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# Coastal waters Black Sea geographic intercalibration group

*Benthic invertebrate fauna ecological assessment methods*

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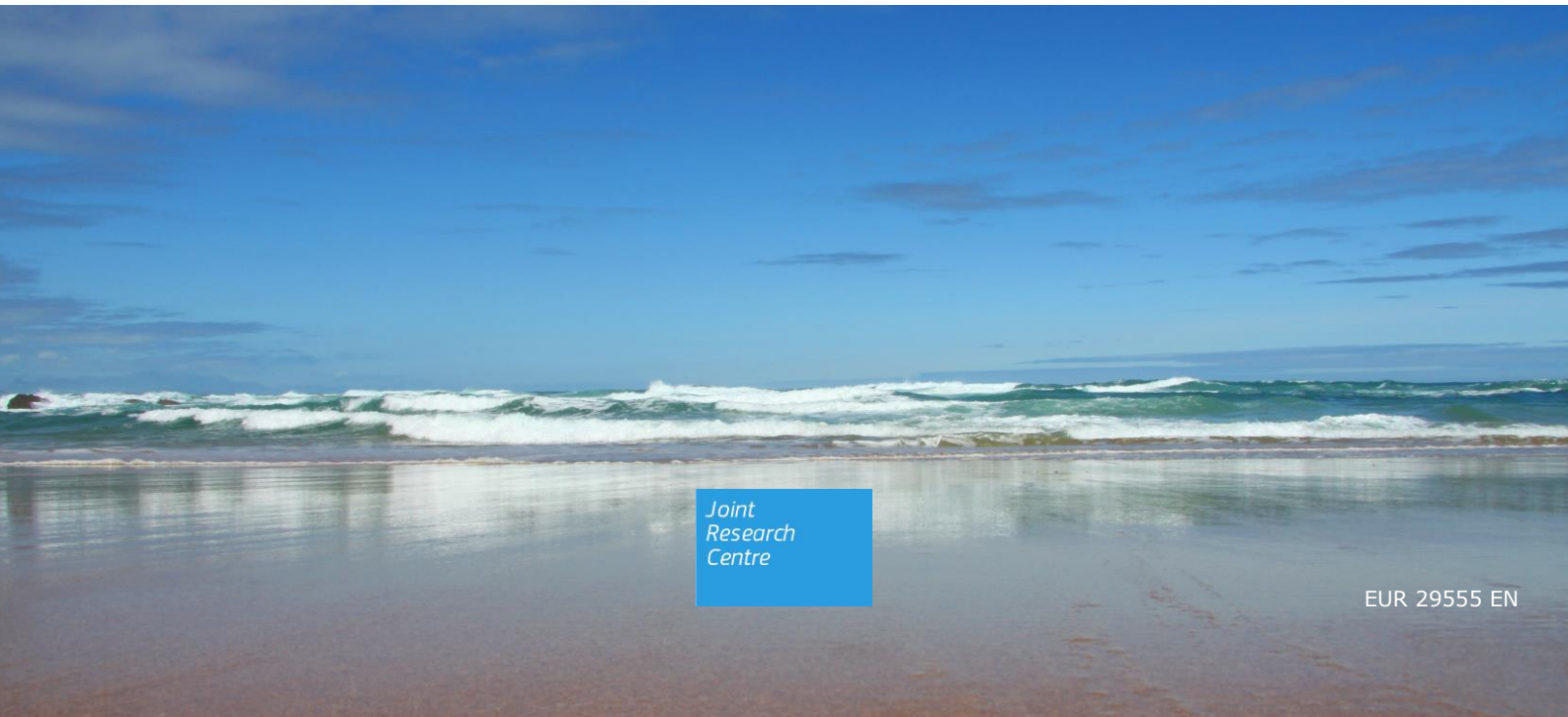
2018



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## Abstract

The European Water Framework Directive (WFD) requires the national classifications of good ecological status to be harmonised through an intercalibration exercise. In this exercise, significant differences in status classification among Member States are harmonized by comparing and, if necessary, adjusting the good status boundaries of the national assessment methods.

Intercalibration is performed for rivers, lakes, coastal and transitional waters, focusing on selected types of water bodies (intercalibration types), anthropogenic pressures and Biological Quality Elements. Intercalibration exercises are carried out in Geographical Intercalibration Groups - larger geographical units including Member States with similar water body types - and followed the procedure described in the WFD Common Implementation Strategy Guidance document on the intercalibration process (European Commission, 2011).

The Technical report on the Water Framework Directive intercalibration describes in detail how the intercalibration exercise has been carried out for the water categories and biological quality elements. The Technical report is organized in volumes according to the water category (rivers, lakes, coastal and transitional waters), Biological Quality Element and Geographical Intercalibration group. This volume addresses the intercalibration of the Coastal Waters-Black Sea Benthic Invertebrates Fauna ecological assessment methods.

# 1. Introduction

This report constitutes a description of the Intercalibration Exercise – Phase 3 (IC3) implemented for benthic invertebrates for Coastal Waters (CW) in the Black Sea. The intention is to fulfil gaps and weaknesses identified by ECOSTAT and the external evaluation panel (Davies 2012) for the previous phase, and contribute to the full acceptance by ECOSTAT of results obtained for the BQE benthic invertebrates during this IC. The report is not a full and detailed description of the Intercalibration process, but it compiles important issues and parts from those reports that are needed to support a better understanding and justification of the issues identified as problematic previous documents.

The final results include EQRs of Bulgaria and Romania benthic invertebrates assessment system for the common intercalibration coastal type CW-BL1.

## 2. Description of national assessment methods

*Table 1 Overview of the national assessment methods*

| Member State | Method | Included in this IC exercise |
|---|---|---|
| Bulgaria | M-AMBI*$_{(n)}$ | Yes |
| Romania | M-AMBI*$_{(n)}$ | Yes |

*Table 2 Overview of the metrics included in the national assessment methods*

| Member State | Full BQE method | Abundance [a] | Disturbance sensitive taxa | (Diversity)* | Combination rule of metrics |
|---|---|---|---|---|---|
| Bulgaria | M-AMBI*$_{(n)}$ | Abundance (ind.m$^{-2}$) | I$^{st}$ ecological group defined as sensitive species in AMBI (Grall and Glémarec, 1997; Borja *et al*. 2000) | Shannon-Wiener diversity index H', Species richness S | Arithmetic average of the min-max normalized AMBI, H', S with (Sigovini *et al*., 2013) |
| Romania | M-AMBI*$_{(n)}$ | Abundance (ind.m$^{-2}$) | I$^{st}$ ecological group defined as sensitive species in AMBI (Grall and Glémarec, 1997; Borja *et al*. 2000) | Shannon-Wiener diversity index H', Species richness S | Arithmetic average of the min-max normalized AMBI, H', S with (Sigovini *et al*., 2013) |

**M-AMBI** (Muxika *et al.*, 2007) is a multimetric index for assessing the ecological quality status of marine and transitional waters. It is based on benthic macroinvertebrates and integrates AMBI, a biotic index based on species sensitivity/tolerance, with diversity and richness, designed in this way to make it compliant with the European Water Framework Directive. It adopts a multivariate (trivariate) approach, integrating the response of three selected metrics, i.e. species richness, the Shannon-Wiener diversity index (Shannon & Weaver, 1949) and the biotic index AMBI (Borja *et al*., 2000).

A user-friendly free software is provided by the authors for direct calculation of the index. However, the exact program code is not accessible and the user is precluded from fully understanding and controlling the algorithm.

