development.
  1) 'Total cover': overall cover of the community representing values up to 100 %, or
  2) 'Cumulated algal cover': summed cover of all individual species, representing values up to several 100 % as the algae may grow in several stories.
- Metric 3 describes the macroalgal composition in rocky littoral and sublittoral zones. The metric includes species richness (or total number of species recorded), proportions of red, green and opportunist, ratio of ephemeral against perennial algae, ratio between Ecological Status Groups I and II (ESG ratio), lower depth extension of a few selected species and their abundance as well as the physical properties of the location. Again this metric is still under development.

2.2.3 Reference conditions and class boundary setting

Metrics -RSL (Reduced Species List) - Rocky Shore Tool**, CFR & P-MarMAT**

CW - NEA1/26a,b,e

Member states involved in intercalibration of rocky shore tools:
Reduced Species List (RSL): (applied only to intertidal areas).
NB: the RSL doesn't incorporate cover:

- UK
- Republic of Ireland
- Norway

CFR: (Applied either for intertidal or subtidal areas).
NB: the CFR incorporates cover:

- Spain
- Portugal

P-MarMAT:
NB: the P-MarMAT incorporates coverage of opportunists:

- Portugal
- Spain

France is yet to decide which metric will be adopted, but this means they will 'automatically' intercalibrate with whichever member state's tool they adopt.

Reference Conditions

General reference conditions are based on the normative definition, which states, "All disturbance-sensitive macroalgal and angiosperm taxa associated with undisturbed conditions should be present and the levels of macroalgal cover and angiosperm abundance should be consistent with undisturbed conditions". The reference values indicated in the nominative definition are calculated as the maximum values possible for the selected indices comprising the macroalgae metric.

The RSL (Reduced Species List) macroalgae metric indices include: Total numerical species richness based on a reduced species list which includes a correction factor based on the shore description, the proportion of red species within the sample, the proportion of green species, the proportion of opportunist species listed as *Blidingia, Chaetomorpha, Enteromorpha, Ulva, Ectocarpus, Pilayella* and *Porphyra*, and the ratio of ESG I/ESG II (ESG 1 – late successionals or perennials and ESG 2 – ephemerals or annuals)

This translates into the following definition (table 2.2.4):

Table 2.2.4: RSL reference values.

Biological element	**Reference value**				
Marine benthic macroalgae	Diverse community of red, green and brown seaweeds with high levels of species richness. Cover variable depending on local physical conditions but species richness relatively constant temporally. Red species present as richest group along with a high proportion of long-lived spp. Opportunist and green species should constitute a lower proportion of the algal present.				
Optimal Metric Scores	Species Richness	% Green Species	% Red Species	ESG Ratio	% Opportunists
Scotland	≥35	≤12	≥55	≥1.0	≤10
England/Wales/RoI	≥35	≤15	≥55	≥1.0	≤10
NI	≥34	≤20	≥45	≥0.8	≤15
Norway	≥33	≤20	≥40	≥0.8	≤15

The reduced species list has a maximum of 70 Species that should be present for UK& IE shores, and 68 for Norway. The actual species within these lists vary to represent regional differences in species composition. These are the reference species numbers for the 'reduced species list' from which species richness is derived.

In terms of the indices agreed for this metric then a reference site should score the maximum possible i.e. 24 points, giving an EQR of 1.0 (or 20points if no shore description is available). The tool now works on an EQR sliding scale and no longer equates to a point system as previously described, the new method is much more accurate and is easier to see when a site is sitting on, or close to, a class boundary.

For UK, IE, and Norway bodies reference values were established using historic data, historic reports and publications and expert judgement. Expert judgement was then used to refine recommended levels of macroalgae species richness to correspond to desired reference conditions of maximum species richness and desirable community composition within natural and undisturbed water bodies.

Spain and Portugal have tested the RSL and found that it was not appropriate for their coasts.

Spain has developed the CFR (Quality of Rocky Bottoms) Index (Juanes *et al.*, 2008), which is composed of three indicators: percentage cover of characteristic macroalgae (Cover), characteristic macroalgae population richness (Richness) and percentage cover of opportunistic species (Opportunists) relative to the total vegetated surface. The reference conditions for this metric are as follows (table 2.2.5):

Table 2.2.5: CFR reference values

Metric	**Ref. Cond.**
Characteristic Macroalgae Cover	70 %
Populations Richness	6
Relative Coverage of Opportunists	9 %

Portugal has developed de P-MarMAT (Portuguese Marine Macroalgae Assessment Tool). Which includes all metrics from RSL (species richness, proportion of greens, proportion of reds, ESG ratio, proportion of opportunists and shore description) and coverage of opportunists (%). The reference conditions for this metric are as follows (table 2.2.6):

Table 2.2.6: P-MarMAT reference values.

Metric	Ref. Cond.
Species richness	25
Proportion of greens	10
Proportion of reds	70
ESG ratio	2.5
Proportion of opportunists	10
Shore description	7
Coverage of opportunists	10

France is yet to decide which metric will be adopted, but this means they will 'automatically' intercalibrate with whichever member state's tool they adopt.

Boundary Setting Protocol

UK, Ireland, Norway:

The Reduced Species List Index has been tested using UK, Irish, Norwegian, Portuguese and Spanish Data. Boundaries have been agreed through data testing and common consensus (expert judgement). As no direct relationship with a pressure gradient could be established, step 8 in the boundary setting procedure was invoked (see Annex C of Milestone 6 report).

The full boundary setting protocol is laid out in the Milestone 6 report Annex C. At present only UK, Ireland, and Norway are fully intercalibrated and have accepted the boundaries agreed for the RSL index.

Spain:

The CFR index was developed in Cantabria (Juanes *et al.*, 2008), region where it has been extensively applied to intertidal and subtidal macroalgae communities (Guinda et al, 2008). This method has been tested as an alternative method to that of the UK, in order to test a more quantitative approach that may reflect, in a homogeneous way, the ecological condition of our hard substrate habitats all through the extent of the water bodies (intertidal+subtidal). Based on independent assessments of the status at different bathymetric levels (intertidal/ depth ranges), the index provides a tool for the consideration of all the possible "coastal reef habitats" that, according to the normative (e.g. Habitat Directive) or the expert judgements, are important in each water body.

Furthermore, the initial formulation of the index (indicators, boundary values) has been intensively discussed by experts before the last version has been adopted.

The last version of the CFR index provides a quantitative approach for reflecting, in a homogeneous way, the ecological condition of hard substrate habitats throughout the extent of the water bodies in the North coast of Spain, based on independent assessment of the quality status at different bathymetric levels (intertidal/subtidal). This metric integrates those features suggested by the WFD for the assessment of seaweed communities, including the composition (Richness, R; presence of Opportunistic species, O) and the abundance (Cover, C). The Richness value (R) evaluates the number of different "characteristic macroalgae" populations that present a significant coverage (ca. >1 %), according to a previously established list for each biogeographic region. Moreover, the Cover score (C) assesses the extent that is occupied by those assemblages, considered all together. The third indicator quantifies the abundance of Opportunistic species (O) in relation to the total vegetated surface, as one of the first symptoms of several anthropogenic disturbances, mainly related to nutrient enrichment.

Further details about theoretical basis and application procedures may be obtained from the previous version of this index (Juanes *et al*., 2008), which included additional assessments of the physiological status.

Different quality thresholds were considered for diverse degrees of exposure (slope) of intertidal sites and depth ranges of subtidal stations. Table 2.2.7 shows the boundaries adopted for each metric.

Table 2.2.7: Value boundaries and scores assigned to each of the three metrics of the CFR index, for its application at different intertidal and subtidal zones.

CFR: Quality of Rocky Bottoms Index

Cover (1)				
Score	Semiexp. Int.	Exposed Int.	5 - 15 m	15 - 25 m
45	70-100%	50-100%	70-100%	50-100%
35	40-69%	30-49%	40-69%	30-49%
20	20-39%	10-29%	20-39%	10-29%
10	10-19%	5-9%	10-19%	5-9%
0	<10%	< 5%	<10%	< 5%

(1) % Cover of Characteristic Macroalgae (CM).

Populations Richness (2)				
Score	Semiexp. Int.	Exposed Int.	5 - 15 m	15 - 25 m
20	> 5	> 3	> 5	> 5
15	4 - 5	3	4 - 5	4 - 5
10	2 - 3	2	2 - 3	2 - 3
5	1	1	1	1
0	0	0	0	0

(2) Characteristic macroalgae populations richness.

Opportunistic species (3)			
Score	Intertidal	5 - 15 m	15 - 25 m
35	<10%	<5%	<5%
25	10-19%	5-9%	5-9%
15	20-29%	10-19%	10-19%
5	30-69%	20-49%	20-49%
0	70-100%	50-100%	50-100%

(3) Relative cover of opportunistic or pollution indicator species respect to the total vegetated surface.

The final CFR value is calculated by adding the scores obtained for each of the three metrics (C+R+O). To obtain the corresponding EQR value ranging between 0 and 1, the CFR/100 value is calculated and the corresponding status is assigned according to the ranges of the next table 2.2.8 (based on REFCOND 2003):

Table 2.2.8: CFR ranges.

CFR Scores	EQR	Status
83-100	0.83-1	High
62-82	0.62-0.82	Good
41-61	0.41-0.61	Moderate
20-40	0.2-0.4	Poor
0-19	0-0.19	Bad

Portugal:

The metrics selected for the Portuguese marine macroalgae assessment tool (P-MarMAT) include the ones from the RSL methodology proposed by UK, Norway and RoI (species richness, proportion of greens, proportion of reds, ESG ratio, proportion of opportunists and shore description), and the coverage of opportunists. A reduced species list was developed to Portugal (keeping the same number of algae from green -8-, red -20- and brown -24- groups initially proposed by other MS), and a new metric was included on the assessment method (coverage of opportunists). The scores for the species richness, proportion of greens, proportion of reds, ESG ratio, and the proportion of opportunists were calculated from the RSL adapted to Portugal (PT-RSL), and the shore description followed the same procedures of the ones proposed earlier by the above mentioned MSs. The coverage of opportunists represents the percentage cover of opportunists (defined from the PT-RSL) on the total area covered by marine macroalgae in the assessed shore. The metrics species richness and % coverage of opportunists have a factor of 2 on the contribution to the total score. The sum of scores is converted in a 0 to 1 scale (EQR according to the WFD definitions), that afterwards allows the determination of the EQS of the shore (according to WFD normative definitions). Table 2.2.9 shows the candidate metrics, the boundaries adopted for each of them to Portugal on this stage of the IC exercise, and provides the way to go from the sum of scores to the EQS.

Table 2.2.9: Candidate metrics comprising the P-MarMAT, their boundaries, and conversion of sum of scores in EQR and EQS classes.

Quality	Bad	Poor	Moderate	Good	High
Species Richness (*)	0 - 5	5 - 8	9 - 16	17 - 24	> 24
Proportion of Greens	40 - 100	30 - 40	20 - 30	10 - 20	0 - 10
Proportion of Reds	0 - 30	30 - 45	45 - 55	55 - 70	70 - 100
ESG Ratio	0 - 1	1 - 1.5	1.5 - 2	2 - 2.5	> 2.5
Proportion of Opportunists	40 - 100	30 - 40	20 - 30	10 - 20	0 - 10
Shore Descriptions	-	15 - 18	12 - 14	8 - 11	1 - 7
Coverage of Opportunists (%) (*)	70 - 100	30 - 70	20 - 30	10 - 20	0 - 10
Sum of scores	0 - 7	8 - 14	15 - 21	22 - 28	29 - 36
EQR	0 - 0.2	0.2 - 0.4	0.4 - 0.6	0.6 - 0.8	0.8 - 1
EQS	**Bad**	Poor	Moderate	Good	High

(*) factor of 2

Metric - MAB (Macroalgal Blooms) - Opportunistic Macroalgae **(NEA1/26/11)**

Reference Conditions

General reference conditions should fulfil the criteria for high status as defined by the Water Framework Directive: There should be no or only very minor anthropogenic alterations to the values of the physico-chemical and hydromorphological quality element relative to those associated with undisturbed conditions. Specifically reference sites should also fulfil the criteria for high status of the quality element 'macroalgae and angiosperms' as defined by the WFD: All disturbance-sensitive macroalgal and angiosperm taxa associated with undisturbed conditions should be present and the levels of macroalgal cover and angiosperm abundance should be consistent with undisturbed conditions. The proposed metrics/indices are % cover and total area of opportunist macroalgae over the available intertidal habitat (AIH – UK/IE), or % cover over the total intertidal (DE), biomass of opportunist macroalgae and presence of entrained algae within the sediment definitions of which are given below in table 2.2.10 (UK/IE):

Table 2.2.10: UK/IE reference values.

Measurement	**Definition**
% cover of AIH	The % cover of algae is estimated as an average over the whole of the available intertidal habitat for the whole waterbody
Total area (ha)	The total extent of algal bloom, measured in hectares and based on the external perimeter of the bloom
Biomass (g m2) of AIH	This is the average biomass of algae per metre squared over the whole of the available intertidal habitat
Biomass (g m2) of affected area	This is the average biomass of algae per metre squared over the affected area only
Presence of entrained algae (% of Quadrats)	This is where algae is seen to be growing > 3cm into the underlying sediment indicating the likelihood of regeneration
% cover of total intertidal	The percentage cover of algae is estimated as an average over the whole intertidal for the waterbody

This translates into the following definition (table 2.2.11):

Table 2.2.11: UK/IE reference value definitions.

Biological element	**Reference value**
Marine benthic macroalgae	Opportunistic macroalgal blooms of anthropogenic origin should be absent or if present should cover less than 5 % of the available intertidal habitat and total biomass of macroalgae per square meter should be less than 100. Total area coverage of opportunist macroalgae should be less than 100 hectares with no effect on quality status. Entrained algae should only present in less than 5 % of quadrats. DE – Opportunistic macroalgal blooms of anthropogenic origin should be absent or if present should cover less than 1 % of the total intertidal of the waterbody. Generally directed at intertidal sedimentary shores in both transitional and coastal waters.

Agreed Indices, Scoring Criteria and Use in Classification

The assessment system uses two metrics, firstly, % cover of opportunist macroalgae which responds directly to increased nutrients, turbidity and reduced salinity and refers to the total area covered by opportunist macroalgae calculated as a percent of the total available intertidal habitat consisting of sedimentary substrate (UK/IE) or to the total intertidal (DE). This metric responds to anthropogenic disturbance by an increased % cover of algae. Secondly, the extent of cover of opportunist macroalgae, which responds as above but is required to account for the total size of the macroalgae bloom with the water body. This metric responds to anthropogenic disturbance by an increased extent of opportunist macroalgae cover.

As nutrient input increases there is often a corresponding increase in turbidity and resulting decrease in light attenuation subsequently providing a habitable environment for those species tolerant to such conditions, these opportunist species take advantage of the condition and dominate the community often to the detriment of other species. This is where macroalgae blooms occur. This biological metric does seem to respond over the whole potential gradient of impact whereby each quality status has defined levels of % cover and total extent of opportunist macroalgae, which correspond directly to the changes in quality. Each quality status has well defined and static class boundary and there is no overlap between classes.

The above applies in full to the metrics developed in the UK and RoI. Portugal is considering using the metrics only in transitional waters. Germany has only extent data (based on 6 year median of *maximum* for year) referenced across the *whole* (as opposed to the *available*) intertidal habitat. (see later sections).

Metric - Subtidal Algae (NEA8/9/10)

Reference Conditions

Conditions should fulfil the general criteria for high status as defined by the Water Framework Directive (WFD): There should be no or only very minor anthropogenic alterations to the values of the physico-chemical and hydromorphological quality element relative to those associated with undisturbed conditions. Sites should also fulfil the criteria for high status of the quality element 'macroalgae and angiosperms' as defined by the WFD: All disturbance-sensitive macroalgal and angiosperm taxa associated with undisturbed conditions should be present and the levels of macroalgal cover and angiosperm abundance should be consistent with undisturbed conditions.

