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# Coastal waters Black Sea geographic intercalibration group

*Macroalgae and angiosperms ecological assessment methods*

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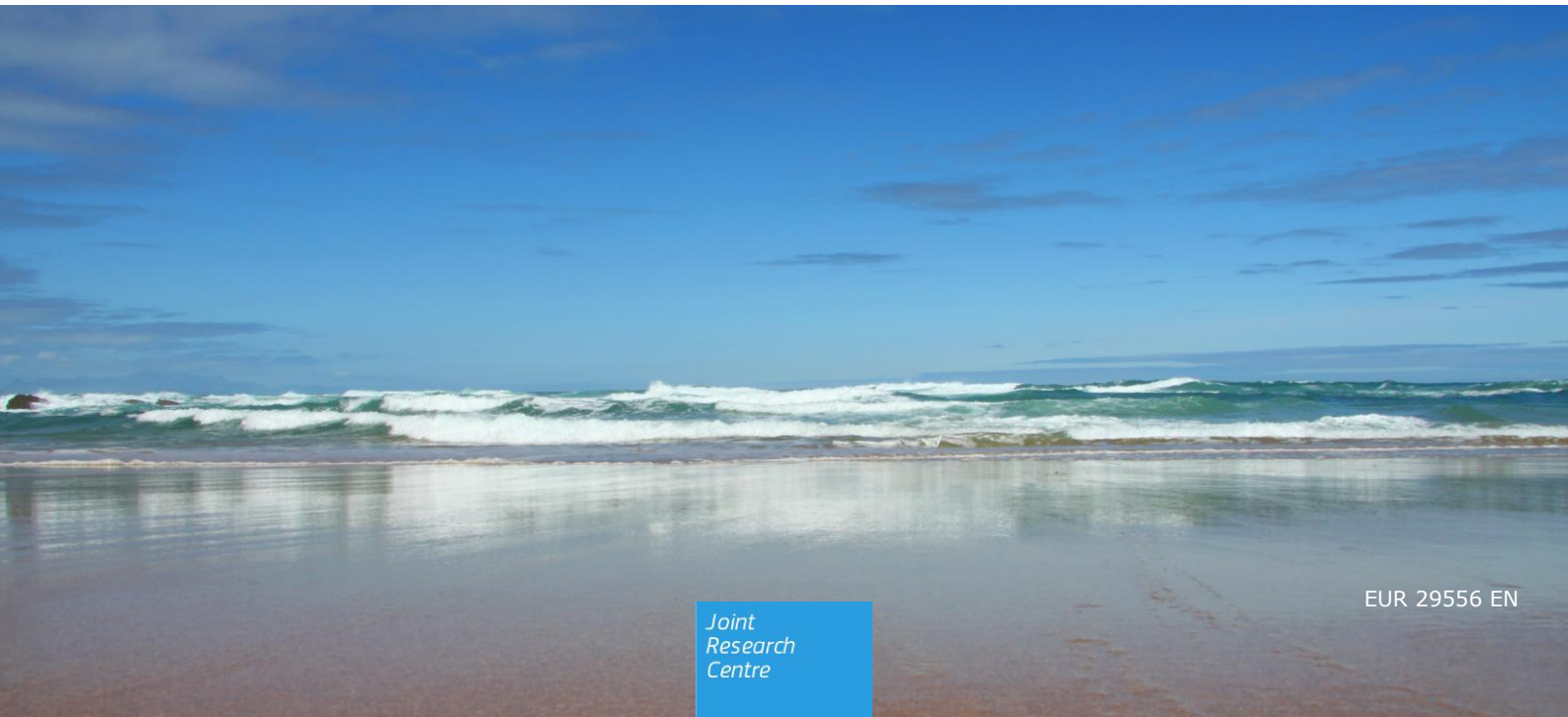

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## Abstract

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 Macroalgae and Angiosperms ecological assessment methods.