**M-AMBI*$_{(n)}$** (Sigovini *et al*., 2013) is a simplified modification of the original method M-AMBI. Sigovini *et al.* argue that factor analysis in M-AMBI should be discarded, since the index does not benefit from it anyway. Moreover, the user shall be able to fully understand and control the calculation procedures. Instead of using factor analysis, M-AMBI*$_{(n)}$ combines the metrics as an arithmetic mean of their normalized values. By substituting standardisation of metrics with their min–max normalisation, the index is transformed into the simple mean of the three equally weighted normalised metrics, therefore, becoming independent of the number of samples. In the normalization procedure, instead of the maximum and minimum of the dataset, the established Reference values of the metrics are used as maximum and the Bad extremum is used as minimum. We support the use of this approach that is more simple, stable, transparent and open.

## 2.1 Sampling and data processing

*Table 3 Overview of the sampling and data processing of the national assessment methods included in the IC exercise*

| | |
|---|---|
| Sampling/survey device | Van Veen grab 0.1 $m^{-2}$ |
| How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? | Once annually. |
| Sampling/survey months | From May to September (spring-summer season). |
| Which method is used to select the sampling/survey site or area? | The sampling sites are selected based on the following criteria:<br>• representativeness of the predominant sediment type within the water body;<br>• within depth range 15-30 m;<br>• within the presumable spatial range of impact of the existing point and/or diffuse sources of pollution;<br>• outside the presumable range of impact of point and/or diffuse sources of pollution for the reference sites. |
| How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? | At least three replicate samples per sampling station. Several sampling stations per water body taking into account the local sources of pollution, the predominant sediment type and the depth variation. |
| Total sampled area or volume, or total surveyed area, or total sampling duration on which ecological quality classification of sampling/survey site or area is based | At least 0.3 $m^2$ per sampling station. |
| Short description of field sampling/ survey procedure and processing (sub-sampling) | Van Veen grab 0.1 $m^2$ is employed as the standard macrozoobenthos sampler. Each sample is sieved through a set of stainless steel gauze sieves with a mesh size of 1.0 x 1.0 mm and 0.5 x 0.5 mm, the residue is collected in specimen containers and fixed in buffered 4-10% formaldehyde: seawater solution stained with Rose Bengal. |

## 2.2 National reference conditions

*Table 4 Overview of the methodologies used to derive the reference conditions for the national assessment methods included in the IC exercise*

| Member State | Type and period of reference conditions | Number of reference sites | Location of reference sites | Reference criteria used for selection of reference sites |
|---|---|---|---|---|
| Bulgaria | Partial reference or BQE benthic invertebrate fauna.<br>Current data (2008, 2012-2014) from real reference sites representative of the common type for zoobenthos defined as fine sand, at shallow (10-30 m) depth. | 2 sites<br>22 samples | Krapetz, Rusalka,<br>Northern Bulgarian coastal marine area | Reference sites have been identified according to the low pressures and impacts they receive in accordance with Annex V of WFD. Criteria used:<br>**Diffuse sources of pollution:**<br>• Irrigated agricultural area = 0 % within 1 km stripe along the coastline.<br>• Industrial area = 0 % within 1 km stripe along the coastline.<br>• Urban area < 10 % within 1 km stripe along the coastline. The largest settlement (Shabla) in the coastal area has a population less than 4000 people.<br>• The population density in coastal municipalities is no more than 30 people per $km^2$.<br>• The main navigation routes are outside the coastal waters, at a distance > 20 km from the reference sites. Pressure from navigation is negligible as compared to the impacted sites (Table 9).<br>• Harbours are absent within at least 20 km along the shoreline.<br>• Tourism is negligible: pressure from tourism (overnights spent per site) is 2 orders of magnitude lower than at the impacted sites (Table 9).<br>**Point sources of pollution:**<br>• Direct waste water discharge in the coastal waters is absent within at least 10 km.<br>• Indirect waste water discharge is absent within at least 5 km.<br>• The loads of pollutants (suspended solids, BOD, heavy metals, total petroleum hydrocarbons, phenols, detergents) from point sources are negligible (2- 3 orders of magnitude lower) than at the impacted sites (Table 9).<br>**Shoreline modifications and hydromorphological alterations:**<br>• Almost complete lack of groynes and other coastal defence constructions, ≤ 0.5 % of the shoreline is modified.<br>• Subsequently of the above changes in deposition/erosion are negligible. |
| Romania | | none | | |

## 2.3 National boundary setting

*Table 5 Explanations for national boundary setting of the national methods included in the IC exercise*

| **Member State** | **Type of boundary setting: Expert judgment – statistical – ecological discontinuity – or mixed for different boundaries?** | **Specific approach for H/G boundary** | **Specific approach for G/M boundary** | **BSP: method tested against pressure** |
|---|---|---|---|---|
| Bulgaria | Statistical boundary setting using EQRs relative to the reference values of the metrics. | **EQR** $_{\mathbf{Ref/High}}$ = 0.9<br>High boundary is set as a very minor deviation from the reference conditions. | **EQR** $_{\mathbf{Ref/Good}}$ = 0.68<br>Good boundary is defined as slight deviation from the reference conditions using equidistant division of the range between High boundary and Bad extremum. | Method is tested using regression analysis of M-AMBI*(n) against PIBS (pressure index for the Black Sea). Logarithmic relationship is demonstrated. |
| Romania | Statistical boundary setting using EQRs relative to reference values. | **EQR** $_{\mathbf{Ref/High}}$ = 0.9<br>As above. | **EQR** $_{\mathbf{Ref/Good}}$ = 0.68<br>As above. | As above. |

## 2.4 Results of WFD compliance checking

*Table 6 List of the WFD compliance criteria and the WFD compliance checking process and results of the national methods included in the IC exercise*

| Compliance criteria | Compliance checking conclusions |
|---|---|
| 1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad). | Yes |
| 2. High, good and moderate ecological status is set in line with the WFD's **normative definitions** (**Boundary setting procedure**). | Yes |
| Scope of detected pressures. | PIBS (Pressure index for the Black Sea) incorporates all relevant pressures including:<br>Diffuse pressure:<br>• urbanization;<br>• land based industries including industrial or commercial units, road and rail networks and associated land, port areas, airports, mineral extraction sites, dump sites, construction sites;<br>• tourism;<br>• navigation.<br>Point sources pressure:<br>• WWTP and coastal emitters discharging urban and industrial waste water directly or indirectly to the sea;<br>• loads from the point sources of BOD, detergents, heavy metals, phenols, suspended solids, total petroleum hydrocarbons. |
| Has the pressure-impact relationship of the assessment method been tested? | Yes. Regression analysis done. |
| Setting of ecological status boundaries: methodology and reasoning to derive and set boundaries. | The boundaries for M-AMBI*$_{(n)}$ were set statistically using the EQR approach as follows:<br>• **Reference conditions** were set as 0.8 percentile of the reference dataset of metrics.<br>• Distinct discontinuity between pressure and response is not identified.<br>• Clear relationship between paired metrics is not found.<br>• **High/Good boundary** is set as EQR=0.9 from reference value, which is deemed as very minor deviation from the values of the index normally associated with that type under undisturbed or nearly undisturbed conditions.<br>• Since there was no discontinuity of the method-pressure relationship, the rest of the boundaries were set by equidistant division of the range High boundary-Bad extremum (0.9/4). Thus:<br>• **Good/Moderate boundary** was set as equal to EQR=0.68 from the reference value, deemed as slight deviation from the values of the index normally associated with that type under undisturbed conditions. |