The best-explored benthic vegetation metric is 'depth limits of selected macroalgal species' and the present protocol analyses this metric for Scandinavia. Further metrics 'macroalgal cover' and 'multimetric macroalgal index' are not yet developed to a state where we can use them in assessment according to the WFD as e.g. reference levels are not known, but the protocol provides an initial description of these metrics.

Hydromorphological conditions
Hydromorphological conditions corresponding to no or very minor anthropogenic alteration in coastal waters involve absence of digging and construction activity.

Physico-chemical conditions
Nutrient concentrations and water transparency are main physico-chemical variables determining the status of the benthic vegetation. We have attempted to identify reference levels for these

variables within the NEA8, NEA9 and NEA10 areas, where vegetation data are available. At present, no Danish coastal areas are considered to represent reference conditions, so we attempt to define reference levels of total-nitrogen concentrations (TN) and water transparency based on historical data and modeling. Suggestions for reference conditions have recently been published for various Danish coastal areas. Reference TN-concentrations of 43 µM have been suggested for the inner parts and 11-19 µM for outer parts of Randers Fjord based on dynamic modeling (Nielsen et al. 2003), concentrations of 50-58 µM have been suggested for inner parts of Roskilde Fjord based on paleoecological reconstruction (Clarke et al. 2003, Andersen et al. 2004), and concentrations of 23-30 µM have been suggested for Limfjorden (Markager et al. in prep. Christensen et al. xx). For Øresund, concentrations in the range 5-19 µM have been suggested based on various sources (Øresundssamarbejdet 2004: Table 1 and references therein).

Reference values for TN and levels of water transparency have been defined for Swedish coastal waters (Hansson och Håkansson 2006). However, too few data exist from the same area as we have no depth distribution data for the vegetation. At present no reference conditions for TN and water transparency exist for Norway within the different areas.

Reference levels can also be defined through modeling. In order to define reference levels of benthic vegetation metrics through modeling there is a need for dose-response relationships between physico-chemical variables (nutrient concentration, transparency) and vegetation metrics. Then reference levels of nutrient concentration and/or transparency are entered in the model and corresponding levels of the vegetation metrics are calculated.

It is also possible to run hind-cast models based on historical as well as today's data for nutrient loads and transparency and adjusting the nutrient input into the model according to the time when one expected reference conditions. The model can then estimate the new transparency or light penetration depths. These can then be related to e.g. depth extension of macroalgae: Today's lower depth extensions of selected macroalgal species as well as today's percentage light penetration at these depths can be obtained from either measurements or the literature. Based on the species minimum requirement for light, new maximum depth extensions can then be calculated for a reference situation. However, at present the reference values for benthic vegetation metrics have been set by use of historical values as well as expert judgement.

Metrics

Metric 1: Depth limit of macroalgal species

This metric describes the depth extension of selected perennial macroalgal species in Scandinavian coastal waters. The species selected for each type are all 1) perennial, 2) commonly occurring in the type and 3) easy to determine. The metric is affected by nutrient concentration and water transparency (Kautsky et al. 2004). Depth limits of macroalgae as a group (Nielsen et al. 2002b) and depth limits of selected macroalgal species (e.g. Kautsky et al. 1986, Kautsky et al. 2004, 2007) have been shown to respond to changes in nutrient concentration and water clarity.

Salinity also affects competition among species and thereby the occurrence and depth distribution of the individual species (e.g. Nielsen et al. 1995. Pedersen and Snoeijs 2001, Torn et al. 2006). In areas containing large salinity gradients, relationships between depth limits and nutrient concentration/water clarity should therefore be developed for specific salinity regimes.

The algae also demand hard substratum for attachment, so the metric is only applicable in areas where hard substratum occurs to the maximum water depth where light allows growth. Moreover, the metric should only be applied in relatively deep areas where water depth in itself does not limit depth penetration of the algae.

We have selected the following macroalgal species for Norwegian and Swedish coastal waters of Skagerrak– Kattegat: The red algae *Chondrus crispus*, *Furcellaria lumbricalis, Coccotylus truncatus/ Phyllophora pseudoceranoides*, Delesseria *sanguinea*, *Phycodrys rubens* and *Rhodomela confervoides* and the brown algae: *Halidrys siliquosa* and *Saccharina latissima.* In Danish coastal waters we have focused on the same species, except for *Delesseria sanguinea* and *Phycodrys rubens*.

Metric 2: Cover of the macroalgal community

This metric describes the cover of the macroalgal community along depth gradients. Algal cover typically peaks at intermediate water depths while cover is lower in shallow and deep water. Physical exposure is likely to limit algal cover in shallow water, whereas light limitation is likely to be responsible for the reduction in algal cover from intermediate depths towards deeper water. With the purpose of increasing the sensitivity of the metric to changes in water quality we only included algal cover data from the 'light regulated part' of the depth gradient, and excluded data from shallow water in the analyses of dose-response relationships. We therefore expect the metric to be primarily regulated by nutrient concentration and water transparency though substratum and salinity also may play regulating roles (Carstensen et al.2005). The cover of the algal community can be expressed in two ways:

1) 'Total cover': overall cover of the community representing values up to 100 %, or
2) 'Cumulated algal cover': summed cover of all individual species, representing values up to several 100 % as the algae may grow in several stories.

Note: This metric is still under development and will be intercalibrated in Phase II.

Metric3: Composite macroalgal index

This metric describes the macroalgal composition in rocky littoral and sublittoral zones. The metric includes species richness (or total number of species recorded), proportions of red, green and opportunist, ratio of ephemeral against perennial algae, ratio between Ecological Status Groups I and II (ESG ratio), lower depth extension of a few selected species and their abundance as well as the physical properties of the location. It is based on many elements of the English metric but includes also sublittoral communities, which are more sensitive to impacts than littoral communities and abundance of a few key species. The littoral zone is usually inhabited by species with a rather high resilience toward all kinds of impact. It has been shown that along the Norwegian coast there has been a change in sublittoral communities within the last 5 years, which has yet to be detected in the littoral zone (Moy & Christie, in press). This emphasizes the importance of including a sublittoral element in the metrics.

The metric is based on a combination of the United Kingdom metric for macroalgae (in NEA1 and NEA26) and the Scandinavian depth limit metric (in NEA8, 9, and 10) and some aspects of the Danish percentage algal cover. This index is still under development.

Note: As still under development intercalibration will be finalized in Phase II.

Boundary Setting

Metrics 1: 'Depth limit of macroalgal species'

Regarding the metric 'depth limit of macroalgal species', the dose-response relationships developed so far seem to show no discontinuities/thresholds, which can be used to define e.g. the good/moderate boundary. The only distinct theoretical threshold is at a very deteriorated stage (e.g. poor/bad boundary) when the benthic vegetation has disappeared and no depth limit exists. No distinct boundaries have been identified based on paired metric assessment. So, for this indicator we should follow the above example approach for defining boundaries. However, in general the dose-response relationships on present-time data do not include a range of nutrient concentrations representing reference levels or high/good boundaries. Reference levels for the benthic vegetation indicators therefore have to be set using historical data, hind-cast modeling and/or expert judgement as discussed in Step 1. As reference levels are likely to vary between areas, we suggest that the levels are defined for each area and that class boundary limits are defined as specified deviations from reference levels.

Depth limits of macroalgal species

Swedish historical data on depth distribution of individual macroalgal species are available for only one waterbody i.e. NEA 9. This data set is from the inner Gullmar Fjorden, in 1941, at the Swedish Skagerrak coast (Eriksson et al. 2002 and references therein). However, Norwegian historical data for lower depth limits were available for the two other water types e.g. NEA 8 and 10. The Swedish dataset is limited both in aerial cover and time but can be used to estimate the maximum historical depth distribution of some commonly occurring, easily recognizable perennial macroalgal species and thereby to define approximate reference depth limits for the type area represented by the data. The historical Norwegian datasets are, however, mainly based on dredging and caution must be exercised. Hence, the historical data were used in combination with modeling (based on Secchi depths), present depth occurrence of the selected species in areas with low nutrient enrichment, and expert judgement to estimate reference depth limits for other water body types. The result is presented in the table 2.2.12, 2.2.13 and 2.2.14. The High/Good boundary for depth limits of nine selected macroalgal species in each salinity regime is reported within the Swedish coastal water types. Information can be found at www.naturvardsverket.se/sv/Lagar-och-andra-styrmedel/Lag-och-ratt/Foreskrifter-och-allmanna-rad/Foreskrifter-utgivningsordning/.

In Table 12 depth limits in NEA9 areas are represented by the only area for which historical data is available, i.e. Gullmar Fjorden, one profile in 1941 and two recent Norwegian surveys. The high-good boundary (= reference condition) for depth limits of nine selected macroalgal species has been defined as a 17-33 % deviation from the estimated reference levels. All depth limits are set by expert judgement for each of the nine selected macroalgal species since the few available data do not allow any good statistical treatment. Similarly, good-moderate boundaries have been set by expert judgement and represent a 42 to 50 % deviation from reference levels.

In table 2.2.13 the lower depth limits have been estimated based on Sundenes historical data sets (Sundene 1953) and several recent data sets. As for NEA9 both the reference values and the boundaries are based on expert judgements. The historical values from Sundene (1953) are based on dredging and are therefore not as reliable as recent surveys carried out by use of diving. Hence, the new estimated reference values are estimated somewhere between Sundenes findings and recent observations. For some species the recent findings of lower depth limits exceeds those found by Sundene. In these cases the recent finding has been used in estimating a new reference value. The High/Good boundaries vary between 18 and 37 % of the estimated reference value and the Good/Moderate boundaries vary between 44 and 58 % of the estimated reference values (Table 2.2.13).

Table 2.2.12: Estimated depth limits for NEA 9 – Skagerrak fjords – in Norwegian and Swedish waters. Historical data by M. Waern 1941 in Eriksson et al. 2002, recent data from Walday et al. 2001ref missing is from Grenlandsfjord 1998-1999, Magnusson et al. 1992 is from Oslofjord 1990.

NEA 9 'Skagerrak fjords	Walday et al. 2001	Magnusson et al. 1992	Eriksson et al 2002	NEW ref.	High / Good	Good / Moderate
Survey nr:	1	2	3			
Species						
CHOCR		5	12.5	12	>10	7
FURLU		3	15	15	>12	8
HALSI			12.5	12	>10	7
LAMSA	7	12	10	12	>8	6
PHYP+COCTR	17	13	11	14	>10	8
RHOCO	12	10	15	15	>12	8
DELSA	17	11	16.6	17	>13	9
PHYRU	14	9	16	16	>13	8

Table 2.2.13. Estimated depth limits for NEA8- Skagerrak inner archipelago - in Norwegian and Swedish waters. (Walday et al. 2001 is from Grenlandsfjord 1998-1999, Magunsson et al. 1992 is from Oslofjord 1990, Pedersen et al., is from Chysochromulina surveys in 1988 and 1989, Moy et al. 2005 is from the Norwegian Coastal Monitoring Program from 1990-2005, Karlsson 1994 -1998 is from the Swedish coastal monitoring program from 1994-1998 and Sundene 1953 is from surveys in outer Oslofjord in 1947 to 1952. New reference is based on expert judgement and all these reports mentioned. * uncertainty of viability as dredging was used in collection the seaweeds.)

Within NEA10 there are several extensive monitoring programs both in Norway and in Sweden that have made it possible to determine a lower depth limits for the same species as in NEA8 and 9. Fredriksen and Rueness (1990) compared the data sets collected at the same stations examined by Sundene in 1947-1952, and used both dredging and diving. The conclusions were that the lower depth limits have been reduced since Sundenes explorations. Both these reports have been compared with recent registrations and new reference levels have been estimated for all species. Within the Coastal Monitoring Program (1990-) some scattered individuals are found even deeper than the new reference values, however, these lower depth limits represent individuals that may not survive the over wintering, hence not a reference level for healthy and reproductive species. The High/Good boundaries vary between 24 and 29 % of estimated reference level and the boundary level of Good/ Moderate between 40 and 50 % of reference levels (Table 2.2.14)

Table 2.2.14: Estimated depth limits for NEA10- Skagerrak exposed - in Norwegian and Swedish waters. (Moy et al. 2005 is from the Norwegian Coastal Monitoring Program from 1990-2005, Fredriksen & Rueness 1990 is from surveys on Sundenes (1953) location in 1989. Karlsson 1994 is from the Swedish coastal monitoring program from 1994-1998 and Sundene 1953 is from surveys in outer Oslofjord in 1947 to 1952. New reference is based on expert judgement and all these reports mentioned. * uncertainty of viability as dredging was used in collection the seaweeds).

NEA10 exposed	Skagerrak	Moy et al. 2005	Fredriksen & Rueness, 1990	Sundene 1953 *	Karlsson 1994-98	NEW ref.	High / Good	Good / Moderate
	Survey nr:	9	4	8	7			
Species								
CHOCR		18		18	16	18	>13	9
FURLU		13	14	20	14	16	>12	9
HALSI		14		8	10	14	>10	8
LAMSA		20	13	16	14	16	>12	9
PHYP+COCTR		30	18	35	16	30	>22	18
RHOCO		24		12	16	16	>12	9
DELSA		30	15	32		30	>22	18
PHYRU		29	27	27		29	>22	17

2.2.4 Results of the comparison

As option 2 has been used the boundaries have been agreed by experts representing all countries in the GIG macroalgae sub-group. Therefore the final agreed results presented below are the results of comparing expert views on what the boundaries should be.

Separate MS data were used (option 3) from Portuguese and Spanish rocky shores representing pressure gradients. Portuguese shores were surveyed on the north coast of Portugal, sites under pressure from the Douro river, and surveyed after Mieres meeting. Data from Spain were collected also from rocky shores under different gradient pressures at Liñera, Usgo and Ontón (for details see earlier reports). To set boundaries, Portugal and Spain used national data from their own pressure sites, and afterwards both assessed data from the other MS to evaluate the output correspondence of different assessment methods. Although not very extent, data from both MSs were used to compare assessment methods and to best fit boundaries to improve EQS classifications.

The Portuguese Marine Macroalgae Assessment Tool (P-MarMAT) operates over a scale range from 0 (impacted) to 1 (non-impacted). Initially, class boundaries were set as equidistant points along the scale (0.2, 0.4, 0.6 and 0.8).

The CFR index operates over a scale range from 0 (impacted) to 100 (non-impacted), but can be easily converted to a 0-1 scale by calculating the CFR/100 value. Class boundaries are set as suggested in REFCOND (2003) 0.2-0.41-0.62-0.83.

These boundaries were then modified to ensure a commitment between the status characteristics as defined in the Normative Definitions and agreeing as much as possible to other MSs thresholds. The process of setting and testing the ecological status boundaries was already described in earlier reports. Portugal and Spain assessed their own data, data from the other MS, and all together from the common dataset, in order to produce a classification result comparable between each other and compliant with the expected to study sites. Figure 2.2.1 illustrates the correlations existent between the compared methods, derived from the process of setting and testing the ecological status boundaries.

Portugal and Spain assessed data from both MSs in order to produce a classification result comparable between each other. From this exercise, a weighted Kappa value of 0.84 was reached, meaning a "very good" correlation between results (Monserud & Leemans, 1992). Table 2.2.15 shows the assessment results from this exercise.

After boundaries optimization, methods showed "excellent" agreement between each other, with a weighted kappa value of 0.89. Table 2.2.16 shows the assessment results from this second part of the IC exercise.

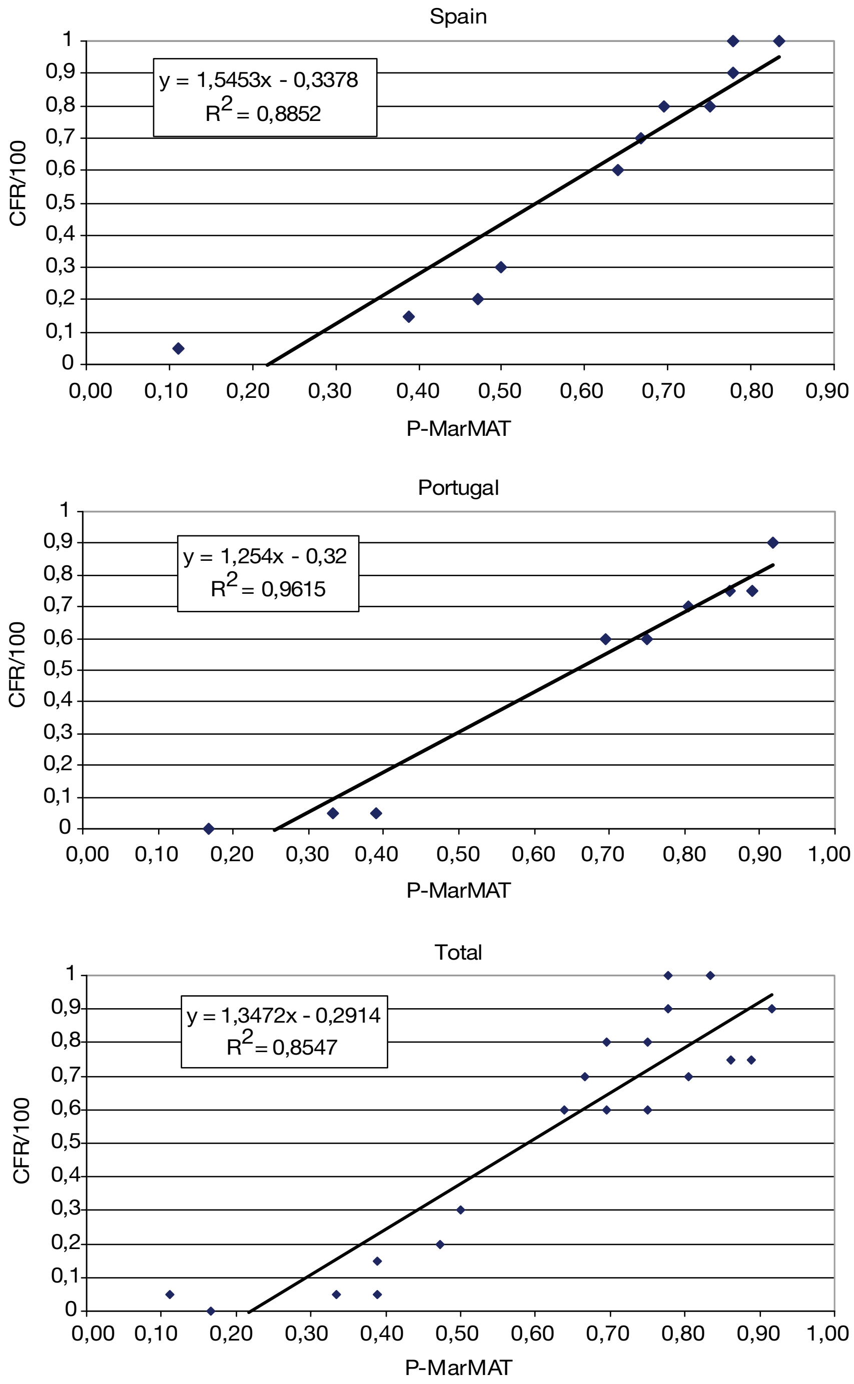


Figure 2.2.1: Correlations between EQR resulted from SP and PT methods.

Table 2.2.15: P-MarMAT and CFR assessment results from data collected in intertidal rocky shores in Portugal and Spain.

	P-MarMAT	CFR B
Usgo 1	0,39	0,15
Usgo 2	0,47	0,2
Usgo 3	0,83	1
Liñera 1	0,64	0,6
Liñera 2	0,67	0,7
Liñera 3	0,75	0,8
Ontón 1.1.	0,11	0,05
Ontón 1.2.	0,50	0,3
Ontón 1.3.	0,78	0,9
Ontón 2	0,69	0,8
Ontón 3	0,78	1
Cabedelo1	0,39	0,05
Cabedelo2	0,17	0
Cabedelo3	0,33	0,05
Salgueiros1	0,81	0,7
Salgueiros2	0,69	0,6
Salgueiros3	0,75	0,6
Aguda1	0,92	0,9
Aguda2	0,86	0,75
Aguda3	0,89	0,75

Table 2.2.16: P-MarMAT and CFR assessment results from data collected in intertidal rocky shores in Portugal and Spain, after boundaries optimization.

	P-MarMAT	CFR
Usgo 1	0,39	0,15
Usgo 2	0,47	0,2
Usgo 3	0,83	1
Liñera 1	0,64	0,6
Liñera 2	0,67	0,7
Liñera 3	0,75	0,8
Ontón 1.1.	0,11	0,05
Ontón 1.2.	0,50	0,3
Ontón 1.3.	0,78	0,9
Ontón 2	0,69	0,8
Ontón 3	0,78	1
Cabedelo1	0,39	0,05
Cabedelo2	0,17	0
Cabedelo3	0,33	0,05
Salgueiros1	0,81	0,7
Salgueiros2	0,69	0,6
Salgueiros3	0,75	0,6
Aguda1	0,92	0,9
Aguda2	0,86	0,75
Aguda3	0,89	0,75

Although the small number of data available and the possibility of further developments in near future, after running the IC exercise and the procedure of setting boundaries the best threshold values for Portugal and Spain are shown in the following table:

From this exercise, it was possible to improve agreement between SP and PT classification methods, resulting a value of 0.89 ("excellent") from Kappa analysis.

Although future improvements and updates surely arise, the class boundaries achieved at the moment to Portugal and Spain are shown in the following table, 2.2.17:

Table 2.2.17: Class boundaries.

	P-MarMAT	**CFR**
H/G	0,816	0,808
G/M	0,638	0,568
M/P	0,460	0,329
P/B	0,282	0,089

2.2.5 Results of the harmonisation – Boundary thresholds and EQR values

Metric - Rocky Shore Macroalgae

CW - NEA1/26a,b,e

UK, Republic of Ireland and Norway

At this time, June 2007, the scoring system for the common indices below have been agreed by Ireland, Norway, and the UK. These common indices for this intercalibration metric describe species richness and composition based on a reduced species list devised specifically for the boundary setting protocol and classification tool development process. There are slightly different between regions due to the varying levels of diversity and composition as shown in table 2.2.18 below.

Table 2.2.18: EQRs.

EQR Quality Class		0.8 - 1.0 High	0.6 - 0.8 Good	0.4 - 0.6 Moderate	0.2 - 0.4 Poor	0 - 0.2 Bad
RSL	Scotland	35-70	25-35	17-25	5-17	0-5
	England/Wales/RoI	35-69	25-35	15-25	5-15	0-5
	NI	34-68	20-34	10-20	3-10	0-3
	Norway	33-68	20-33	10-20	4-10	0-4
Greens	Scotland	0-12	12-20	20-30	30-80	80-100
	England/Wales/RoI	0-15	15-20	20-25	25-80	80-100
	NI	0-20	20-30	30-45	45-80	80-100
	Norway	0-20	20-30	30-45	45-80	80-100
Reds	Scotland	55-100	45-55	35-45	15-35	0-15
	England/Wales/RoI	55-100	45-55	40-45	15-40	0-15
	NI	45-100	35-45	25-35	10-25	0-10
	Norway	40-100	30-40	22-30	10-22	0-10
ESG	Scotland	1.0-1.2	0.8-1.0	0.7-0.8	0.2-0.7	0-0.2
	England/Wales/RoI	1.0-1.2	0.8-1.0	0.55-0.8	0.2-0.55	0-0.2
	NI	0.8-1.2	0.6-0.8	0.4-0.6	0.2-0.4	0-0.2
	Norway	0.8-1.2	0.6-0.8	0.4-0.6	0.2-0.4	0-0.2
Opportunist	Scotland	0-10	10-15	15-25	25-50	50-100
	England/Wales/RoI	0-10	10-15	15-25	25-50	50-100
	NI	0-15	15-25	25-35	35-50	50-100
	Norway	0-15	15-25	25-35	35-50	50-100

A 'de-shoring factor' has been incorporated to adjust the level of species richness according to the overall description of the shore. This uses an exponential-type model of the form:

$RICHNESS = a + b \exp(cSHORE)$

where a, b and c are parameters to be estimated from the data. Using least squares, these parameters were estimated to be:

$a = 14.210$
$b = 4.925$
$c = 0.108$

The final metric system works on a sliding scale to enable an accurate EQR value to be calculated for each of the different parameters, an average of these values is then used to establish the final classification status.

Where a shore description is not available the uncorrected level of species richness is to be put into the final metric, although the level of confidence in the overall result may be reduced slightly.

EQRs

The above scoring system for the indices for this metric gives EQR boundaries of:

H/G 0.80
G/M 0.60
M/P 0.40
P/B 0.20

The average EQR is used to classify this metric.
The overall scoring and EQR's show above are currently under review.

Spain have developed preliminary boundary classifications for their CFR metric, which are still to be fully tested before they are accepted. Details of which can be found in the detailed Milestone 6 Report.

Spain and Portugal:

As explained before, the class boundaries achieved at the moment to Portugal and Spain are shown in the following table, 2.2.19:

Table 2.2.19: ES/PT EQRs.

	P-MarMAT	CFR
H/G	0,816	0,808
G/M	0,638	0,568
M/P	0,460	0,329
P/B	0,282	0,089

Metric - Opportunistic Macroalgae (NEA1/26a,b,c,e/11)

The Opportunistic Macroalgal bloom tool shown below has been agreed if only by the UK and Ireland, table 2.2.20 below. Germany is also considering using this tool, full information on German macroalgae intercalibration can be found in: German Macroalgae & Angiosperm Annex-IC-Report Macroalgae and Angiosperms NEA GIG - Germany_05-2007.doc (Kolbe, 2007). Portugal is only considering opportunistic macroalgae in transitional waters.

Other countries are considering if this tool could be used in their waters. Denmark will intercalibrate at a later date, post June 2007.

The tool is a combination of percent cover by opportunistic green algae, the density of growth with a defined area and the presence of entrained algae.

The final metric system works on a sliding scale to enable an accurate EQR value to be calculated for each of the different parameters, an average of these values is then used to establish the final classification status.

Table 2.2.20: EQRs.

Quality Status	High	Good	Moderate	Poor	Bad
EQR	0.8 - 1.0	0.6 - 0.8	0.4 - 0.6	0.2 - 0.4	0.0 - 0.2
% Cover of AIH	0 - 5	5 - 15	15 - 25	25 - 75	75 - 100
Area (ha)	0 - 100	100 - 500	500 - 1000	1000 - 2500	>2500 (- 6000)
Biomass (g m^2) of AIH	0 - 100	100 - 500	500 - 1000	1000 - 3000	>3000 (- 6000)
Biomass (g m^2) of affected area	0 - 100	100 - 500	500 - 1000	1000 - 3000	>3000 (- 6000)
Presence of entrained algae (% of Quadrats)	0 - 5	5 - 20	20 - 50	50 - 75	75 - 100

The above scoring system for the indices for this metric gives EQR boundaries of:

H/G 0.80
G/M 0.60
M/P 0.40
P/B 0.20

Portugal has a long time series of data for one particular estuary and tested the metrics of total area, percentage and biomass in different combinations. The results are very comparable and comply with the expert judgement of Portuguese scientists

Currently Germany only has data for the areal cover of opportunistic macroalgal growth on soft sediments in the intertidal. A recent report has tried to compare this metric to part of the UK/RoI tool (see below). (Classification tools for biomass and for taxonomic composition might be designed after further investigations in the field).
A recent German report considered that "UK intercalibration tool on the whole cannot be applied to the German dataset. Moreover model calculations already show that the class boundaries of the UK proposal do not reflect the heavy disturbances in algal growth that have been reported from the German water bodies in the 1990s (ADOLPH 2007)." Therefore, Germany are still in the process of intercalibrating their data, the progress of which is detailed below.

Currently Germany have described their references conditions as:
Opportunistic macroalgae blooms of anthropogenic origin should be absent or if present should cover less than 1 % of the total intertidal of the water body.

Coverages with densities ≥1 % rarely ever exceed 15 % of the intertidal; normalised to 100 % density they stay below 5 % in most of the cases. It is very likely, that differences in morphological and hydrodynamic conditions between the British and the German water bodies are the underlying reason for these mismatches.

The class boundaries proposed by Germany are based on the 6 year median of the annual maximum, defined as the acreage of algal cover (monitored in km^2; area with green algal density ≥1 %) as a percent of the total intertidal in a water body at the time of maximal extent. This was monitored along the entire coast in the respective year. Currently the class boundaries are calculated separately for each waterbody (based on historical reference values and recent information from problem areas). The EQRs that match the percentage cover data are calculated by means of linear interpolation within the respective equidistant EQR class ranges (KOLBE 2007). Germany's EQRs were not downgraded if total acreage exceeded a certain total patch size. See below table 2.2.21.

Table 2.2.21: DE EQRs.

Metric	High	Good	Moderate	Poor	Bad
Percentage intertidal area overgrown with opportunistic macroalgae (green algae) (density ≥1 %)	0 - 1 % EQR 1 – 0.8	1.1 - 1.5 % EQR 0.799 – 0.6	1.6 - 5 % EQR 0.599 – 0.4	5.1 -20 % EQR 0.399 – 0.2	**> 20 %** **EQR** **0.199 – 0**

In an attempt to compare with parts of the UK/RoI method, a "normalization" process to 100 % density, and a downgrading option have been applied to German data. After normalization these data were classified using the German class boundaries. The resulting EQRs were downgraded according to the UK-method if the total acreage exceeded a certain total patch size.

The results of this analysis were compared with the results of the German method. It was shown that the use of the UK density basis but DE-class boundaries can, if downgraded, may lead to the same outcome as the DE-tool. This only holds true for 6 (NEA 26 and 4) of the 13 German water bodies tested (46 %).

For 7 (NEA 1, 3, 11) of the 13 water bodies (54 %) the classification lead to a status rank which is still one or two classes higher than the De-tool and does not reflect the eutrophication status in those water bodies (KOLBE 2007).

Future information and work will assist in completing the intercalibration. The intercalibration therefore has been completed only for the sub-types 1/26a, b, e.

Metric - Subtidal Algae (NEA8/9/10)

Metric 1: 'Depth limit of macroalgal species'

After the intercalibration procedure where new values for references conditions, and the depth limit between High/Good and Good/Moderate ecological status for the selected number of macroalgal species was agreed upon the following EQR –calculations for area NEA 8, 9 and 10 and the following scoring system was used.

For the depth limit of the selected set of easily determined species the following expert EQR-scale was decided on the areas intercalibrated as well as nationally:

1.0 - 0.81 = High status
0.80 - 0.61 = Good status
0.60 - 0.41 = Moderate status

To calculate and intercalibrate the ecological status, i.e. the EQR values in NEA 8, 9 and 10 we have agreed to use the intercalibrated depth limits given in tables 12, 13 and 14 respectively.

An EQR-value is calculated by transforming the depth value (in meter) to a classification value (score) for each of the included species, (i.e. 5, 4 or 3. where 5 corresponds to reference/high status , 4 to a depth distribution above Good status and 3 to a depth distribution in the studied transect above Moderate status. After giving each of the occurring species the appropriate score the mean value is calculated and divided by 5, i.e. the reference condition. For example, if 3 species score 5, 2 species score 4 and 2 species score 3; the total is 32 giving an average score of 4; which when divided by 5 gives an EQR of 0.8; thus equating to Good Status.

Table 2.2.2: Depth limits (m) for a set of selected macroalgal species used in the intercalibration and Calculation of EQR for NEA 8, 9 and 10.