| | |
|---|---|
| | • **Moderate/Poor boundary** is equal to EQR=0.45 from reference value, deemed as moderate deviation from the values of the indices normally associated with that type under undisturbed conditions.<br>• **Poor/Bad boundary** is equal to EQR=0.23 from the reference value, deemed as major alteration from the type specific values. |
| Boundary setting procedure in relation to the pressure:<br>Which amount of data/pressure indicators have been related to the method and what was the outcome of the relation? | The PIBS (Pressure index for the Black Sea) was calculated using PCA and multiple regression to define the weights of different pressures. PIBS integrates data for Urban and Industrial area from CORINE landcover; Loads from point sources of biological oxygen demand, detergents, heavy metals, phenols, suspended solids, total petroleum hydrocarbons; Tourism – overnights spent; Navigation – density of AIS positions data as an indicator for the shipping intensity.<br>There is logarithmic relationship established between PIBS and M-AMBI*(n).<br>Since discontinuity of the relationship between PIBS and M-AMBI*(n) was not detected the boundaries were set using the EQR approach and equidistant division of the High-Bad range. |
| Reference and Good status community description:<br>Is a description of the communities of reference/high–good–moderate status provided? Not only a formula or an EQR value, but the range of values for the different parameters included in the method that result in high-good-moderate status. | Communities description is provided of reference/high–good–moderate–poor status (See Chapter 6).<br>The range of values for the different parameters (H', S, AMBI) included in the method (M-AMBI*$_{(n)}$) that result in high-good-moderate status are provided (See Table 14). |
| 3. **All relevant parameters** indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole. | Abundance, sensitive and pollution indicative groups (AMBI) and diversity measures (H', S) are covered.<br>The combination rule is defined in the calculation of M-AMBI*$_{(n)}$. |
| Complete list of biological metric(s) used in assessment. | Taxonomic list<br>Abundance per species.<br>S Species richness<br>H' Shannon-Wiener diversity index<br>Each species assigned to one of 5 ecological groups: sensitive, indifferent, tolerant, opportunists 1st order, opportunists 2nd order (Grall and Glémarec, 1997).<br>AMBI (Borja *et al.*, 2000)<br>M-AMBI*$_{(n)}$ (Sigovini *et al.*, 2013) |
| Data basis for metric calculation. | **In total 63 sampling dates** and 33 site-years including:<br>• RO: 41 sampling dates from 2003-2009<br>• BG: 22 sampling dates from (2008)2012-2014 |

| | |
|---|---|
| | • 1 – 4 sampling occasions per site-year.<br>**In total 97 taxa**<br>Per sampling date:<br>• Species list<br>• Abundance (raw data matrix)<br>• AMBI<br>• 5 AMBI sensitivity classes<br>• Shannon-Wiener diversity H'<br>• Taxa richness S<br>• M-AMBI* $_{(n)}$. |
| Combination rule for multimetrics. | M-AMBI*$_{(n)}$ calculated as the arithmetic mean of the min-max normalized AMBI, H and S. (Sigovini *et al*., 2013). |
| 4. Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT. | The assessment is adapted to common intercalibration type that is defined as **fine sand, shallow.** The substrate type and the depth are identified as the principal drivers of the zoobenthic communities. The common type is also characterised as mesohaline. Regarding the exposure conditions the common type is defined as very exposed in Bulgaria, while moderately exposed in Romania. Regardless of the different exposure definitions in the two member states, there is no actual exposure difference since all sampling sites included in the intercalibration exercise are situated along open coasts facing into the predominant winds and waves from North-East direction. |
| Is the assessment method applied to water bodies in the whole country? | Yes, the assessment method is applied to all water bodies belonging to the common type. |
| Specify common intercalibration types. | In terms of the benthic invertebrate fauna GIG Black Sea identified one common type in the coastal waters described according to the factors for System B as: microtidal, mesohaline, very exposed (open coast facing into the predominant East and North-East winds), shallow, fine sand. |
| Does the selection of metrics differ between types of water bodies? | No |
| 5. The water body is assessed against **type-specific near-natural reference conditions.** | Yes |
| Scope of reference conditions. | Type specific reference conditions for shallow coastal waters with predominant fine sand. |
| Key source(s) to derive reference conditions. | Monitoring data of IO-BAS at reference sites from recent period (2008, 2012-2014). |
| Number of sites, location and geographical coverage of sites used to derive reference conditions. | 2 sites, Krapetz and Rusalka, Northern Bulgarian Black Sea coastal marine waters.<br>22 sampling dates, 4 sampling years. |
| Time period (months+years) of data of sites used to derive reference conditions. | October 2008, July 2012, June 2013, July 2014 |

| | |
|---|---|
| Reference site characterisation: criteria to select them. | See Table 4 above. |
| Is a true reference used for the definition of High status or an alternative benchmark estimation? | Partial reference sites for the invertebrate benthic fauna. |
| 6. Assessment results are expressed as **EQRs:**<br>- Are the assessment results expressed as Ecological Quality Ratios (EQR)? | Yes |
| 7. Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time.**<br>See info from WISER Questionnaires: | Yes |
| Has the uncertainty of the method been quantified and is it regarded in the assessment? | Yes |
| Specify how the uncertainty has been quantified and regarded. | Sampling replication per site, several sites per water body, calculation of mean and StDev statistics to demonstrate the variation. |
| 8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure.** | Yes |
| 9. Selected taxonomic level achieves adequate **confidence and precision** in classification. | Yes |
| Minimum size of organisms sampled and processed. | 1 mm |
| Record of biological data: level of taxonomical identification – what groups to which level. | The three major taxonomic groups in the Black Sea macrozoobenthos – Polychaeta, Mollusca and Crustacea are identified to the species level. These taxa are generally dominant in the abundance and/or biomass. Anthozoa, Echinodermata, Cephalochordata, Phoronidea and Pantopoda are also identified to the species level, since sufficient taxonomic expertise and keys are available. Nemertini, Turbellaria and Oligochaeta, are identified to higher taxonomic level (Phylum or Class), generally considered acceptable for routine monitoring purposes. |

**General conclusion of the compliance checking:**

WFD compliance criteria are met. M-AMBI*(n) meets the requirements stated in the WFD IC Guidance (2.1. WFD compliance criteria). Good ecological status boundary for M-AMBI*(n) complies with the WFD normative definitions.

# 3. Results IC feasibility checking

## 3.1 Typology

The Intercalibration is feasible in terms of typology? Yes

| Method | Appropriate for IC types/subtypes | Remarks |
|---|---|---|
| M-AMBI*$_{(n)}$ | The method was applied for the IC Type defined according to the factors for System B as: coastal waters - Black Sea, microtidal, mesohaline, very exposed (open coast facing into the predominant East and North-East winds), shallow, fine sand. | **Correction of the common type CW-BL1 is required previously described as "moderately exposed, mixed substratum".** |

## 3.2 Pressures addressed

*Table 7 Pressures addressed by the national methods and overview of the relationship between national methods and the pressures*

| Member State | Method/ Metrics tested | Pressure | Pressure indicators | Amount of data | Strength of relationship |
|---|---|---|---|---|---|
| Bulgaria and Romania | M-AMBI*$_{(n)}$ | Diffuse: urbanization, land based industries, tourism, navigation, Point: WWTP and coastal emitters of waste water and pollutants. | Urban and industrial area % within 1 km stripe of coastal territory from CORINE land cover.<br>Loads from point sources of suspended solids, BOD, heavy metals, phenols, total petroleum hydrocarbons and detergents.<br>Overnights spent by tourists.<br>Shipping intensity measured as density of AIS data points.<br>The pressure indicators were combined in a Pressure Index for the Black Sea using PCA and multiple regression analyses. | 62 paired data of PIBS- M-AMBI*$_{(n)}$ | At sample level:<br>$R^2$ = 0.3351<br>Averaged at the year/site level:<br>$R^2$ = 0.4518<br>Averaged at the site level:<br>$R^2$ = 0.8359 |

The dataset of pressures used in the IC is presented in Table 8. Mean data per site (years not separated, n = 8) were used. The following pressures and pressure indicators were addressed:

- Point sources: **annual mean loads** for BOD, suspended solids (SS), total heavy metals (HMET), detergents (DET), phenols (PHE), total petroleum hydrocarbons (TPH).
- Diffuse sources: **land use based on CORINE landcover** within 1000 m stripe of coastal territory including:

  **Urban area:** includes the categories 111 Continuous urban fabric, 112 Discontinuous urban fabric.

  **Industrial area:** includes the categories 121 Industrial or commercial units, 122.

  Road and rail networks and associated land, 123 Port areas, 124 Airports, 131

  Mineral extraction sites, 132 Dump sites, 133 Construction sites.
- Diffuse sources: **tourism** – input data are overnights spent in the coastal municipalities; relative pressure values at the monitoring sites were calculated in GIS using Spatial Analysis Tool.
- Diffuse sources: **navigation** – input data is density map of AIS positions data (available at http://www.marinetraffic.com), relative pressure values were calculated in GIS using Spatial Analysis Tool.