NEA 8	**Species**	**Reference value**	**Score 5 if >than**	**Score 4 if >than**	**Score 3 if >than**
	Furcellaria lumbricalis	16	10	7	4
	Phyllophora pseudoceranoides	22	18	12	6
	Rhodomela confervoides	16	12	7	4
	Chondrus crispus	12	8	5	3
	Delesseria sanguinea	25	18	12	6
	Halidrys siliquosa	10	8	5	3
	Saccharina latissima	16	10	7	4
	Phycodrys rubens	22	15	10	5
NEA 9					
	Furcellaria lumbricalis	15	12	8	4
	Phyllophora pseudoceranoides	14	10	8	4
	Rhodomela confervoides	15	12	8	4
	Chondrus crispus	12	10	7	4
	Delesseria sanguinea	17	13	9	5
	Halidrys siliquosa	12	10	7	4
	Saccharina latissima	12	8	6	3
	Phycodrys rubens	16	13	8	4
NEA 10					
	Furcellaria lumbricalis	16	12	9	5
	Phyllophora pseudoceranoides	30	22	18	9
	Rhodomela confervoides	16	12	9	5
	Chondrus crispus	18	13	9	5
	Delesseria sanguinea	30	22	18	9
	Halidrys siliquosa	14	10	8	4
	Saccharina latissima	16	12	9	5
	Phycodrys rubens	29	22	17	9

An EQR-value is calculated by transforming the depth value (in meter) to a classification value (score) for each of the included species, (i.e. 5, 4 or 3. where 5 corresponds to reference/high status, 4 to a depth distribution above Good status and 3 to a depth distribution in the studied transect above Moderate status. After giving each of the occurring species the appropriate score the mean value is calculated and divided by 5, i.e. the reference condition. For example, if 3 species score 5, 2 species score 4 and 2 species score 3; the total is 32 giving an average score of 4; which when divided by 5 gives an EQR of 0.8; thus equating to Good Status.

2.2.6 Open issues and need for further work

Gaps for the future:

- Subtidal macroalgae tools are to be developed
- Spain will attempt to separate macroalgae and seagrass from their macrophyte tool, for intercalibration purposes
- Questions of how to combine different sub-metrics into a higher level overall EQR.
- Development of macroalgae tool for transitional waters (or use of the existing Spanish tool) and intercalibration
- Completion of the intercalibration process for subtype 1/26c.

3 Discussion

3.1 Comparability between GIGs

Metrics

Intercalibration for the macroalgae quality element has been successfully performed by all of the four Coastal Waters GIGs, using indices (MEDGIG) or a selection of unaggregated metrics (NEAGIG).

Types

Almost all types for each GIG have been covered.

Option

Option 2 (MS agreed on the common metrics, created data sets relating MSs assessment methods to the common metrics, agreed on High/Good and Good/Moderate class boundaries and established relationships between common and national metrics) approach has been adopted for the intercalibration.

Boundary Setting Procedure

All Coastal Waters GIGs are providing results for the H/G and G/M boundaries (at least), applying similar approaches based on the expert supervised selection of appropriate percentiles as boundaries best fitting with evident changes in ecological status (90^{th} percentile is the favorite choice).

Reference Condition

There’s no shared view among GIGs (but also among MSs) on how to fix Reference Conditions: in some cases real reference sites have been identified and selected as a reference, in some others the maximum possible values for the selected indices/metrics have been used.

3.2 Open issues and need for further work

A shared task for the next intercalibration round is to refine typology (or include types non considered at this stage) in order to maximize the representativeness towards different waterbodies and ecological status of the Quality Element.

4 References

Adolph, W., 2007. Classification of opportunistic Macroalgae blooming in Germany compared to the UK-Intercalibration proposal. Report for Intercalibration, Mieres, 2007.

Andersen JH, Conley DJ, Hedal S. 2004. Paleoecology, reference conditions and classification of ecological status: the EU Water Framework Directive in practice. Marine Pollution Bulletin 49: 283-290.

Arévalo R., Pinedo S., Ballesteros E. 2007. Changes in the composition and structure of Mediterranean rocky-shore communities following a gradient of nutrient enrichment: descriptive study and test of proposed methods to assess water quality regarding macroalgae. Marine Pollution Bulletin 55: 104-113.

Ballesteros E. 1988. Estructura y dinámica de la comunidad de *Cystoseira mediterranea* Sauvageau en el Mediterráneo noroccidental.Investigación Pesquera 52: 313-334.

Ballesteros E. 1992. Els vegetals i la zonació litoral: espècies, comunitats i factors que influeixen en la seva distribució. Arxius Secció Ciències I.E.C. 101, 1-616.

Ballesteros E., Pérez M., Zabala M. 1984. Aproximación al conocimiento de las comunidades algales de la zona infralitoral superior en la costa catalana. Collect. Bot. 15: 69-100.

Bellan G., Bellan-Santini D. 1972. Influence de la pollution sur les peuplements marins de la région de Marseille. In: Ruivo M. (Ed.) Marine Pollution and Sea Life, Fishing News Ltd. Survey, 396-401.

Bellan-Santini D. 1965. Étude quantitative du peuplement à *Mytilus galloprovincialis* Lamarck en eau moyennement polluée. Rapports Commission International de la Mer Méditerranée 18: 85-89.

Bellan-Santini D. 1966. Influence des eaux polluées sur la faune et la flora marines benthiques dans la région Marsellaise. Techniques et Sciences Municipales 61: 285-292

Bellan-Santini D. 1968. Influence de la pollution sur les peuplements benthiques. Rev. Intern. Oceanogr. Med. 10: 27-53.

Belsher T. 1977. Analyse des répercussions de pollutions urbaines sur le macrophytobenthos de Méditerranée (Marseille, Port-Vendres, Port-Cros). Thèse Doctorat. Université d'Aix-Marseille II, Marseille, pp. 287.

Boudouresque C.F. 1969. Étude qualitative et quantitative d'un peuplement algal à *Cystoseira mediterranea* dans la région de Banyuls sur Mer. Vie Milieu 20, 437-452.

Boudouresque C.F. 1971. Contribution à l'étude phytosociologique des peuplements algaux des côtes varoises. Vegetatio 22: 83-184.

Carstensen J, Krause-Jensen D, Dahl, K, Middelboe AL. 2005. Development of macroalgal indicators of water quality *In* Dahl, K., Andersen, J., Krause-Jensen, D., Josefson, A., Kjerulf-Petersen, J., Hansen, O.S., Middelboe A.L. (Eds) Scientific and technical background for intercalibration of Danish coastal waters. National Environmental Research Institute, Denmark xx pp. – NERI Technical report no. 563. http://technical-reports.dmu.dk

Clarke A, Juggins S, Conley DJ. 2003. A 150-year reconstruction of the history of coastal eutrophication in Roskilde Fjord, Denmark. Marine Pollution Bulletin 46: 1615-1629.

Coppejans, E., 1980. Phytosociological studies on mediterranean algal vegetation: Rocky surfaces of the photophilic infralittoral zone. In:Price, J.H., Irvine, D.E.G., Franham, W.E. (Eds.), The shore environment, Ecosystems, Vol. 2. Academic, London, pp. 371-393.

Cottalorda JM., Meinesz A., Thibaut T., Chiaverini D. 2004. Représentation cartographique de l'abondance de quelques algues et invertébrés sur le littoral des îlots du Rascas et de la Gabinière (Parc National de Port Cros, Var, France). Sci. Rep. Port-Cros Natl. Park, Fr. 20: 195-209.

Edwards, P., 1975. *An assessment of possible pollution effects over a century on the benthic marine algae of Co. Durham, England.* Bot. J. Linn. Soc. 70, 269-305.

Eriksson KB, Johansson G, Snoeijs P. 2002. Long–term changes in the macroalgal vegetation of the inner Gullmar fjord, Swedish Skagerrak coast. Journal of Phycol. 38: 284-296.

Feldmann J. 1937. Recherches sur la ve´ge´tation marine de la Me´diterrane´e. La coˆ te des Albères. Revue Algologique 10: 73-254

Giaccone G. 1993. The vertical zonation along the phytal system in the Mediterranean sea and the effects of municipal and industrial wastewater disposal on phytobenthos communities. Fifth Optima Meeting, Istanbul 5: 47-56.

Gibert A. 1918. Flora algològica marina de les aigües i costes occidentals de Catalunya. Publicacions de l'Agrupacio´ Excursionista, pp. 65.

Golubic S. 1970. Effect of organic pollution on benthic communities. Marine Pollution Bulletin 1: 56-57.

Gros C. 1978. Le genre *Cystoseira* sur la côte des Albères. Répartition – Écologie–Morphogenèse. Thése Doctorat. Université P. et M. Curie, Paris VI, pp. 115.

Guinda, X., Juanes, J.A., Puente, A., Revilla, J.A., 2008. Comparison of two methods for quality assessment of macroalgae assemblages, along different pollution gradients. Ecological Indicators 8 (5), 743-753.

Hoffmann L., Clarisse S., Detienne X., Goffart A., Renard R., Demoulin V. 1988. Evolution of population of *Cystoseira balearica* (Phaeophyceae) and epiphytic Bangiophyceae in the Bay of Calvi (Corsica) in the last eight years. Bulletin de la Socie´te´ Royale de Liège 4-5: 263-273.

Jaklin, S., Petersen, B., Adolph, W., Petri, G. & W. Heiber (2006). Aufbau einer Matrix für die Gewässertypen nach EG-WRRL im Küstengebiet der Nordsee, Schwerpunkt Flussgebietseinheiten Weser und Elbe. Abschlussbericht Teil 1: Nährstoffe, Fische, Phytoplankton, Makroalgen und Seegras. Berichte des NLWKN 2006.

Juanes, J.A., Guinda, X., Puente, A., Revilla, J.A., 2008. Macroalgae, a suitable indicator of the ecological status of coastal rocky communities in the NE Atlantic. Ecological Indicators 8 (4), 351-359.).

Kautsky L, Andersson C, Dahlgren S. 2004. Framtagning av nya bedömningsgrunder för kust och hav enligt ramdirektivets krav. Förslag till Ekologisk Kvalitetskvot (EQR) för den biologiska parametern – makrovegteation. Report to the Swedish EPA. In Swedish. 23pp.

Kautsky N, Kautsky H, Kautsky U, Waern M. 1986. Decreased depth penetration of *Fucus vesiculosus* L. since the 1940s indicate eutrophication of the Baltic Sea. Mar. Ecol. Prog. Ser. 28: 1-8.

Kautsky, L, Wibjörn, C, Kautsky, H. 2007. Bedömningsgrunder för kust och hav enligt krav i ramdirektivet vatten – makroalger och några gömfröiga växter. Report to the Swedish EPA, in Swedish, 50 pp.

Kolbe, K. (2006). Bewertungssystem nach WRRL für Makroalgen und Seegräser der Küsten- und Übergangsgewässer der FGE Weser und Küstengewässer der FGE Elbe. Studie im Auftrag des Niedersächsischen Landesbetriebs für Wasser-, Küsten- und Naturschutz, Oldenburg/Brake, 99 S.

Kolbe, K., 2007. Assessment of German Coastal Waters (NEA 1/26, NEA 3/4) and Transitional Waters (NEA 11) by Macroalgae and Angiosperms. Intercalibration Report (NEA GIG).- (postal to D. Coates, D. Jowett).

Lipej L., Mozetič P., Orlando-Bonaca M., Mavrič B., Šiško M., Bettoso N. 2006. Evaluation of the Ecological Status of Coastal Waters in accordance with the European Water Framework Directive (Water Framework Directive, 2000/60/EC). Final national report in Slovenian, Marine Biology Station Piran, National Institute of Biology, October 2006, 180 pp.

Littler M.M., Littler D.S. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. American Naturalist 116: 25-44.

Littler M.M., Murray S.N. 1975. Impact of sewage on the distribution, abundance and community structure of rocky intertidal macroorganisms. Marine Biology 30: 277-291

MacArthur R.H, Wilson E.O. 1967. The theory of island biogeography. Princeton University Press, Princeton.

Magnusson J, Johnsen T.M, Beyer F, Gjøsæter J, Lømsland E.R, Sollie, Aa, 1999. or 1992 som i tabellen vilket år skall det vara?? Pollution monitoring in the Inner Oslofjord in 1998. NIVA-report. OR-4058. 63pp. In Norwegian (http://www.niva.no/)

Mangialajo, L., Ruggieri, N., Asnaghi, V., Chiantore, M., Povero, P., Cattaneo-Vietti, R., 2007. Ecological status in the Ligurian Sea: The effect of coastline urbanisation and the importance of proper reference sites. Marine Pollution Bulletin 55: 30-41.

Meinesz A., Javel F., Cottalorda J.M., Susini M.L., Capiomont A., Levi F., Robert A. 2004. Cartographie des espèces médiolittorales et infralittorales supérieures des falaises de Bonifacio (Corse du Sud) – Mission 2003

– Rapport intermédiaire. Contrat Office de l'Environnement de la Corse & Laboratoire Environnement Marin Littoral. Laboratoire Environnement Marin Littoral publ., Nice: 1-14.

Meinsez A., Javel F., Chiavérini D., Cottalorda J.M., Giletta L. 2004. Représentation cartographique de l'abondance de quelques algues et invertébrés du littoral de la facez ouest du Parc National de Port-Cros. Laboratoire Environnement Marin Littoral publ., Nice: 1-26.

Monserud, R., Leemans, R., 1992. Comparing global vegetation maps with the Kappa statistic. Ecological modelling 62, 275-293.

Murray S.N., Littler M.M. 1978. Patterns of algal succession in a perturbated marine intertidal community. Journal of Phycology 14: 506-512.

NFS: Information can be found at www.naturvardsverket.se/sv/Lagar-och-andra-styrmedel/Lag-och-ratt/Foreskrifter-och-allmanna-rad/Foreskrifter-utgivningsordning/.

Nielsen R, Kristiansen A, Mathiesen L, Mathiesen H. 1995. Distributional index of the benthic macroalgae of the Baltic Sea area. Acta Bot Fenn 155: 1-51.

Nielsen SL, Sand-Jensen K, Borum J, Geertz-Hansen O. 2002b. Depth colonization of eelgrass (*Zostera marina*) and macroalgae as determined by water transparency in Danish coastal waters. Estuaries 25: 1025-1032.

Nielsen SL, Sand-Jensen K, Borum J, Geertz-Hansen,O. 2002a. Phytoplankton, nutrients and transparency in Danish coastal waters. Estuaries 25: 930-937.

NIENBURG, W. (1927): Zur Ökologie der Flora des Wattenmeeres I. Teil. Der Königshafen bei List auf Sylt. - Wiss. Meeresunters. (Abteilung Kiel) Neue Folge, 20(2): 145-196.

Opportunistic Green Macro algae References (Types NEA 1/26a,b,c, NEA11, NEA3/4).

Orfanidis S. 2007. Comments on the development of new macroalgal indices to assess water quality within the Mediterranean Sea. Marine Pollution Bulletin (in press).

Orfanidis S., Panayotidis P. 2005. Implementation of Water Framework Directive (WFD) for coastal waters by using the Ecological Evaluation Index-EEI: the case of Kavala's and Maliakos Gulfs, Greece. Proceedings 12th Panhellenic Congress Ichthyologists, Drama, Greece, pp. 237-240.

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

Orfanidis S., Panayotidis P., Stamatis N. 2003. An insight to the ecological evaluation index (EEI). Ecological Indicators 3: 27-33.

Orfanidis S., Stamatis N., Tsiagga E. 2005. Ecological status assessment of Delta Nestos Lagoons by using biological and chemical indicators in agreement to Water Framework Directive (WFD 2000/60). Proceedings 12th Panhellenic Congress of Ichtyologists: 245-248 (In Greek with English summary).

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

Orlando-Bonaca M., Lipej L., Orfanidis S. 2008. Benthic macrophytes as a tool for delineating, monitoring and assessing ecological status: the case of Slovenian coastal waters. Marine Pollution Bulletin 56 (4): 666-676.

Panayotidis P., Montesanto B., Orfanidis S. 2004. Use of low-budget monitoring of macroalgae to implement the European Water Framework Directive. Journal of Applied Phycology 16: 49-59.

Panayotidis P., Orfanidis S., Tsiamis K. 2007. *Cystoseira crinita* community in the Aegean Sea. Rapp. Comm. int. Mer Médit., 38 (in press).

Panayotidis P., Siakavara A., Orfanidis S., Haritonidis S. 2001. Identification and description of habitat types at sites of interest for conservation. Study 5: Marine habitats. Final Technical Report, Athens October 2001.

Pedersen M, Snoeijs P. 2001. Patterns of macroalgal diversity, community composition and long-term changes along the Swedish west coast. Hydrobiol. 459: 83-102.