*Table 8 Dataset of pressures*

| Station | ID | BOD | DET | HMET | PHE | SS | TPH | Tourism | Navigation | Urban | Industrial |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cazino Mamaia | 1 | 0.0009172 | 0.0000107 | 0.0023302 | 0.0000003 | 0.0009984 | 0.0000011 | 1797 | 1.0 | 19.3 | 0.1 |
| Est Constanta | 2 | 0.0007104 | 0.0000083 | 0.0000083 | 0.0000002 | 0.0007677 | 0.0000003 | 2078 | 0.9 | 24.3 | 51.9 |
| Eforie Sud | 3 | 0.0030261 | 0.0001160 | 0.0052376 | 0.0000006 | 0.0043884 | 0.0000131 | 5618 | 0.0 | 13.2 | 25.9 |
| Costinesti | 4 | 0.0001056 | 0.0000077 | 0.0000984 | 0.0000000 | 0.0001670 | 0.0000003 | 3974 | 0.4 | 17.1 | 1.5 |
| Mangalia | 5 | 0.0018098 | 0.0000709 | 0.0029425 | 0.0000003 | 0.0012699 | 0.0000048 | 4292 | 0.5 | 6.7 | 19.4 |
| Vama Veche | 6 | 0.0008775 | 0.0000344 | 0.0014267 | 0.0000001 | 0.0006158 | 0.0000024 | 2690 | 0.0 | 3.5 | 15.9 |
| Krapets | 7 | 0.0000034 | 0.0000001 | 0.0000582 | 0.0000000 | 0.0000103 | 0.0000020 | 56 | 0.0 | 10.0 | 0.0 |
| Rusalka | 8 | 0.0000000 | 0.0000000 | 0.0000000 | 0.0000000 | 0.0000000 | 0.0000000 | 75 | 0.4 | 8.1 | 0.0 |

Loads of BOD – Biological oxygen demand, DET – detergents, HMET – heavy metals, PHE – phenols, SS – suspended solids, TPH – total petroleum hydrocarbons. Color corresponds to pressure intensity. Tourism – relative values, calculated in GIS using information on nights spent; Navigation – relative value, calculated in GIS using density map of AIS positions data (available at http://www.marinetraffic.com/en/ais/home/ :7647f63edf677cc913b6af1b46b8ad49); Urban and Industrial = Corine Land Cover.

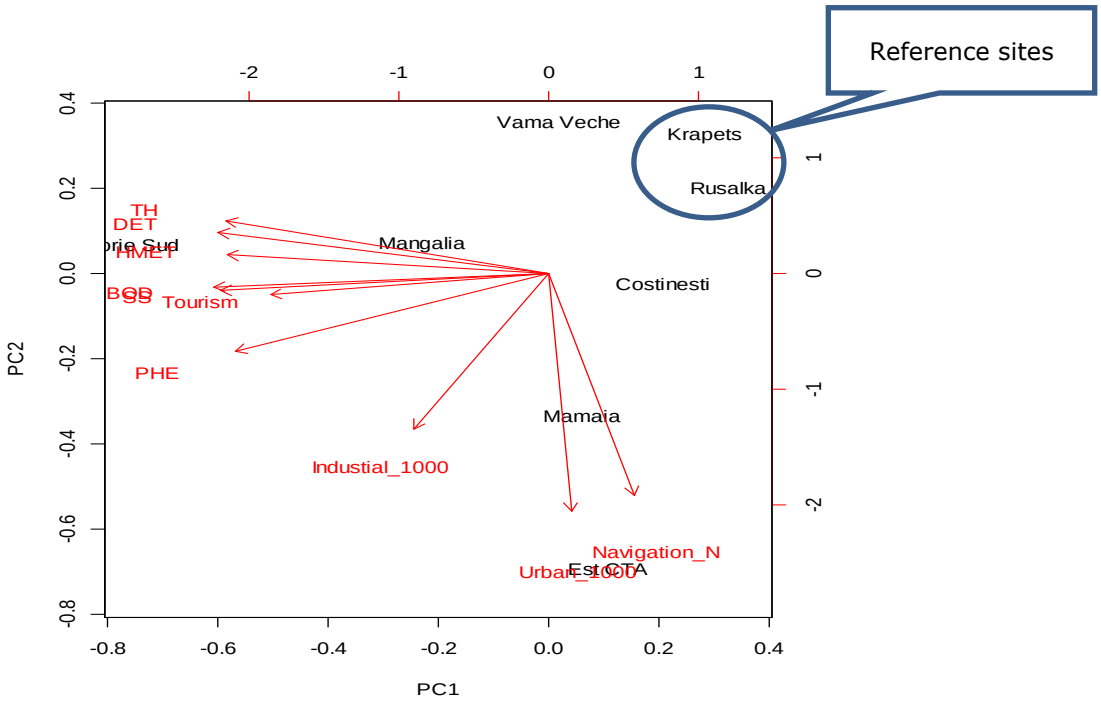


*Figure 1 PCA of the pressures at sites. Arrows indicate the direction of increasing pressures*

PCA was performed on the standardized pressure data. PCA analysis of the pressures validates Krapets and Rusalka as reference sites with the lowest level of pressure (Figure 1).

The pressures were combined based on the Spearman correlation with PCA axis 1 and 2 (Table 9). Thus 2 pressure metrics were generated: "pressure_point" combines the pressures with high correlation with PCA axis 1 (Loads from point sources of pollution, Tourism and Industrial area), while "pressure_diffuse" combines the pressures with high correlation with PCA axis 2 (Navigation and Urban area). Multiple regression of the 2 pressure metrics with M-AMBI*$_{(n)}$ was performed and used to set weights according to coefficients in the regression. A final Pressure Index for the Black Sea (PIBS) was calculated according to the equation shown in Table 9.

Regression of M-AMBI*$_{(n)}$ against PIBS at the level of samples, year-site averaged data and site averaged data was performed, the results shown on Figures 2-4. A logarithmic relationship was demonstrated. $R^2$ increases from sample to year-site and site averaged data. This can be explained with the fact that PIBS was also calculated using averaged data for the loads, while urban and industrial areas from Corine Land Cover are invariable, therefore do not reflect the temporal trends for the diffuse pressures.