Polo L. 1978. Estudio sobre las algas bentónicas de la costa catalana. Tesis Doctoral, Universidad de Barcelona, pp. 222

REFCOND, 2003. Guidance on establishing reference conditions and ecological status class boundaries for inland surface waters. Working group 2.31 Reference conditions for inland surface (REFCOND), Common Implementation Strategy of the Water Framework Directive, European Commission.

Rodríguez-Prieto C., Polo L. 1996. Effects of sewage pollution in the structure and dynamics of the community of *Cystoseira mediterranea* (Fucales, Phaeophyceae). Scientia Marina 60: 253-263.

Sauvageau C. 1912. Á propos des *Cystoseira* de Banyuls et de Guéthary. Bulletin de la Station Biologique d'Arcachon 14: 133-556.

Scanlan, C.M., Foden, J., Wells, E. and Best, M.A., 2007. The monitoring of opportunistic macroalgal blooms for the water framework directive. *Marine Pollution Bulletin* 55: (1-6): 162-171.

Seoane-Camba J.A. 1975. Algas bentónicas españolas en los herbarios Thuret-Bornet y Sauvageau del Museum National d'Histoire Naturelle de Paris. II. Anales Instituto Botánico Cavanilles 32: 33-51.

Soltan D., Verlaque M., Boudouresque C.F., Francour P., 2001. Changes in macroalgal communities in the vicinity of the Mediterranean sewage outfall after the setting up of a treatment plant. Marine Pollution Bulletin 42: 59-70.

Sundene O, 1953. The algal vegetation of Oslofjord. Mat. – Naturv. Klasse. No. 2 244 pp.

Thibaut T., Hereu B., Susini M. L., Cottalorda J.M. 2005. Inventaire et cartographie des Fucales du Parc National de Port-Cros. Contrat Parc National de Port-Cros et Laboratoire Environnement Marin Littoral. Laboratoire Environnement Marin Littoral publ., Nice: 1-30.

Thibaut T., Pinedo S., Torras X., Ballesteros E. 2005. Long term decline of the populations of Fucales (*Cystoseira*, *Sargassum*) in the Albères coast (northwestern Mediterranean). Marine Pollution Bulletin 50: 1472-1489.

Torn K, Krause-Jensen D, Martin G. 2006. Present and past depth distribution of bladderwrack (*Fucus vesiculosus*) in the Baltic Sea. Aquatic Botany 84: 53-62.

Wells, E. and Wilkinson, M., 2002b. *Intertidal seaweed biodiversity in relation to environmental factors – a case study from Northern Ireland.* Marine Biodiversity in Ireland and Adjacent Waters, Ulster Museum, Belfast.

Wells, E. and Wilkinson, M., 2003. *Intertidal seaweed biodiversity of Orkney.* Coastal Zone Topics. 5, 25-30

Wells, E., 2002a. *Seaweed Species Biodiversity on Rocky Intertidal Seashores in the British Isles*. Ph.D. Thesis, Heriot-Watt University, Edinburgh.

Verlaque, M., Tine, J., 1979. Végétation marine de la rade de Toulon (Var, France): la Rade-abri. Revue de Biologie et Ecologie Méditerrannée 5, 67-86.

Wilkinson, M. and Tittley, I., 1979. *The marine Algae of Elie: A reassessment.* Botanica Marina. 22, 249-256.

Wilkinson, M., Fuller, I.A., Telfer, T.C., Moore, C.G. and Kingston, P.F., 1988. *A Conservation Oriented Survey of the Intertidal Seashore of Northern Ireland.* Institute of Offshore Engineering, Heriot-Watt University, Edinburgh.

Øresundsvandsamarbejdet - Öresundsvattensamarbetet. 2004. Jämförelse mellan 'urtida' och 'nutida' näringsnivåer i Öresund – beräkningar utförda med MIKE3-modell. www.oresundsvand.dk

Section 5 – Angiosperms

1 Introduction

National classification tools for the angiosperms quality element have been intercalibrated in two coastal GIGs. Intercalibration of angiosperm-specific assessment tools was possible to carry out between Denmark and Germany in the Baltic Sea GIG, and between Germany, Ireland, Netherlands, and UK in the North East Atlantic in the North East Atlantic GIG. For these GIGs a sub-set of common types was selected where angiosperms were present. Information about the types and countries with each type in each GIG is described in the sections below.

The intercalibration of the angiosperm quality element for the Mediterranean GIG was not finalised during the first phase and will be continued in the future.

The Option 2 was chosen in this phase of the intercalibration process. Member States participating in the intercalibration have developed metrics as part of their own classification schemes. Selected metrics have been chosen for assessment and agreement of boundaries in this phase of the intercalibration process. At this stage not all the metrics that make up Member States' schemes can be intercalibrated, so it is not possible to produce EQRs for the whole quality element. Therefore boundaries have been agreed for the selected metrics.

2 Methodology and Results

2.1 Baltic GIG

2.1.1 Intercalibration approach

The Baltic Sea Geographical Intercalibration Group (GIG) includes the whole or parts of the coastline of the following countries: Germany, Denmark, Estonia, Finland, Latvia, Lithuania, Poland and Sweden (Table 2.1.1).

The common coastal water types are characterised by the descriptors of the System B typology. The typology factors are based on the common typology framework presented in the guidance on the typology for the coastal and transitional waters[27]. In the Baltic Sea GIG, the common intercalibration types were characterized using basic salinity and exposure with further delineation based on depth and number of ice cover days (Table 2.1.1). One transitional water type (TW B 13) was identified. All countries agreed to focus the intercalibration on the quality elements that are sensitive to eutrophication pressures.

The common intercalibration types were characterized by the following descriptors:

- Salinity (using practical salinity scale): low (0,5-3) and high (3-6) oligohaline, mesohaline (6-22)

[27] Guidance document No. 5 *'Transitional and Coastal Waters - Typology, Reference conditions, and Classification systems'*. Common Implementation Strategy of the Water Framework Directive, Available at: http://forum.europa.eu.int/Public/irc/env/wfd/library

- Depth: all shallow (<30 m)
- Exposure (using agreed Pan-European scale[28]): exposed, sheltered and very sheltered
- Duration of ice cover: >150 days/ year, 90-150 days/ year, no or very short ice cover

Table 2.1.1: Description of Baltic Sea Common intercalibration types that are included in the intercalibration exercise.

Type	Salinity psu	Exposure	Depth	Ice days	Other Characteristics
CW B0	0.5- 3	Sheltered	Shallow	> 150	Sites in Botnian Bay (Northern Quark)
CW B2	3-6	Sheltered	Shallow	> 150	Sites in Bothnian Sea
CW B3 a	3-6	Sheltered	Shallow	90-150	Sites in the area extending from the southern Bothinian Sea to the Archipelago Sea and the western Gulf of Finland
CW B3 b	3-6	Exposed	Shallow	90 -150	
CW B12 a Eastern Baltic Sea	5-8	Sheltered	Shallow	-	Sites in the Gulf of Riga
CW B12 b Western Baltic Sea	8 - 22	Sheltered	Shallow	-	Sites at the Southern Swedish coast and the South western Baltic Sea open coast along Denmark and Germany
CW B13	6-22	Exposed	Shallow	-	Sites along the coast of the Estonia, Latvia and Lithuania, the Polish coast and the Danish island "Bornholm"
CW B 14	6-22	Sheltered	Shallow	-	Lagoons
TW B 13	6-22	Exposed	Shallow		Transitional water. Sites along the coast of Lithuania and Poland

Countries sharing types that have been intercalibrated:
Types CWB0, CWB2, CWB3a, CWB3b: Finland, Sweden.
Type CWB12a: Estonia
Type CWB12b: Germany, Denmark, Sweden.
Type CWB13: Denmark, Estonia, Lithuania, Latvia, Poland.
Type CWB14: Denmark, Poland
Type TWB13: Lithuania, Poland.

Angiosperms were only intercalibrated between Denmark and Germany for the type B12b. Angiosperms are not intercalibrated in the other types, because the vegetation is scarce and distribution scattered. For the angiosperms quality element a hybrid approach between option 2 and 3 was agreed bilaterally between Denmark and Germany. Reference levels are based on historical data, expert judgment and modeling. Two approaches have been used for classification: 1) percent deviation from reference conditions (3 scenarios) and 2) modeling. Denmark used the maximum depth of 5 % eelgrass (*Zostera marina*) cover to define the depth limit. Germany used historical records of *Zostera marina* depth limit to define reference and light modeling to define depth limits

[28] According to the definitions of the common European exposure categories; Guidance document No. 5

2.1.2 National methods that were intercalibrated

Increased nutrient concentrations stimulate the growth of phytoplankton and opportunistic macroalgae which cause light attenuation in the water column to increase. Angiosperms like seagrasses but also perennial macroalgae and eventually the entire benthic vegetation become shaded and vegetation cover, biomass and depth distribution decline (Duarte 1991, Nielsen et al 2002, Valiela et al. 1997). Moreover, increased nutrient load is expected to increase the abundance of opportunistic algae, which may further shade seagrasses. A reduction in the vertical and horisontal distribution of eelgrass beds vegetation is likely to cause increased resuspension of bottom sediments, which may further shade the remaining benthic vegetation. Increased nutrient load also tends to increase the risk of anoxic events, which may further harm the benthic vegetation (e.g. Duarte 1995).

The selected metric is 'depth limit of eelgrass'. It describes the depth extension of eelgrass (*Zostera marina)*, which is the dominant seagrass in Scandinavian coastal waters. The metric is affected by nutrient concentration and water transparency (Nielsen et al. 2002, Krause-Jensen et al. 2005).

A large compilation of environmental data on eelgrass distribution from Danish coastal waters demonstrated that eelgrass depth limits increase significantly as nitrogen concentrations decline and water clarity increases (Nielsen et al 2002, Boström et al. 2003). Similar trends have been shown for other sea grass species in a worldwide compilation (Duarte 1991). Examples of these relationships are illustrated by Nielsen et al. 2002 (see Figures 3 and 4, in Nielsen et al. 2002). It should be noted that in order to illustrate the relationship between depth limit and nitrogen concentration a double logarithmic scale was used denoting that depth limits vary considerably for given levels of nutrients or Secchi depths (Nielsen et al. 2002). This means that the models may be useful to describe general relationships but cannot be used to predict depth limits precisely in specific areas.

2.1.3 Reference conditions and class boundary setting

Reference conditions

Modelling

At present, no Danish and German coastal water bodies are considered to represent reference conditions, so we attempt to define reference levels of total-nitrogen concentrations (TN) and water transparency based on historical data and modeling.

In order to define reference levels of benthic vegetation metrics by modeling there is a need for dose-response relationships between physico-chemical variables (nutrient concentration, transparency) and vegetation metrics. The reference levels of nutrient concentration and/or transparency are entered in the model and corresponding levels of the vegetation metrics are calculated. In this approach the reference levels of nutrient concentration and transparency has been identified within Danish waters, where vegetation data are available i.e. Øresund.

The model by Nielsen et al. (2002) was used to hind-cast reference depth limits based on reference TN levels. If reference TN levels are defined as e.g. 14 µM along open Danish coasts, then the corresponding reference eelgrass (*Zostera marina)* depth limits are 7.7 m. If the high-good boundary is defined as 25 % of reference levels, then the high/good boundary for eelgrass is 5.8 m.

However, historical data suggest differences in reference depth limits between various coastal waters/estuaries so it may be an advantage to use historical information on reference depth limits or to use area-specific values of reference total-N to model reference depth limits.

Historical data

The reference depth limit is defined as the values >90 % of the historical values. Furthermore, it is assumed that the depth limit represents the eelgrass (*Zostera marina)* main distribution rather than the maximum depth limit.

The most extensive historical data set of eelgrass depth distribution was reported by Ostenfeld (1908) around year 1900. This dataset contains raw data on eelgrass depth distribution in Danish coastal waters representing samples taken from a ship with information on location, water depth and whether or not eelgrass was present. Based on these raw data Ostenfeld assessed the depth limit of eelgrass in various Danish coastal waters (e.g. Kattegat, Øresund, Little Belt) as the maximum depth where eelgrass was observed. We defined the reference depth limit for eelgrass as equaling the high-good boundary and defined this to represent 90 % of the historical maximum. This definition thus assumes that values above 90 % of the historical maximum represent a high ecological status/ reference situation.

It is observed that for the reference conditions there is a reasonable compliance between the historically-based and the modeled values, but there is a tendency that the modeled values are a bit lower than the data based on history (Table 2.1.2).

German data show reference values of a “closed Zostera vegetation” at depth of 8.0-10.0 m

Table 2.1.2: Historical level and the high/good boundary of the eelgrass (Zostera marina) depth limits defined as reference condition in Danish and German Waters in the Baltic Sea. The historical depth limits are based on Ostenfeld (1908) - for the western part of the Baltic Sea it is assumed that the depth limit is the same as for Kattegat, and we have excluded the Ostenfelds data from the Bøgestrømmen as the depth limit as it seems to be determined by the maximal limit of the area. The reference depth limits are defined as the values >90 % of the historical values.
German values are after Schories et al. (2006)

	Zostera marina **depth limit (m)**	
Danish and German water areas In the Baltic Sea GIG	**Historical level**	**Class boundary High/good** (Reference)
Storebælt and Langelandsbælt	10,4	9,4
Lillebælt	8,5	7,7
Smålandshavet – open part	8,5	7,7
Østersøen, Fakse Bay	7,5	6,7
Hjelm Bay	10,4	9,4
German coast (German type B3)	10,0	8,0

Boundary setting

Denmark:

The good-moderate boundary for eelgrass depth limits in Danish coastal waters has been calculated in three scenarios representing a 15 %, a 20 % and a 25 % deviation from reference levels (Krause-Jensen 2005). Based on an expert judgment the 15 % and the 20 % deviations are the best scenarios to comply with the normative definition of good ecological state (Krause-Jensen 2006). Populations of eelgrass growing at depths of 4.4 – 4.0 m in Limfjorden and a population of eelgrass in Kattegat growing at 8.1-7.6 m depth are examples of good growing conditions. Accordingly, it was concluded that 25 % deviation from the reference condition is not congruent with “slight changes of disturbance”.

To underpin which of the 3 scenarios describing the boundary between good and moderate is most appropriate, a comparison with a model scenario was carried out. The model scenario enters the total nitrogen concentration, believed to represent the boundary between good and moderate into the empirical relationship between nutrient concentration and eelgrass depth limit (Nielsen et al. 2002). Jacob Carstensen (NERI) estimated the total nitrogen concentrations (TN) describing the reference condition and the good/moderate boundary for a number of Danish coastal waters. Similar values have been calculated for the German coast. The empirical relationship (Nielsen et al. 2002) is based on average TN-concentrations for the period March-October, while the Carstensen's TN-concentrations are from the period January-June. Therefore, to correct the period a reduction is made (reducing the January-June data with 22.5 % compared to the March-October concentrations, Table 2.1.3).

Table 2.1.4 compares the reference depth limits and the depth limits for good/moderate state calculated partly by the historical data and the modeled TN-concentrations. The empirical model used is not area-specific, but a general relationship for all Danish coastal waters, i.e. it does not take into account differences in the relationship between TN and depth limit between areas.

Table 2.1.3: Total nitrogen concentrations, which define respectively, reference condition and the boundary between high/good and the good/moderate state. Danish data: Average values for the period January-June from Carstensen et al. (2006). German data from sub-working group "Physico-chemical quality elements" of Bund-Länder-Messprogramm (BLMP) Working group "WFD". The good status was estimated as 50 % increase of reference.

Baltic Sea GIG – Type B 12	**Total Nitrogen concentrations (µM)**		
Water	**Reference**	**Class boundary (high/good)**	**Class boundary (good/moderate)**
Fakse Bay	16.6	17.6	19.6
Hjelm Bay	16.6	17.7	19.8
German coast	9	12	16

Table 2.1.4: Eelgrass (*Zostera marina) r*eference depth limit and depth limit for good/moderate state. Calculations based on historical eelgrass-data and modeled values derived from TN-concentrations. The empirical relation origins from Nielsen et al. (2002): $\ln Z_c = 6.039 - 0.755 * \ln TN$, where Z_c is the eelgrass depth limit and TN represent the period March-October. TN-values are calculated by Carstensen et al. (2006) – corrected by 22.5 % to adjust to March-October level.

Baltic Sea GIG – Type B 12	***Zostera marina* depth limit (m) based on historical data**				**Zostera marina depth limit (m) modeled based on TN-concentration**		
Danish Coastal waters	**Ref.**	**Good/moderate (% of ref)**			**Ref.**	**High/good**	**Good/moderate**
		15 %	20 %	25 %			
Fakse Bay	>6.7	5.7	5.4	5.0	8.3	8.0	7.3
Østersøen Falster/Hjelm Bay	>9.4	8.0	7.5	7.1	8.3	7.9	7.3
German coast (German type B3)					10.0	8.0	7.0

In Danish and German coastal waters, the dose-response relationships on present-time data do not include a range of nutrient concentrations representing reference levels or high/good boundaries. Reference levels for the benthic vegetation indicators therefore have to be set using historical data, hind-cast modeling and/or expert judgment. Reference levels have been shown to vary between areas in Denmark (Table 2.1.5), so we suggest that reference levels are defined for each area and that class boundary limits are defined as specified deviations from reference levels.

The good/moderate boundary representing a 25-30 % deviation from reference levels and the resulting depth limits are shown in Table 2.1.6.

Table 2.1.5: Eelgrass depth limit in the Danish coastal waters in the Baltic Sea: The historical depth limits are based on Ostenfeld (1908) - for the western part of the Baltic Sea it is assumed that the depth limit is the same as for Kattegat, and we have excluded the Ostenfelds data from the Bøgestrømmen as the depth limit as it seems to be determined by the maximal limit of the area. The reference depth limits are defined as the values >90 % of the historical values.
For the Danish data the class boundary between good and moderate is described in 3 scenarios: 15 %, 20 % and 25 % of the reference value (reference Krause-Jensen (2005)) corresponding to EQR values = 0.85, 0.80 and 0.75.
In addition a fourth scenario representing 30 % deviation was included.

Danish water areas In the Baltic Sea GIG	***Zostera marina* depth limit (m)**					
	Historical level	**Class boundary High/good (Reference)**	**Class boundary good/moderate**			
			15 % of ref.	**20 % Of ref.**	**25 % of ref.**	**30 % of ref.**
Storebælt and Langelandsbælt	10,4	9,4	8,0	7,5	7,1	6,6
Lillebælt	8,5	7,7	6,5	6,2	5,8	5,4
Smålandshavet – open part	8,5	7,7	6,5	6,2	5,8	5,4
Østersøen, Fakse Bay	7,5	6,7	5,7	5,4	5	4,7
Hjelm Bay	10,4	9,4	8,0	7,5	7,1	6,6

Table 2.1.6: Eelgrass (Zostera marina) depth limits (m) for the reference conditions and for the good-moderate boundary in the Danish Baltic Sea intercalibration sites. The good-moderate boundary is defined as a 25-30 % deviation from reference conditions.

Coastal area	**Reference**	**Good/moderate boundary**
Baltic Sea GIG- Type B 12b Danish intercalibration sites	**Depth limit (m)**	**Depth limit (m)**
Baltic Sea – Fakse Bay	6,7	5 – 4,7
Baltic Sea – Hjelm Bay	9,4	7,1 – 6,6

Germany

In Germany from historical records eelgrass "stands" were defined as >50 shoots/m², which is the minimum end of a range of 50->2500 shoots/m² for the Baltic Sea (Schories et al. 2006). The historical depth limit of *Zostera marina* was assessed as 10 m for stands, while for single plants few records of deeper occurrence exist (down to 17 m). However, it has to be noted that seeds of *Zostera* may germinate at depths where germination is possible due to stored energy, but persisting occurrence of adult plants is not. Also, as in Denmark, the genuine historical depth limit of single plants remains unclear, since data were obtained with historical techniques.10 m is thus assumed as the historical depth limit.

Through light-modelling (compare Blümel et al. 2002) and under the assumption that 10 % of the incident light is necessary to maintain *Zostera* stands, Schories et al. (2006) calculated depth limit for the WFD classes. Here they arbitrarily set certain percentages of light reduction (compared to pristine conditions) by enhanced attenuation and calculated border depth limits for *Zostera* stands and single plants (see Table 2.1.7).

Schories et al. 2006 have for the German coast defined the eelgrass depth limit for good status to 7.0 – 8.0 m and moderate status to 4.5 – 7.0 m for the intercalibrated type B12 (German type B3) with an application of the Danish 90 % rule to historical data, these values show a good fit to the Danish boundaries.

For the German coastal waters, intercalibration type B12b, TN concentrations representing High status were estimated to vary between 9 and 12 µM, and for Good status between 11 and16 µM (sub-working group "Physico-chemical quality elements" of Bund-Länder-Messprogramm (BLMP) Working group "WFD"). The good status was estimated as 50 % increase of reference.

Table 2.1.7: German depth limits of Zostera marina from Schories et al. (2006) under the assumption of 10 % incident light necessary to maintain Zostera. As basis attenuation coefficient, K = 0.23025 was used.

Classes	**Reduction of light, single plants**	**Depth limits of single *Zostera* plants**	**Tolerance reduction of *Zostera* stands**	**Depth limits of stands**
High	0 – 1 %	9.63 – 10.00	0 – 6 %	8.12 – 10.00
Good	1 – 5 %	8.39 – 9.62	6 – 10 %	7.21 – 8.11
Moderate	5 – 25 %	4.89 – 8.38	10 – 30 %	4.32 – 7,20
Poor	25 – 75 %	1.11 – 4.88	30 – 80 %	0.63 – 4.31
Bad	75 – 100 %	0.00 – 1.10	80 – 100 %	0.00 – 0.63

4.1.4 Results of the comparison

Comparison of Danish and German values based on experts' opinions:
Eelgrass depth limits for reference status and good-moderate boundary in the Danish and German Waters in the Baltic Sea.

Coastal area	**Reference**	**Good/moderate boundary**	**Preliminar EQR values**
Danish Baltic 12b- GIG areas			
Baltic Sea – Fakse Bay	6,7 m	5 – 4,7 m	0.75 – 0.70
Baltic Sea – facing Falster	9,4 m	7,1 – 6,6 m	0.75 – 0.70
German Baltic 12b- GIG areas			
Geltinger Birk	8-10* m	7* m	0.88
Darß-Zingst-Außenküste	8-10* m	7* m	0.88

* These values represent the recommended thresholds for field monitoring, where the estimation of depth to the first or second decimal is futile. They are rounded and thus slightly different from those in Table 7.

4.1.5 Results of the harmonisation – Boundary EQR values

INTERCALIBRATION RESULTS					
Biological Quality Element			**Angiosperms**		
Results **BALTIC SEA GIG: Angiosperms intercalibration results.** Depth limit eelgrass *Zostera marina*					
	Reference conditions m	**Boundary H/G m**	**Boundary G/M M**	**EQR H/G**	**EQR G/M**
Type B12 Denmark Germany Open coast	9.4 (8 – 10.4)	8,5 (8 – 9.4)	7 (6.6 – 7.1)	0.90	0.74

Final Results

Biological Quality Element			Angiosperms	
Angiosperms: parameter indicative of abundance (Depth limit of eelgrass *Zostera marina*) **Results: Ecological quality ratios and parameter values**				
Type and country	**Ecological Quality Ratios for the national classification systems**		**Parameter values/ranges Depth limit (m) eelgrass *Zostera marina***	
	High-Good boundary	***Good-Moderate boundary***	***High/-Good boundary***	***Good-Moderate boundary***
CW B 12 b Denmark and Germany Open coast	0.90	0.74	8.5 (8.0 – 9.4)	7 (6.6 – 7.1)

4.1.6 Open issues and need for further work

- Results have been achieved at parameter level (depth limit of eelgrass, *Zostera marina*) for coverage and density by two countries (DK and DE) covering one type.
- The metric used for angiosperms is not relevant for other countries in the GIG
- More widely usable methods for coverage and density should be explored in most types.
- The need for assessment methods related to taxonomic composition should be analysed.

2.2 *NE Atlantic GIG*

2.2.1 Intercalibration approach

In the NE Atlantic seven basic intercalibration types have been agreed. These are shown in table 1 below:

Table 2.2.1: NEA GIG typology.

New Type ID	*Name*	*Salinity (PSU)*	*Tidal range (m)*	*Depth (m)*	*Current velocity*	*Exposure*	*Mixing*	*Residence time*
CW –NEA 1/26a,b,c,d,e	Exposed or sheltered, euhaline, shallow	Fully saline (> 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or sheltered	Fully mixed	Days
CW – NEA3/4	Polyhaline, exposed or moderately exposed (Wadden Sea type)	Polyhaline (18 - 30)	Mesotidal (1 - 5)	Shallow (< 30)	Medium (1 - 3 knots)	Exposed or moderately exposed	Fully mixed	Days
CW – NEA7	Deep, low current, sheltered	Fully saline (> 30)	Mesotidal (1 - 5)	Deep (> 30)	low (< 1 knot)	Sheltered	Fully mixed	Days
CW – NEA8	Polyhaline, microtidal, sheltered, shallow (Skagerrak inner arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Shallow (< 30)	low (< 1 knot)	Sheltered	Partially Stratified	Days-Weeks
CW – NEA9	Fjord with a shallow sill at the mouth with a very deep maximum depth in the central basin with poor deepwater exchange.	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	low (< 1 knot)	Sheltered	Permanently Stratified	Weeks
CW – NEA10	Polyhaline, microtidal exposed, deep (Skaggerak outer arc type)	Polyhaline (18 - 30)	Microtidal (< 1)	Deep (> 30)	low (< 1 knot)	Exposed	Permanently Stratified	Days
TW – NEA11 (will be split into sub-types)	Transitional waters	Oligo-Euhaline (0 - 35)	Mesotidal (1 – 5)	Shallow (< 30)	Medium	Sheltered or moderately Exposed	Partially- or Permanently Stratified	Days-Weeks

The above types occur in Member State's waters as detailed below in table 2.2.2:

Table 2.2.2: Member States sharing types.

Type	**BE**	**DK**	**FR**	**DE**	**IE**	**NL**	**NO**	**PT**	**ES**	**SE**	**UK**
CW - NEA1/26a			X		X		X		X		X
CW - NEA1/26b	X		X			X					X
CW- NEA1/26c		X		X							
CW- NEA1/26d		X									
CW-NEA1/26e								X	X		
CW – NEA3/4				x		x					
CW – NEA7							x				**X**
CW – NEA8		x					x			X	
CW – NEA9							x			x	
CW – NEA10							x			x	
TW – NEA11	**X**		X	X	X	X		X	X		X

Option 2 has been used in this phase of the intercalibration process. Two metrics have been selected, Intertidal Seagrass: Abundance (Areal extent and density) and species composition (in types NEA1/26a,b,c/3/4/11) and Subtidal Seagrass: Abundance (Areal extent and density) and species composition (in type NEA8). Boundaries have been agreed for some countries for each metric where applicable. This is described below.

2.2.2 National methods that were intercalibrated

Table 2.2.3 below indicates the components of the national methods that were considered for intercalibration for intertidal seagrass.

It is important to note that:

- Member states have different seagrass species and different numbers of species. Member states are considering standardising their percentage loss descriptors.
- The Netherlands considers waterbody types 1 and 26 separately. Type 26 represents saline lakes.
- Spain might only able to intercalibrate on taxonomic composition (from the three metrics proposed) because the Spanish area and density (% cover) metrics incorporate actual data (for seagrass, saltmarsh and macroalgae) not potential or historical data.
- German seagrass data are derived from aerial monitoring and ground truth investigations. At present no differentiated data are available concerning species composition and coverage/ density (per species). Accordingly only the bed extent of intertidal seagrass is ready to be classified. Future monitoring programs will help to close this gap.

Table 2.2.3: National Methods.

Member states	**Angiosperm metric**			**Notes**
	Change in taxonomic composition	**Change in density**	**Change in bed extent**	
UK	Yes	Yes	Yes	Intertidal seagrass *Zostera noltii* and *Z. angustifolia* & *Ruppia* sp.
Republic of Ireland	Yes	Yes	Yes	Intertidal seagrass *Zostera noltii* and *Z. angustifolia* & *Ruppia* sp.
Netherlands	Yes	Yes	Yes	Intertidal seagrass beds; Z*ostera noltii* & intertidal *Z. marina*
Germany			Yes	*Intertidal seagrass beds; Zostera noltii & intertidal Z. marina*
Spain	Under consideration			Habitat code 1110-A, 1110-B & 1140
Portugal	Under consideration			
France	Under consideration			

Below is a summary, table 4, of member states and waterbody types for intertidal seagrass intercalibration.

Denmark and Sweden have assessed subtidal seagrass bed depth limits in the NEA8 typology (see below).

Table 2.2.4: Member States sharing types relevant to intertidal seagrass

	UK	Republic of Ireland	Netherlands	Germany	Spain	Portugal (for future intercalibration)
Waterbody types	CW-NEA1/26a, b CW-NEA7 TW-NEA11	CW-NEA1/26a, b TW-NEA11	CW-NEA1/26b CW-NEA4 TW-NEA11	CW-NEA1/26c CW NEA 4 TW-NEA11	TW-NEA11	CW-NEA1/26e TW-NEA11

2.2.3 Reference conditions and class boundary setting

The following intercalibration work between Netherlands, Ireland and the UK has been published by Foden and de Jong (2007).

Metric - Intertidal Seagrass: Abundance (Areal extent and density) and species composition (NEA1/26a,b,c/3/4/11)

This metric is only being fully intercalibrated between the Netherlands, Ireland, and UK. Germany currently only has bed extent data and is currently only intercalibrating on Metric 2 (Seagrass Abundance: acreage/bed extent), however when species data is available Germany should be able to participate in all the metrics. Spain have developed a separate metric which has not yet been intercalibrated, this will be completed Phase II of Intercalibration (see end of section for more details). This metric has been declared as not applicable by the other GIG Member States.

Reference Conditions

The reference conditions for seagrass for each of the chosen sub-metrics in the co-operating countries are defined as below. The assumption is made that these occur in unimpacted areas with unpolluted water quality and no hydromorphological alterations to the shore or seabed. Dutch waterbodies are embanked and may be classed as heavily modified. Although the waterbodies are managed and protected by engineering works, habitats such as seagrass beds have established naturally within them. Potential Reference Conditions (P-REF) and Potential Good Ecological Status (P-GES) are the highest two classes heavily modified waterbodies can attain, and scientists in the Netherlands have set values for these by focusing on the current situation in the waterbodies concerned (de Jong, 2004).

German Reference conditions can be found in German Macroalgae & Angiosperm Annex: IC-Report Macroalgae and Angiosperms NEA GIG - Germany_05-2007.doc (Kolbe, 2007)

Metric 1. Species Composition (NL, UK, IE)

Table 2.2.5: NL species composition.

	REF	GES	Moderate	Poor	Bad
Number of seagrass species	2 species	1 species	-	-	-

Table 2.2.6: UK and IE species composition.

Number of seagrass species	High/Ref	Good	Moderate	Poor	Bad
	No loss of species	¼ to ⅓ loss of species	Loss of ½ of species.	Loss of ⅔ to ¾ of species	**Loss of all species**

Metric 2. Seagrass Abundance: acreage/bed extent Composition (NL, UK, IE, DE)

Table 2.2.7: NL seagrass acreage (hectares).

Water type	Waterbody	REF	GES	Moderate	Poor	Bad
				<25 % below GES	**25-50 % below GES**	**>50 % below GES**
CW-NEA4	Wadden Sea total	250	150	>112.5	112.5- 75	**< 75**
CW-NEA4	Oosterschelde	1000	750	>563	563-375	**< 375**
TW-NEA11	Ems-Dollard	100	50	>37.5	37.5-25.0	**< 25.0**
TW-NEA11	Westerschelde	3	2	>1.5	1.5-1.0	**< 1.0**

Table 2.2.8: UK AND IE seagrass bed extent: historical data and expert judgement establish the reference conditions for a seagrass bed to be compared with its current extent.