*Table 9 Steps in the calculation of the Pressure Index for the Black Sea*

| correlation coefficients (Spearman) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **BOD** | **DET** | **HMET** | **PHE** | **SS** | **TH** | **Tourism** | **Navigation** | **Urban** | **Industrial** |
| **PC1** | -0,976 | -1,000 | -0,905 | -0,881 | -0,929 | -0,857 | -0,810 | 0,073 | 0,143 | -0,707 |
| **PC2** | -0,214 | -0,071 | -0,024 | -0,429 | -0,381 | 0,286 | -0,167 | -0,805 | -0,833 | -0,419 |

| rounded to 1 digit | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **PC1** | -1 | -1 | -0,9 | -0,9 | -0,9 | -0,9 | -0,8 | | | -0,7 |
| **PC2** | | | | | | | | -0,8 | -0,8 | |

**combined to 2 pressure 'metrics'**

**pr_point = (1 BOD + 1 DET + 0.9 HMET + 0.9 PHE + 0.9 SS + 0.9 TH + 0.8 Tourism + 0.7 Industrial) / 7.1**

**pr_diff = (0.8 Navigation + 0.8 Urban) / 1.6**

| weights from multiple regression | | | |
|---|---|---|---|
| | regression using: | samples | siteyears | sites |
| **pr_point** | | -0,079 | -0,078 | -0,049 |
| **pr_diff** | | -0,050 | -0,044 | -0,038 |
| **R2** | | 0,29 | 0,38 | 0,32 |
| **adj. R2** | | 0,27 | 0,34 | 0,05 |
| **p** | | <0,001 | <0,001 | >0,05 |

**final Pressure Index for the Black Sea**

**PIBS (8 pr_point + 5 pr_diff) / 13**

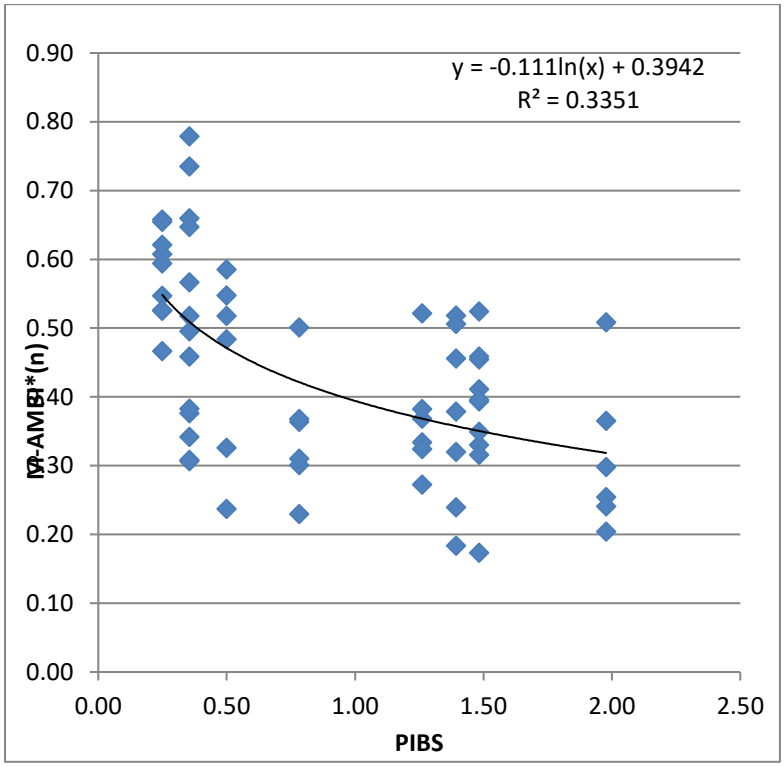


*Figure 2 PIBS to M-AMBI*$_{(n)}$ relationship calculated at the samples level*

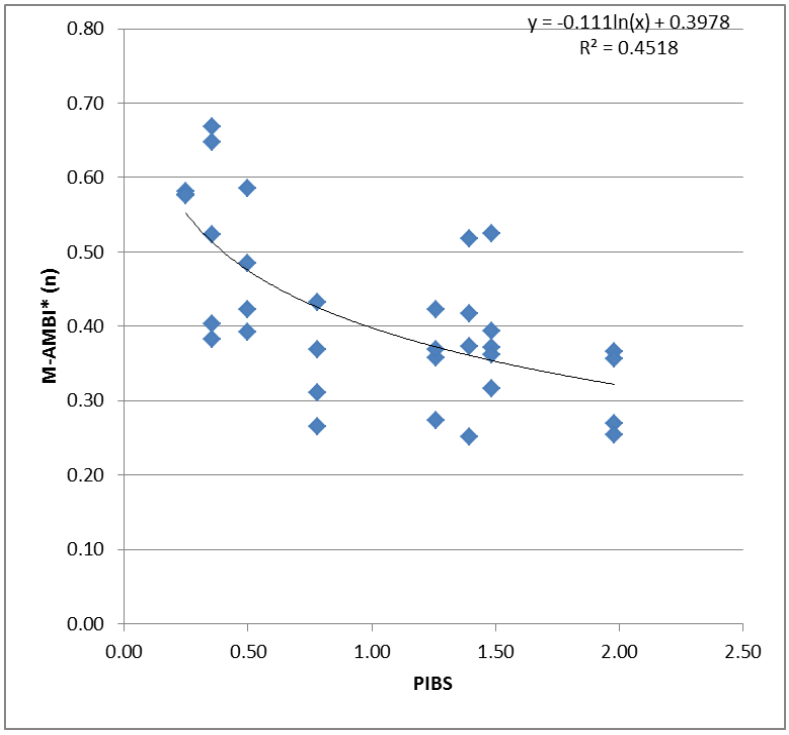


*Figure 3 PIBS to M-AMBI*$_{(n)}$ relationship calculated at the year-site level averaged data*

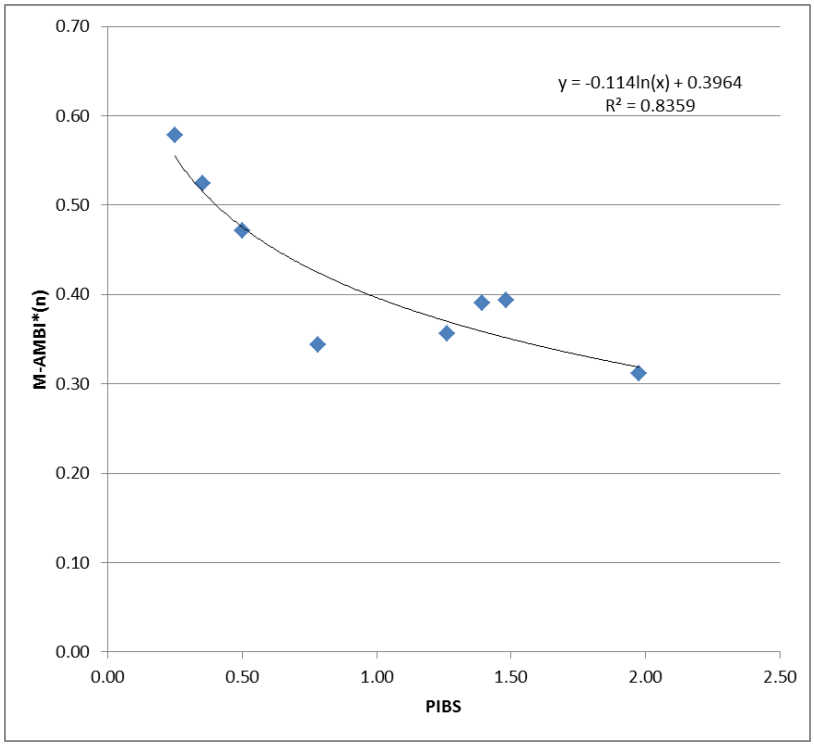


*Figure 4 PIBS to M-AMBI*$_{(n)}$ relationship calculated at the site level averaged data*

| Method | Pressure |
|---|---|
| M-AMBI*$_{(n)}$ | PIBS (Pressure index for the Black Sea) |
| **Conclusion**<br>The Intercalibration is feasible in terms of **pressures** addressed? Yes. M-AMBI*$_{(n)}$ shows clear relationship with the gradient of the overall relevant pressures reflected in PIBS that is calculated in the same way for the Bulgarian and Romanian sites. | |

## 3.3 Assessment concept

| Method | Assessment concept |
|---|---|
| M-AMBI*$_{(n)}$ | The overall assessment concept is based on the principles that disturbance –sensitive taxa decrease, while tolerant and opportunistic species increase along the increasing pressure gradient, coupled with decrease in species richness and evenness of distribution. These two aspects of the invertebrate community change are reflected by AMBI and S/H' respectively, combined in a composite index M-AMBI*$_{(n)}$. |

**The Intercalibration is feasible in terms of assessment concept?**

Yes, the same method is used by Bulgaria and Romania and therefore, the intercalibration is feasible in terms of assessment concept.

# 4. Collection of IC dataset and benchmarking

## 4.1 Dataset description

*Table 10 Description of the data collection within the GIG*

| | |
|---|---|
| Size of common dataset: total number of sites | 8 |
| Number of Member States | 2 |
| Repackage/disaggregation of samples/ WB results? | No |
| Gradient of ecological quality | from High to Poor status |
| Coverage per ecological quality class | 16% of the dataset at High status, 35% at Good status, 40% at Moderate status, 10% at Bad status |

*Table 11 Overview of the data set*

| **Member State** | **Number of sites or samples or data values** | | |
|---|---|---|---|
| | **Biological data** | **Physico- chemical data** | **Pressure data** |
| Bulgaria | 2 sites<br>22 samples from (2008) | Not available | Mean data for 2 sites:<br>Loads – 12 data values;<br>Landuse – 4 data values;<br>Tourism – 2 data values;<br>Navigation – 2 data values<br>Table 8 |
| Romania | 6 sites<br>41 samples | Not available | Mean data for 6 sites:<br>Loads – 36 data values;<br>Tourism – 6 data values;<br>Navigation – 6 data values<br>Table 8 |

## 4.2 Data acceptance criteria

*Table 12 List of data acceptance criteria used for the data quality control and describe the data acceptance checking process and results*

| **Data acceptance criteria** | **Data acceptance checking** |
|---|---|
| Data requirements (obligatory and optional) | Obligatory:<br>Data shall come from fine sand, at depth 10-30 m; Samples are collected in spring-summer season.<br>Sample size is 0.1 $m^{-2}$.<br>Abundance data is standardized to $m^2$.<br>The main Black Sea taxonomic groups Polychaeta, Mollusca and Crustacea are identified to the species level.<br>Nomenclature is according to WoRMS.<br>Metadata are provided including the sampling date, site name, geographic coordinates, depth and substrate type. |
| The sampling and analytical methodology | The same sampling and analytical methodology is applied (Todorova and Konsulova, 2005). |

| Data acceptance criteria | Data acceptance checking |
|---|---|
| Level of taxonomic precision required and taxa lists with codes | The same level of taxonomic precision is applied. The three major taxonomic groups in the Black Sea macrozoobenthos – Polychaeta, Mollusca and Crustacea are identified to the species level. Anthozoa, Echinodermata, Cephalochordata, Phoronidea and Pantopoda are also identified to the species level. Nemertini, Turbellaria and Oligochaeta, are identified to higher taxonomic level (Phylum or Class), generally considered acceptable for routine monitoring purposes. Taxa lists are provided with nomenclature according to WoRMS (http://www.marinespecies.org). |
| The minimum number of sites / samples per intercalibration type | At least two sites per country. |
| Sufficient covering of all relevant quality classes per type | High to poor ecological classes are covered. |

## 4.3 Common benchmark: IC reference conditions or alternative benchmark

Common reference conditions have been defined. The common dataset contains 2 sites in near-natural conditions.

- Summary of the common approach for setting reference conditions (true reference sites or indicative partial reference sites, see Annex III of the IC guidance):

Both reference sites are situated along the northern Bulgarian coast. The coastal area is sparsely populated, the arable land is not irrigated, industrial area is absent, and tourism is very limited in terms of overnights spent and touristic season duration (3 months). Due to absence of commercial harbours the main navigation routes do not cross the coastal waters.

The reference conditions are considered as partial with regards to only the BQE benthic invertebrate fauna. Since the northern Bulgarian Black Sea is influenced by the Danube waters, the phytoplankton community may demonstrate moderate ecological quality due to increased nutrient levels, especially in spring. However, since the mid-1990s, there has been enough scientific evidence of the Black Sea ecosystem recovery from the anthropogenic eutrophication experienced in the 1970-1980s due to the "green revolution" in agriculture within the catchments of the rivers discharging in the North-Western Black Sea. Moreover, during the recent period used as reference, hypoxia in the bottom waters associated with phytoplankton blooms and consequent mortality of benthic invertebrates have not been observed, therefore the conditions are believed to have recovered to near-natural with regards to zoobenthos. Romania also uses the Bulgarian reference sites for developing the classification system in the common type of coastal waters.

- Detailed description of **reference criteria** for screening of sites in near-natural conditions (abiotic characterisation, pressure indicators):

Reference sites have been identified according to the low pressures and impacts they receive in accordance with Annex V of WFD. The Criteria and indicators used include the following:

**Diffuse sources of pollution:**

- Irrigated agricultural area = 0 % within 1 km stripe along the coastline.
- Industrial area = 0 % within 1 km stripe along the coastline.
- Urban area < 10 % within 1 km stripe along the coastline.
- The population density in the coastal municipalities is ≤ 30 people per $km^{-2}$.

- The main navigation routes are outside the coastal waters, at a distance > 20 km from the reference sites. Pressure from navigation is negligible as compared to the impacted sites (Table 8).
- Harbours are absent within at least 20 km along the shoreline from the reference site.
- Tourism is negligible: pressure from tourism (overnights spent per site) is 2 orders of magnitude lower than at the impacted sites (Table 8).

**Point sources of pollution:**

- Direct waste water discharge in the coastal waters is absent within at least 10 km from the reference site.
- Indirect waste water discharge is absent within at least 5 km from the reference site.
- The loads of pollutants (suspended solids, BOD, heavy metals, total petroleum hydrocarbons, phenols, detergents) from point sources are negligible (2- 3 orders of magnitude lower) than at the impacted sites (Table 8).

**Shoreline modifications and hydromorphological alterations:**

- Less than 0.5 % of the shoreline is modified due to groynes and other coastal defence constructions.
- Subsequently of the above, changes in deposition/erosion are negligible.
- Identification of the **reference sites:** Two monitoring sites along the northern Bulgarian coast have been identified as suitable reference sites for the IC type shallow fine sand: Krapets (43.5875 N; 28.5917E) and Rusalka (43.4243N; 28.5534 E).
- Description of **setting reference condition**s (summary statistics used):

The reference conditions for AMBI, S, H and M-AMBI*$_{(n)}$ were calculated from the reference sites dataset (n=22) as the percentile 0.2 for AMBI and the percentile 0.8 for S, H and M-AMBI*$_{(n)}$. The following statistics were calculated for reference conditions:

AMBI = 3
H = 3
S = 27
M-AMBI*$_{(n)}$ = 0.97

**Description of the biological communities at reference sites:**

The reference benthic invertebrates' community in fine sand at depth 10-20 m in the Black Sea is characterized by the occurrence of species belonging to all five ecological groups: sensitive, indifferent, tolerant, and opportunist of 1$^{st}$ and 2$^{nd}$ rank. The characteristic sensitive species include the bivalves *Chamelea gallina, Tellina tenuis, Paphia aurea, Spisula subtruncata, Parvicardium exiguum*, *Acanthocardia paucicostata,* the gastropods *Retusa umbilicata* and the polychaetes *Aricidea claudiae*. These are suspension feeders and grazers feeding on the primary producers phytoplankton and benthic microalgae respectively. Typical indifferent to organic enrichment species include the bivalves *Lentidium mediterraneum, Pitar rudis*, the polychaete *Nephtys hombergii*, the amphipods *Ampelisca diadema* and *Perioculodes longimanus*. These are filter feeders or predators feeding on primary producers or other animals. Overall, the sensitive and indifferent benthic invertebrates belong to the grazing food chain that is based upon the primary producers and is usually indicative of oligo- to mesotrophic conditions. The two groups combined comprise on the average around 25% of the community total abundance.