High/Ref	Good	Moderate	Poor	Bad
No loss in seagrass bed extent – at maximum potential and in equilibrium (within natural variability)	Extent < 30 % loss from highest recorded	30 – 50 % loss of bed extent	50 – 70 % loss of bed extent	**> 70 % loss of bed extent**

Metric 3. Seagrass Abundance: coverage/density Composition (NL, UK, IE)

Table 2.2.9: NL seagrass coverage as mean % cover per species. The present positions of the waterbodies are indicated (WZ = Wadden Sea, OS = Oosterschelde, ED = Ems-Dollard, WS = Westerschelde)

	REF	GES	Moderate	Poor	Bad
Common Eelgrass (Zostera marina)	>/= 30	>/= 20	>/= 10	>/= 5	**< 5**
Dwarf grass-wrack (Zostera noltii)	>/= 60	>/= 40	>/= 30	>/= 20	**< 20**
Water		WS	OS, ED	WZ	

Table 2.2.10: UK and IE seagrass density (% cover).

High/Ref	Good	Moderate	Poor	Bad
Bed density at or above ~highest previously recorded	Density <30 % loss	Density <50 %	Density <70 %	**Density >70 %**

Sub-metric to support Metrics 2 & 3 *Trends in seagrass abundance*

Table 2.2.11: NL trends in seagrass acreage (bed extent) and coverage (% density). The present trends of the waterbodies are indicated (WZ = Wadden Sea, OS = Oosterschelde, ED = Ems-Dollard, WS = Westerschelde).

	REF/GES	**Moderate**
Trend	Positive/neutral	Negative
Water	ED WZ	WS

UK (Foden & Brazier, 2007)

Classification status for density is determined by the underlying trend over a period of 5-6 years, where data exist, to coincide with the WFD's reporting cycle. The trend for an individual bed and the loss or gain, as compared with a maximum recorded density, can be used to identify whether the seagrass bed is in a state of degradation or recovery.

Boundary Criteria

Metric 1. Species Composition

Most seagrass beds in the UK will comprise 1 or 2 species. Consequently, the NL and UK metrics are similar. The main difference is that no distinction can be made between High/Ref and Good for the UK metric because, for example, there are sublittoral beds of *Z. marina* that are naturally mono-specific and are at High status. The NL's metric is not able to define conditions less than GES, whereas the UK metric has boundaries between Good and Moderate, and between Moderate and Poor/Bad. As the tool testing examples show (below) in most cases the outcomes of the NL metric and UK metric are generally the same.

Metric 2. Seagrass Abundance: acreage/bed extent

There are significant similarities between the NL and UK metric boundary conditions between each ecological status class for seagrass acreage/bed extent. With only four waterbodies the NL have been able to use modeling and expert judgement to set precise bed areas for REF and GES for each of those waterbodies. The average difference between REF and GES is ~30 % which is broadly in line with the UK's more generalised boundary of a 30 % decrease in bed extent between High/Ref and Good. The mean difference between the NL's Moderate and REF for all four waterbodies is ~50 %, between Poor and REF is ~70-75 % and between Bad and REF is >70 %. All of these boundaries are broadly in common with the UK/IE and Germany metric's boundary conditions (see section 5.3.5).

Metric 3. Seagrass Abundance: coverage/density

As with bed extent, there are significant similarities between the NL and UK metric boundary conditions between each ecological status class for seagrass coverage/density. With only four waterbodies the NL have been able to use modeling and expert judgement to set precise density ranges for *Z. noltii* and *Z. angustifolia*, for REF and GES. The difference between NL's REF and GES for both species is ~30 % which is broadly in line with the UK boundary of a 30 % difference between High/Ref and Good. For *Z. noltii* the difference between The NL's Moderate and REF is 50 %, which corresponds with the 50 % difference between Moderate and High for the UK metric. For *Z. angustifolia* there is a greater difference between The NL's Moderate and REF (⅔) than between Moderate and High for the UK metric. However, only the Ems-Dollard waterbody will be assessed against this criterion because the other 3 waterbodies either comprise solely of *Z. noltii* or *Z. noltii* is the dominant species present.

There is a difference of ⅔ between NL's Poor and REF and >⅔ between Bad and REF, for *Z. noltii*. These boundaries are broadly in common with the UK metric's boundary conditions of <70 % loss of seagrass for Poor and >70 % loss for Bad.

Sub-metric to support Metrics 2 & 3 Trends in seagrass abundance

Both NL and UK agree that the underlying trend in seagrass abundance should show a stable seagrass bed (at the maximum potential identified for that site/waterbody). If abundance is less than would be expected for High/Reference conditions then abundance should show a positive underlying trend, indicative of recovery. Conversely, a negative trend in seagrass abundance is undesirable, indicative of degradation, and would signal a potential deterioration in ecological class.

Furthermore, the Member States agree that the ideal period over which to consider the trend in abundance is ~6 year, designed to coincide with the WFD reporting cycles.

Testing Dutch waterbody data against NL and UK metrics; data for 2004

NB. The analysis of German metric against NL and UK metrics information can be found in German Macroalgae & Angiosperm Annex: IC-Report Macroalgae and Angiosperms NEA GIG - Germany_05-2007.doc

Wadden Sea, NL

Table 2.2.12: Wadden Sea CW-NEA4.

	1 – species composition	**2 – acreage/bed extent**	**3 – coverage/bed density (%)**
NL REF	2 species	250 ha.	*Z. marina* var. *angustifolia* = ≥30 Z. noltii = ≥*60*
NL GES	1 species	150 ha.	*Z. marina* var. *angustifolia* = ≥20 *Z. noltii* = ≥40
Current situation	2 species	47 ha.	*Z. noltii* ≈40 %
NL outcome	REF	**Bad**	GES
UK outcome	No species lost = High	**19 % of reference conditions = Bad**	Previous highest recorded density was 58 % in 1997. = Good

The outcomes for the NL and UK metrics are comparable (Table 12). The only difference is for species composition where the loss of *Z. angustifolia* (littoral eelgrass) indicates a Moderate outcome for the UK metric rather than a GES outcome. The underlying trends in both seagrass bed extent (acreage) and density could be described as positive or neutral (Figure 1); so although the size of the bed is very small compared with the REF of 250 ha. It is possibly in a recovery phase.

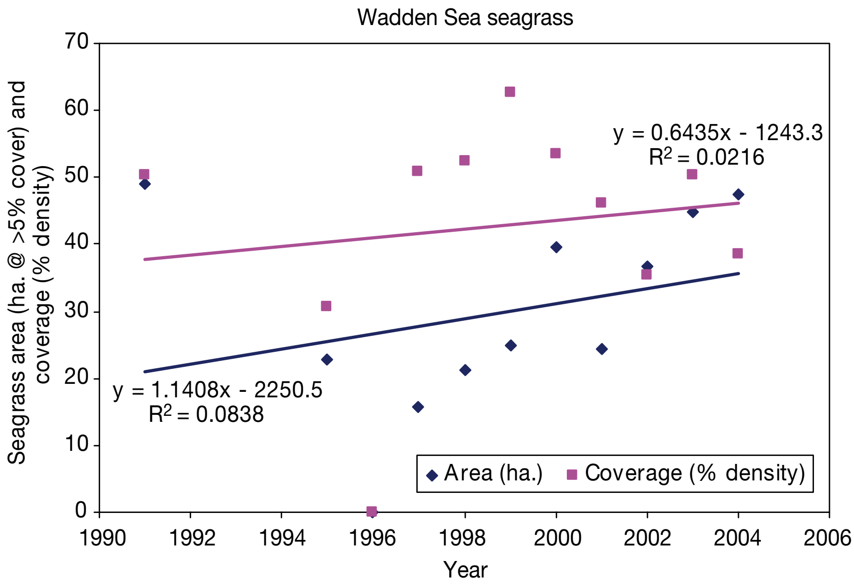


Figure2.2.1: Temporal trends in Wadden Sea seagrass acreage (bed extent) and coverage (% density).

Oosterschelde, NL

Table 2.2.13: Oosterschelde CW-NEA4.

	Species composition	Acreage/bed extent	Coverage/bed density (%)
NL REF	2 species	1000 ha.	*Z. marina* var. *angustifolia* = ≥30 *Z. noltii* = ≥60
NL GES	1 species	750 ha.	*Z. marina* var. *angustifolia* = ≥20 *Z. noltii* = ≥40
Current situation	2 species	94 ha.	*Z. marina* var. *angustifolia* = ≥30 in Roggenplaat, <10 % elsewhere *Z. noltii* ≈ 62 %
NL outcome	REF	**Bad**	GES/Moderate boundary
UK outcome	No species lost = High	**~10 % of reference conditions = Bad**	Previous highest density was 50 % in 1979-1983; decline of ~35 % = Moderate

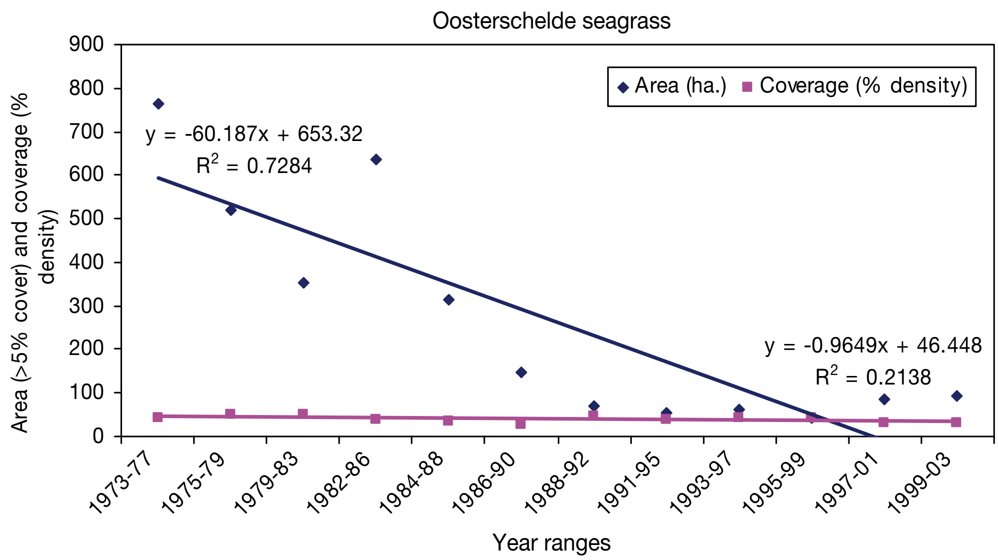


Figure 2.2.2: Temporal trends in Oosterschelde seagrass acreage (bed extent) and coverage (% density).

The outcomes for the NL and UK metrics are broadly the same for the three metrics (Table 13). Both *Z. noltii* and *Z. angustifolia* are present. The underlying trend in seagrass bed extent (acreage) is strongly negative between 1973 and the beginning of the 1990s (Figure 2). Since then it has become neutral, with possibly a very slight increase in recent year. *Z. noltii* is the species considered for the density metric as it dominates the species composition; *Z. angustifolia* (littoral eelgrass) constitutes a very small proportion of the overall seagrass area at very low density therein. The most recent mean density calculation (62 %) lies on the boundary between the NL's GES and Moderate. The trend in density could be described as neutral as there has been consistently low percentage cover (Figure 2).

Ems-Dollard, NL

Table 2.2.14: Ems-Dollard TW-NEA11.

	Species composition	Acreage/bed extent	Coverage/bed density (%)
NL REF	2 species	100 ha.	*Z. marina* var. *angustifolia* = ≥30
NL GES	1 species	50 ha.	*Z. marina* var. *angustifolia* = ≥20
Current situation	1 species	14 ha.	Z. *marina* var. *angustifolia* ≈ 13 %
NL outcome	GES	**Bad**	Moderate
UK outcome	(No record of *Z. noltii*) = High	**~15 % of reference conditions = Bad**	Previous highest density was 30 % in 1988; decline of ~55 % = Moderate

The outcomes for the NL and UK metrics are the same in all three instances (Table 14). *Z. noltii* has not been recorded in this waterbody and the overall seagrass area is comprised of *Z. angustifolia* (littoral eelgrass) at low densities. The underlying trend in seagrass bed extent (acreage) is positive from 1986 to 2003, but drops sharply in 2004. The trend in density could be described as negative with the 2004 figure being one of the lowest since 1988.

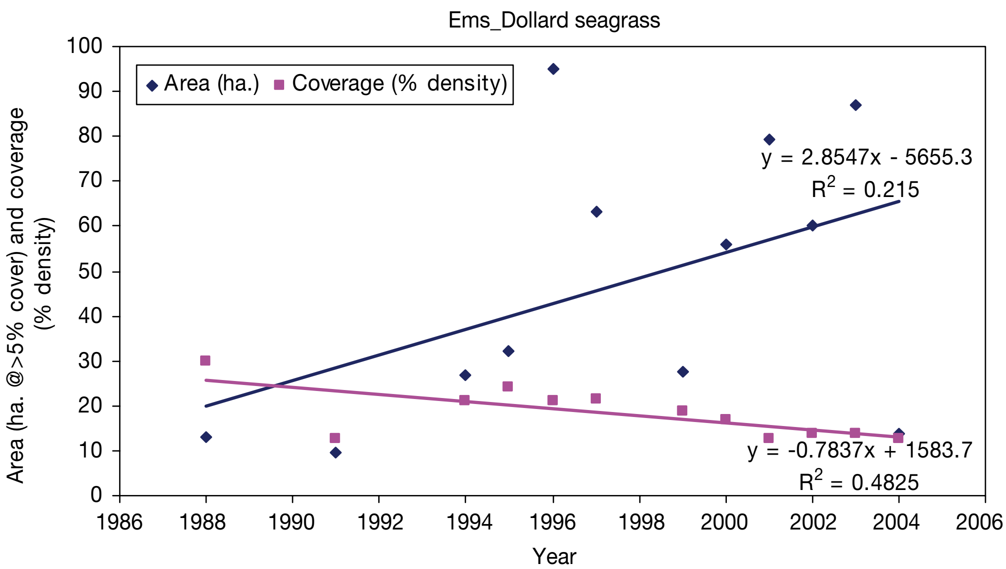


Figure 2.2.3: Temporal trends in Ems-Dollard seagrass acreage (bed extent) and coverage (% density).

Westerschelde, NL

The *Z. noltii* bed is a small bed, situated in the harbour of Flushing-east, with an uncertain history. It might be that *Z. noltii* was present in the past (before the construction of the harbour) in the inlet (Sloe) between the two islands Walcheren (western side) and South Beveland (eastern side), but no records are available. Equally it might that it appeared there after the construction of the harbour.
It is only mapped when a vegetation map is made of the small salt marsh that is present there. The cover is low, as it is a patchy bed in between the *Spartina* tussocks on the seaward side of the marsh. Estimated mean cover is between 5 and 20 %.

Table 2.2.15: Westerschelde TW-NEA11.

	Species composition	Acreage/bed extent	Coverage/bed density (%)
NL REF	2 species	3 ha.	*Z. marina* var. *angustifolia* = ≥30 *Z. noltii* = ≥60
NL GES	1 species	2 ha.	*Z. marina* var. *angustifolia* = ≥20 *Z. noltii* = ≥40
Current situation	1 species	2 ha.	*Z. noltii* ≈ 5-20 %
NL outcome	GES	GES	**Poor (possibly Bad)**
UK outcome	(No record of *Z. marina* var. *angustifolia*) = High	~66 % of reference conditions = Good	Previous highest density data unavailable, but cover is patchy. Therefore less than ‘Good’ – precautionary Moderate or Poor Moderate or **Poor**

The outcomes for the NL and UK metrics are the same for species composition and acreage (bed extent), but differ for bed density (Table 15). In fact this latter difference is of minor significance because; (a) there is a lack of raw data to allow a more confident setting of class, and (b) both The NL and UK outcomes are less than Good, meaning a programme of investigative measures would be undertaken. The absence of raw data prevents a temporal trend plot over recent years for the Westerschelde.