Considerable proportion (around 30%) of tolerant to organic enrichment invertebrates may occur in the community structure at reference conditions. The tolerant to organic enrichment species, typical of the reference sites comprise mainly surface deposit

feeders and facultative filter feeders such as: the bivalves *Abra prismatica* and *Cerastoderma glaucum,* the polychaetes *Melinna palmata* and *Spio filicornis.* Being part of the detritus food chain the tolerant species indicate slight/moderate organic enrichment of the sediments. The reference community is also characterized by relatively high species richness S ($S_{reference}$=27) and community diversity H' ($H'_{reference}$=3) as compared to the impacted sites.

**Description of boundary setting procedure set for the common IC type:**

**Step 1**

The pressure criteria and specific values for type-specific reference conditions are described in detail above.

Two reference sites – Krapets and Kaliakra were identified in the Northern Bulgarian coastal marine area. These are used as reference for both Bulgaria and Romania, since the Romanian coastal waters did not meet the agreed pressure criteria for reference conditions.

The biological dataset from the reference sites comprises n=22.

**Reference conditions** were quantified by using the following statistic:

- the percentile 0.8 from the reference sites dataset for S, H and M-AMBI*$_{(n)}$;
- the percentile 0.2 from the reference sites dataset for AMBI.

The following values were calculated for the reference conditions:

AMBI = 3

H = 3

S = 27

M-AMBI*(n) = 0.97

**High/Good boundary**

High/Good boundary is set as EQR=0.9 from reference value for each of the metrics used S, H', AMBI and M-AMBI* (n)

EQRs of 0.9 is deemed as **very minor deviation** from the values of the indices normally associated with that type under undisturbed or nearly undisturbed conditions.

Since AMBI=3 corresponds to $EQR_{AMBI}$=1, and AMBI=6 corresponds to $EQR_{AMBI}$=0, a linear relationship is established between AMBI and $EQR_{AMBI}$ according to the equation:

$$EQR_{AMBI}=-0.3333*AMBI+2$$

Thus, the class boundaries for AMBI are set following the equation above.

**Step 2**

Description of the zoobenthos biological community and a conceptual model how it changes along the pressure gradient:

**At Reference/High status** the benthic invertebrate community is characterised by high species richness and diversity, high abundance, significant proportion of sensitive to pollution species (predators and filter feeders), and dominance of tolerant to organic enrichment species (surface deposit feeders), due to naturally mesotrophic conditions in the North-Western Black Sea (Figure 5).

The characteristic sensitive species include the bivalves *Chamelea gallina, Tellina tenuis, Paphia aurea, Spisula subtruncata, Parvicardium exiguum*, *Acanthocardia paucicostata,* the gastropods *Retusa umbilicata* and the polychaetes *Aricidea claudiae*.

**At Good status** the community is characterised by presence of sensitive to pollution species, although their relative proportion decreases. Indifferent species (e.g. *Lentidium mediterraneum, Pitar rudis*, *Nephtys hombergii*, *Ampelisca diadema, Perioculodes longimanus*) and tolerant to organic enrichment species (e.g. *Abra prismatica, Cerastoderma glaucum, Melinna palmata, Spio filicornis, Prionospio cirrifera*) become dominant in the abundance structure (Figure 5). Diversity and abundance are high.

**At Moderate status** a major functional shift in the community structure is evident, notable by the disappearance of the sensitive species. The indifferent species are still present. The tolerant species (e.g. *Alitta succinea, Prionospio cirrifera, Ampelisca diadema*) and the opportunists (e.g. *Polydora cornuta, Capitella capitata*, Oligochaeta) become dominant in the abundance structure. Richness and diversity decrease, while abundance is still high.

**At Poor status** the indifferent species disappear as well. The abundance is distributed between only tolerant and opportunistic species, the latter being dominant. Richness and diversity are low. The abundance decreases as well.

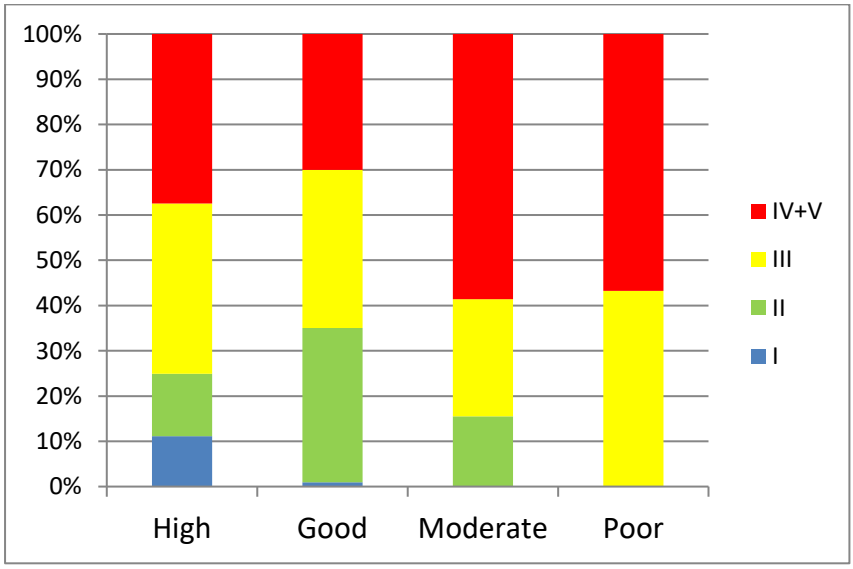


*Figure 5 Proportion of the ecological groups abundance in the benthic community structure at high, good, moderate and poor status*

**Step 3**

M-AMBI* (n) is selected as a suitable composite metric of the BQE benthic invertebrate fauna since it reflects the changes in the relative proportion of sensitive to tolerant/opportunistic species on the one hand and the richness/biodiversity changes on the other hand in response to increasing pressure.

A clear relationship between the metric and the pressure gradient is demonstrated (Figures 2-4).

**Step 4.** No clear and significant discontinuities were observed in the relation between pressure (PIBS) and response (M-AMBI*$_{(n)}$) as evident on Figures 2-4.

**Step 6.** Paired metrics analysis gave unclear results, not convincing to set boundaries.

**Step 8.** Since the relationship between the M-AMBI*(n) and the pressure gradient is a continuum and Step 6 failed to identify boundaries based on paired metric assessments the last option was used - dividing the continuum of metric values between the High/Good boundary established in Step 1 and the Bad extrema (0 for S, H', and M-AMBI $*_{(n)}$ and 6 for AMBI) into four equal width classes. Using the EQR approach the equidistant boundaries were set by dividing EQR=0.9/4.

**Good/Moderate boundary**

EQR=0.68 from reference is set as Good/Moderate boundary.

EQR of 0.68 is deemed as **slight deviation** from the values of the indices normally associated with that type under undisturbed conditions.

**Moderate/Poor boundary**

EQR=0.45 from reference value is set as Moderate/Poor boundary.

EQR of 0.45 is deemed as **moderate deviation** from the values of the indices normally associated with that type under undisturbed conditions.