Testing UK waterbody data against NL and UK metrics

Strangford Lough, UK (Portig, 2004)

Historical data are scarce, but there has clearly been a marked decline in the distribution of seagrasses in Northern Ireland since 1930s. This has been coupled with a change in the dominant *Zostera* spp. present in the intertidal areas with *Z. marina* in its perennial form dominant in the 1930s being replaced by *Z. noltii* and *Z. angustifolia* by 1970.
There has been a general improvement in the status of *Zostera* spp. in the northern end of Strangford Lough during the last 10 years. The necessary data are lacking, however, to determine whether these changes are part of ongoing cyclical processes or longer term changes. The mean seagrass density for all N.I. (Northern Ireland) loughs is 52 %

Table 2.2.16: Strangford Lough CW-NEA26 (CW8).

	Species composition	Acreage/bed extent	Coverage/bed density (%)
Current situation	*Z. marina* var. *angustifolia*, *Z. noltii*, *Z. marina*, *Ruppia* spp.	924 ha. Literature and qualitative sources suggest the beds are in a 'recovery' phase; i.e. expanding, but at less than maximum potential; assume >50 % and <70 % of maximum.	53 % mean density for all species. No baseline data are available, but this figure is comparable with N.I. records of seagrass bed densities in other loughs.
NL outcome	REF	Moderate	REF
UK outcome	High	Moderate	High

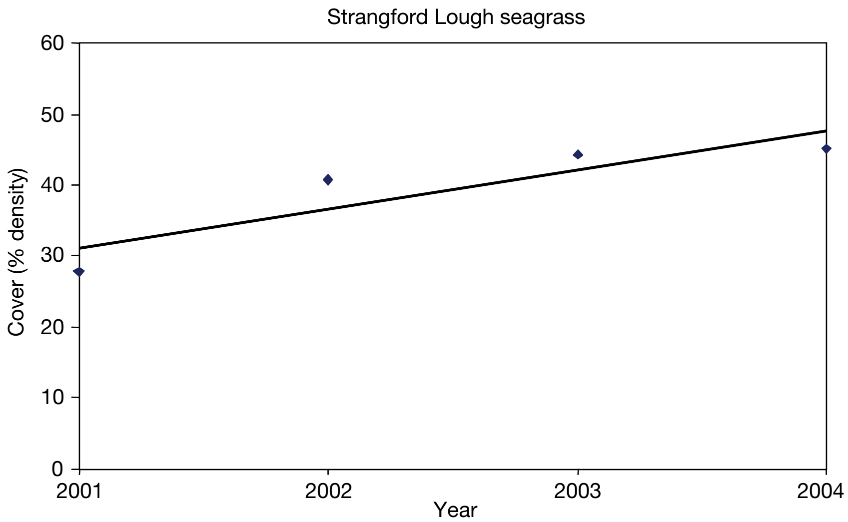


Figure 2.2.4: Temporal trends in Strangford Lough seagrass % density.

The outcomes for the NL and UK metrics are the same in all three instances (Table 16). The lack of raw data makes precise statements regarding seagrass acreage (bed extent) and coverage (density) difficult. The underlying trend in seagrass density is positive over the 4 survey years, confirming the judgement of the seagrass being in a 'recovery' phase.

Fleet lagoon, UK (Bunker et al., 2004)

Distribution changes in seagrass species of Fleet Lagoon, as surveyed in 2002 (Bunker *et al*., 2004):

- *Z. marina*; Lost from Swannery Basin since 1983, but a north-westward extension of range in West Fleet since 1999
- *Z. noltii*; Lost from Swannery Basin since 1983, but qualitative reports of unchanged distribution since 1999.
- *Ruppia* spp.; Lost from Swannery Basin and west of Berry Coppice since in 1983, but distributions in the rest of the Fleet remain broadly unchanged since 1999.

Variability in the data is very high because of the transect survey method and absence of mapping. There are three issues that affect the final decision of Fleet's ecological status:

1. Standard Deviation at some stations was almost as large as the mean of the 12 quadrats (Figure5)

2. Power analysis shows a high % change in density (>50 % at the majority of stations) would have to occur to detect a statistically significant change; analysed for Type I and Type II errors (percentage change in mean density required in order to be 95 % confident that a statistically significant change has occurred).
3. Previous surveys do not provide raw quadrat data so statistical comparison between data sets is not possible.

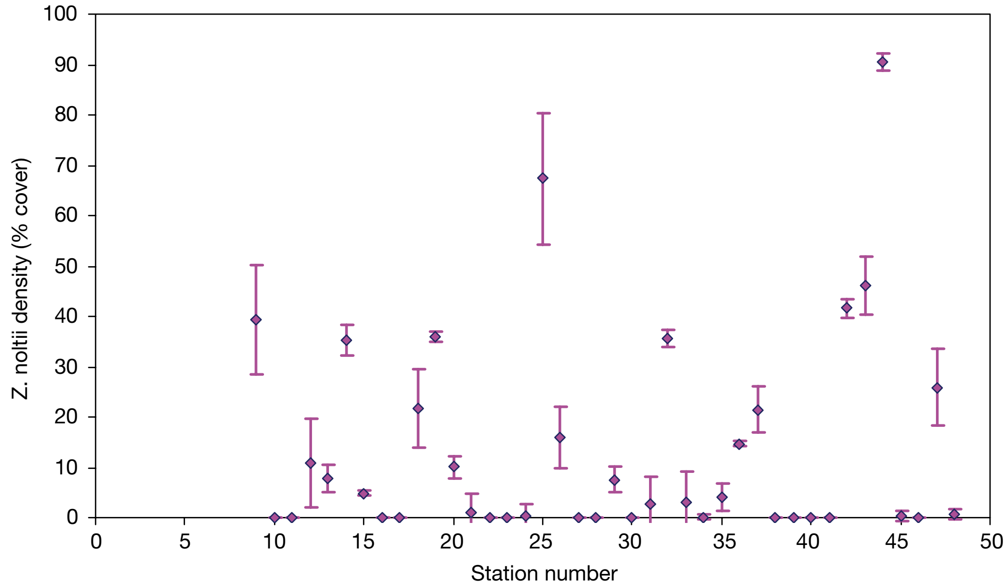


Figure 2.2.5: Standard deviation examples.

2.2.4 Results of the comparison

As option 2 has been used the boundaries have been agreed by experts representing all countries in the GIG angiosperms sub-group. Therefore the final agreed results presented below are the results of comparing expert views on what the boundaries should be.

2.2.5 Results of the harmonisation – Boundary thresholds and EQR values

Metric - Intertidal Seagrass: Abundance (Areal extent and density) and species composition (NEA1/26a,b/3/4/11)

The Netherlands, Ireland and the UK have agreed a common matrix for allocating status to intertidal seagrass assessments on the basis of the table below. This matrix combines both loss of species and degradation in the % cover (measured as the number of seagrass shoots in a quadrat or % cover of seagrass within a quadrat). The matrix covers both situations where naturally either two or three species of seagrass are found within either a type or where there are differences within types in specified geographic areas. The appropriate selection from the matrix is made at the waterbody level.

Table 2.2.17: Intertidal Seagrass: Abundance and Species composition classification boundary matrix.

Density (% cover)		0 - 10 % lost	10 - 30 % lost	30 - 50 % lost	50 - 70 % lost	>70 % lost
Species	No spp. lost	High	Good	Moderate	Poor	**Bad**
	1 spp. lost, 2 remain	Good	Good	Moderate	Poor	**Bad**
	1 spp. lost, 1 remains	Good	Moderate	Poor	**Bad**	**Bad**
	2 spp. lost, 1 remains	Moderate	Moderate	Poor	**Bad**	**Bad**
	All spp. lost, therefore 0 % cover	**Bad**				

The above matrix does not allow the true mathematical derivation of EQRs so the generic approach of allocating equal EQR ranges for status classes (as allowed by the boundary setting protocol) will apply; the EQR boundaries and ranges are shown below.

Status	**EQR**
High	0.9 (0.8 – 1.0)
Good	0.7 (0.6 – 0.8)
Moderate	0.5 (0.4 – 0.6)
Poor	0.3 (0.2 – 0.4)
Bad	0.1 (0.0 – 0.2)

France and Portugal are to consider using the above matrix and hope to decide by June 2007.

Germany's seagrass data are derived from aerial monitoring and ground truth investigations. At present no differentiated data are available concerning species composition and coverage/density (per species). Consequently Germany cannot yet participate in the species composition component. Future monitoring programs will help to close this gap.

Metric - Intertidal seagrass area (acreage/bed extent) (NEA1/26a,b,c/3/4/11)

This scheme has been agreed by UK, IE, NL and DE and is tabulated below:

Table 2.2.18: Intertidal seagrass area (acreage/bed extent) classification boundary values.

Member states	Description of seagrass		High (REF in NL)	Good (GES in NL)	Moderate	Poor	Bad
UK	Intertidal seagrass beds	Change in area	0 - 10 % loss	11 - 30 % loss	31 - 50 % loss	51 - 70 % loss	>70 % loss
Republic of Ireland	Intertidal seagrass beds	Change in area	0 - 10 % loss	11 - 30 % loss	31 - 50 % loss	51 - 70 % loss	>70 % loss
Netherlands	*Z. noltii* and mixed beds	Change in area	0-10 % loss (allowing for natural variation)	11-30 % loss	31 - 50 % loss from REF	51 - 70 % loss from REF	>70 % loss from REF
Germany	*Z. noltii* and mixed beds	Change in area	0 - 10 % loss	11 - 30 % loss	31 - 50 % loss	51 - 70 % loss	>70 % loss

The values in table 17 translate into the EQR boundary values and ranges shown below.

Status	EQR
High	0.9 (0.8 – 1.0)
Good	0.7 (0.6 – 0.8)
Moderate	0.5 (0.4 – 0.6)
Poor	0.3 (0.2 – 0.4)
Bad	0.1 (0.0 – 0.2)

Spain:

Have developed an Angiosperm Intertidal/Subtidal Quality Index for their NEA Type 11 waters. Other countries have not yet tested the Spanish Angiosperm quality index and no comparison between the developed metrics has been made. Therefore the intercalibration for Spain will not be complete until testing has been carried and boundaries agreed for June 2007. Details of the metric proposed can be found in the detailed Milestone 6 Report.

2.2.6 Open issues and need for further work

Gaps for the future:

- Spain will attempt to separate macroalgae and seagrass from their macrophyte tool, for intercalibration purposes
- Development of saltmarsh tools is needed for most member states.
- Questions of how to combine different sub-metrics into a higher level overall EQR.
- Spain has to decide the metric to use for seagrass and improve it
- Germany has to test and to decide using the combined metric intertidal seagrass abundance (density)/species composition

Intercalibration for Saltmarsh will need to be undertaken at a future date.

3 References

Blümel C, Domin A, Krause J, Schubert M, Schiewer U, Schubert H (2002). Der historische Makrophytenbewuchs der inneren Gewässer der deutschen Ostseeküste. Rostock. Meeresbiol. Beitr. 10:5-111.

Boström C, Baden SP, Krause-Jensen D. 2003 The seagrasses of Scandinavia and the Baltic Sea. In Green EP, Short FT (Eds) World atlas of seagrasses. California University Press. 310 pp.

Bunker, F., T. Mercer & C. Howson, 2004. Fleet lagoon and tidal rapids survey 15th–22nd July 2002. Report to English Nature, 80 pp.

Carstensen, J., Conley, D. J., Andersen, J., Ærtebjerg, G. (2006) Coastal eutrophication and trend reversal: a Danish case study. Limnology and Oceanography 51, pp. 398-408

de Jong, D.J., 2004. Water Framework Directive: determination of the reference condition and Potential-REF/Potential-GES and formulation of indices for plants in the coastal waters CW-NEA3 (K1), CWNEA4 (K2), CW-NEA1 (K3), transitional water, TW-NEA11 (O2), and large saline lakes, NEA26 (M32), in The Netherlands. (Working document RIKZ/OS/2004.832.x; final draft).

Duarte CM. 1991. Seagrass depth limits. Aquat. Bot. 40: 363-377.

Duarte CM. 1995. Submerged Aquatic Vegetation in Relation to Different Nutrient Regimes. Ophelia 41: 87-112.

Foden, J. and Brazier, D.P., 2007. Angiosperms (seagrass) within the EU water framework directive: A UK perspective. Marine Pollution Bulletin 55: 181–195.

Foden, J. and de Jong, D.J., 2007. Assessment metrics for littoral seagrass under the European Water Framework Directive; outcomes of UK intercalibration with the Netherlands. Hydrobiologia 583(1): 383-383

Hansson M, Håkansson B. 2006 Förslag till Vattendirektivets bedömningsgrunder för pelagiala vintertida näringsämnen och sommartida effektrelaterade i kust- och övergångsvatten

Krause-Jensen D. 2005. Scenarier for klassifikation af kystvande vha. ålegræssets dybdegrænse

Chapter 3.4 in Dahl K, Andersen JH, Riemann B (Eds) Carstensen J, Christiansen T, Krause-Jensen D, Josefson AB, Larsen MM, Petersen JK, Rasmussen MB, Stand J 2005: Redskaber til vurdering af miljø- og naturkvalitet i de danske farvande. Typeinddeling, udvalgte indikatorer og eksempler på klassifikation. Danmarks Miljøundersøgelser. 158 p. – Technical report from NERI no. 535. (In Danish) http://technical-reports.dmu.dk

Kolbe, K., 2007. Assessment of German Coastal Waters (NEA 1/26, NEA 3/4) and Transitional Waters (NEA 11) by Macroalgae and Angiosperms. Intercalibration Report (NEA GIG).- (postal to D. Coates, D. Jowett).

Krause-Jensen D, Greve TM, Nielsen K. 2005. Eelgrass as a bioindicator under the Water Framework Directive. Water Resources Management 19: 63-75.

Magnusson J, Johnsen T.M, Beyer F, Gjøsæter J, Lømsland E.R, Sollie, Aa, 1999. Pollution monitoring in the Inner Oslofjord in 1998. NIVA-report. OR-4058. 63pp. In Norwegian (http://www.niva.no/).

Nielsen SL, Sand-Jensen K, Borum J, Geertz-Hansen O. 2002. Depth colonization of eelgrass (*Zostera marina*) and macroalgae as determined by water transparency in Danish coastal waters. Estuaries 25: 1025-1032.

Ostenfeld DH 1908.'Ålegræssets (*Zostera marina*) udbredelse I vore farvande', in CGJ Petersen (ed), Beretning til landbrugsminiteriet fra den danske biologiske station XVI, Copenhagen, Centraltrykkeriet.

Portig, A.A., 2004. The distribution of intertidal Zostera spp. in Northern Ireland, 2003. Report for Environment and Heritage Service p. 84.

Schories D, Selig U, Schubert H. 2006. Testung des Klassifizierungsansatzes Mecklenburg-Vorpommern (innere Küstengewässer) unter den Bedingungen Schleswig-Holsteins und Ausdehnung des Ansatzes auf die Außenküste. Küstengewässer-Klassifizierung deutsche Ostsee nach EU-WRRL. Teil A: Äußere Küstengewässer.

Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnol. Oceanogr. 42:1105-1118.

Øresundsvandsamarbejdet - Öresundsvattensamarbetet. 2004. Jämförelse mellan 'urtida' och 'nutida' näringsnivåer i Öresund – beräkningar utförda med MIKE3-modell. www.oresundsvand.dk

European Commission

EUR 23838 EN/3 – Joint Research Centre – Institute for Environment and Sustainability
Water Framework Directive intercalibration technical report - Part 3: Coastal and Transitional waters
Alessandro Carletti, Anna-Stiina Heiskanen
Luxembourg: Office for Official Publications of the European Communities
2009 – 240 pp. – 21 x 29,7 cm
EUR – Scientific and Technical Research series – ISSN 1018-5593
ISBN 978-92-79-12568-3
DOI 10.2788/19561

Abstract
This Technical Report gives an overview of the technical and scientific work that has been carried out in the intercalibration of coastal and transitional waters ecological classification systems across the European Union as required by the Water Framework Directive (WFD). The results of this exercise were published in the Official Journal of the European Union as Commission Decision 2008/915/EC of 30 October 2008.

14

LB-NC-23838-EN-C

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

JRC
EUROPEAN COMMISSION

ISBN 978-92-79-12568-3

9 789279 125683

Publications Office
Publications.eu.int