**Poor/Bad boundary**

EQR=0.23 from reference value is set as Poor/Bad boundary.

EQR of 0.23 and below is deemed as **major alteration** from the type specific values of the metrics.

Any values below these boundaries are considered as a **severe alteration** from the type specific values and qualified as bad status.

The classification system for the metrics derived as described above is summarized in Table X-3.

The boundaries themselves are allocated to the upper class, e.g. EQR 0.9 = High, EQR 0.68 = Good, etc.

*Table 13 Classification system with boundary values and EQRs*

| | EQR | AMBI | H | S | M-AMBI*(n) |
|---|---|---|---|---|---|
| **Reference** | **1** | **3** | **3.0** | **27** | **0.97** |
| High/Good | 0.9 | 3.30 | 2.70 | 24 | 0.87 |
| Good /Moderate | 0.68 | 3.96 | 2.04 | 18 | 0.66 |
| Moderate/Poor | 0.45 | 4.65 | 1.35 | 12 | 0.44 |
| Poor/Bad | 0.23 | 5.31 | 0.69 | 6 | 0.22 |

## 4.4 Benchmark standardisation

No biogeographical differences. Benchmark standardization is not necessary. Common dataset has been used for the establishment of common boundaries.

# 5. Comparison of methods and boundaries

## 5.1 IC option and common metrics

Which IC option did you use?

Option 1 has been used. The choice of the IC option is based on the premises that the two member states Bulgaria and Romania in GIG Black Sea acquire data on the BQE benthic invertebrate fauna in the same way including the sampling, sample analyses and the numerical evaluation procedures and use a common assessment method. A common dataset was established for the BQE benthic invertebrate fauna and for the relevant pressures. The assessment method used by both member states is the composite metric M-AMBI*$_{(n)}$. Reference conditions based on agreed criteria were set for the invertebrate fauna. An agreed boundary setting procedure was applied. Good ecological status class boundary values were accepted for M-AMBI*$_{(n)}$.

# 6. Final results to be included in the EC

## 6.1 Table with EQRs

*Table 14 Overview of the IC results for the national methods.*

| Biological Quality Element | Benthic invertebrate fauna | | |
|---|---|---|---|
| **Results coastal waters: Ecological quality ratios of national classification systems** | | | |
| **Country** | **National classification systems intercalibrated** | **Ecological Quality Ratios** | |
| | | **High-Good boundary** | **Good-Moderate boundary** |
| Bulgaria | M-AMBI*$_{(n)}$ | 0.9 | 0.68 |
| Romania | M-AMBI*$_{(n)}$ | 0.9 | 0.68 |

## 6.2 Correspondence common types versus national types

The common type corresponds to the following national types:

| Country | Code | Type | Remark |
|---|---|---|---|
| BG | CW1N | Mesohaline, very exposed, shallow, sand | The correspondence between the national types and the common IC type refers only to the quality element benthic invertebrate fauna in fine sand. |
| BG | CW3.1 | Mesohaline, very exposed, shallow, mixed (hard, sand) | |
| BG | CW3.2 | Mesohaline, very exposed, shallow, mixed (hard, sand, mud) | |
| BG | CW3.3 | Mesohaline, very exposed, shallow, mixed (hard, sand, coarse sediments) | |
| BG | CW5.1 | Mesohaline, moderately exposed, shallow, mixed (hard, sand, mud) | |
| BG | CW5.2 | Mesohaline, moderately exposed, shallow, mixed (hard, sand, coarse sediments) | |
| RO | RO_CT01 | Mesohaline, moderately exposed, shallow, sand | Correspondence between national types and common IC type refers to the quality element benthic invertebrate fauna in fine sand that constitutes the most part of the sediment in the coastal water bodies defined in Romania. |
| RO | RO_CT02 | Mesohaline, moderately exposed, shallow, mixed (hard, coarse sediments, sand) | |

# 7. Ecological characteristics

## 7.1 Description of reference of alternative benchmark communities

The reference benthic invertebrate community is characterised by high species richness and diversity, high abundance, significant proportion of sensitive to pollution species (predators and filter feeders), and dominance of tolerant to organic enrichment species (surface deposit feeders), due to naturally mesotrophic conditions in the North-Western Black Sea.

The characteristic sensitive species include the bivalves *Chamelea gallina, Tellina tenuis, Paphia aurea, Spisula subtruncata, Parvicardium exiguum*, *Acanthocardia paucicostata,* the gastropods *Retusa umbilicata* and the polychaetes *Aricidea claudiae*.

## 7.2 Description of good status communities

**At Good status** the community is characterised by presence of sensitive to pollution species, although their relative proportion decreases. Indifferent species (e.g. *Lentidium mediterraneum, Pitar rudis*, *Nephtys hombergii*, *Ampelisca diadema, Perioculodes longimanus*) and tolerant to organic enrichment species (e.g. *Abra prismatica, Cerastoderma glaucum, Melinna palmata, Spio filicornis, Prionospio cirrifera*) become dominant in the abundance structure. Diversity and abundance are high.

# 8. Conclusion

The method proposed by the Romania and Bulgaria meets the WFD compliance criteria, and responds to the general degradation.

A proposal for common class boundaries has been established in basis on a common dataset built for the BQE benthic invertebrate fauna and for the relevant pressures.

The class boundaries will be applied for the establishment of high and good ecological status in the water bodies of the national types included in the common Intercalibration types.

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## List of abbreviations and definitions

### *Key Terms:*

Assessment method: The biological assessment for a specific biological quality element, applied as a classification tool, the results of which can be expressed as EQR.

Biological Quality Element (BQE): Particular characteristic group of animals or plants present in an aquatic ecosystem that is specifically listed in Annex V of the Water Framework Directive for the definition of the ecological status of a water body (for example phytoplankton or benthic invertebrate fauna).

Class boundary: The Ecological Quality Ratio value representing the threshold between two quality classes.

Common Intercalibration type: A type of surface water differentiated by geographical, geological, morphological factors (according to WFD Annex II) shared by at least two Member States in a GIG.

Compliance criteria: List of criteria evaluating whether assessment methods are meeting the requirements of the Water Framework Directive.

Ecological Quality Ratio (EQR): Calculated from the ratio observed value/reference value for a given body of surface water. The ratio shall be represented as a numerical value between zero and one, with high ecological status represented by values close to one and bad ecological status by values close to zero.

Geographic Intercalibration Group (GIG): Organizational unit for the intercalibration consisting of a group of Member States sharing a set of common intercalibration types.

Intercalibration: An exercise facilitated by the Commission to ensure that the high/good and good/moderate class boundaries are consistent with Annex V Section 1.2 of the Water Framework Directive and comparable between Member States.

IC Option: Option to intercalibrate (IC) different national assessment methods.

Method Acceptance Criteria: List of criteria evaluating whether assessment methods can be included in the intercalibration exercise.

Pressure: Human activities such as organic pollution, nutrient loading or hydromorphological modification that have the potential to have adverse effects on the water environment.

Reference/Benchmark sites: Reference sites meet international screening criteria for undisturbed conditions. Benchmark sites meet a similar (low) level of impairment associated with the least disturbed or best commonly available conditions.

Water Framework Directive: Directive 2000/60/EC establishing a framework for Community action in the field of water policy.

### *Abbreviations:*

AIS: Automatic Identification System

BOD: Biological oxygen Demand

BG: Bulgaria

DET: Detergents

EQR: Ecological Quality Ratio

GIG: Geographic Intercalibration Group

GIS: Geographical Information System

HMET: Heavy metals

IC: Intercalibration

MS: Member State

POCA: Principal Component Analysis

PHE: Phenols

PIBS: Pressure index for the Black Sea

RO: Romania

SS: Suspended Solids

TPH: Total petroleum hydrocarbons

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