# 1. Introduction

This report constitutes a description of the Intercalibration Exercise – Phase 3 (IC3) implemented for macroalgae and angiosperms 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 macroalgae and angiosperms 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 macroalgae and angiosperms 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 | Ecological Index (EI) | Yes |
| Bulgaria | Ecological Evaluation Method cont. formula (EEI-c) | No |
| Romania | Ecological Index (EI) | Yes |

## 2.1 Methods and required BQE parameters

* EI method description (Dencheva&Doncheva, 2014):

Similarly to the Ecological Evaluation Index (Orfanidis *et al*., 2011), several ecological groups were differentiated taking into account the peculiarities of species structure in the Black Sea. Low temporal stability of the environment and physical disturbances explain the lower complexity of benthic macroalgal communities in the near shore Black Sea region. Biotic interactions (grazing press) play a minor role in controlling benthic communities and the community dynamics is mainly due to abiotic forcing. Eutrophication is ranked among the most serious threats to species diversity. In high eutrophication conditions, macrophytobenthic communities obtain very simplified patchy structure, with monospecific character and prevalence of tolerant species.

Species are classified in two main groups, divided in 7 categories: ESG I (sensitive, slow-growing, perennial species) with three subcategories, and ESG II (tolerant, fast-growing opportunistic species) with 4 subcategories. Sensitivity of species was determined based on literature data (Berov, 2013; Berov *et al*., 2012; K. Dencheva, 2008; Diez *et al*., 2012; Kalugina-Gutnik, 1975; Marin *et al*., 2013; Minicheva, 1998; Minicheva *et al*., 2003; Orfanidis *et al*., 2003; Pinedo *et al*., 2007) and authors' own observation and experience. Main criteria in differentiating the species into sensitivity groups was species morphology, biology and growth rates, as well as experimental and observational evidence of their sensitivity to eutrophication, based on experimental results on their distribution along the eutrophication gradient. Salinity, light and temperature adaptation of macrophytes were also taken into account.

ESG I comprises species with thick or calcareous thallus, low growth rates and long life cycles (perennials, some annuals), whereas ESG II includes sheet-like and filamentous species with high growth rates and short life cycles (annuals, seasonal) (Orfanidis *et al*. 2011). ESG I and ESG II were divided into subgroups as follows:

1. ESGIA – *Cystoseira bosphorica, Zostera marina,* form one group that represents slow-growing, sun adapted species with a thick, differentiated and angiosperm thallus and long life histories. They form late-successional communities, mainly in pristine and high irradiance environments, due to their high demands for light (*Cystoseira bosphorica*). *Phillophora crispa*, shade adapted scyophylic red macroalgae, with fleshy thallus is also classified in this group.
2. ESGIB include species with faster than the ESGIA species growth rates, with a coarsely branched, fleshy thallus, with lower adaptive plasticity, with less sensitivity to eutrophication pressures. These include - *Cystoseira barbata*, *Stilophora* spp.- *Laurencia* spp.*., Nemalion helmintoides.*They form communities in pristine environments. The non-calcareous crusts such as *Ralfsia* can be classified within this group also. In the specific ecological conditions of the Black Sea *Cystoseira barbata* has higher tolerance towards eutrophication impacts in the Black sea than *C. bosphorica*, hence it was placed in this category (see Kalugina-Gutnik, 1975; Berov *et al*., 2012; Berov, 2013) . The perennial angiosperms *Zannichelia palustris* and *Zostera noltii* are alsoclassified within this group.

3. EGIC - Corallina spp., *Halipthon virgatum, Hildenbrandia rubra, Gelidium spinosum form* one group that represents slow growing, shade-adapted calcareous jointed and crustose species as well as coarsely branched fleshy species. (ESG IC). They form late-successional communities in pristine and rarely are spread in moderately degraded coasts. Phymatholiton, *Pterocladiella capillacea and* Zanardinia *typus* are also classified in this group.
4. ESGIIA Polysiphonia spp.*, Lomentaria clavellosa,* Gracilaria spp. form group of faster growing species than ESGI, adapted to limited eutrophication impacts. They are also sun and shade adapted, coarsely branched filamentous and sheet like species which can grow in pristine and moderately degraded environments and rare in low conditions. They are with high adaptation abilities.
5. ESGIIB - Ceramium spp.*,* Acrochaetium spp., representatives of this group are sun and shade adapted, fast growing, filamentous species. Some of them grow in all environments; however the species are with high abundances in waters of high, good and moderate conditions.
6. The angiosperm *Ruppia maritima*, which is perennial, with slow growth rate and grows in all the environments, found with high abundance in more eutrophicated areas with different salinity (14-17 psu) is classified in this group also.
7. ESGIICa – *Chaetomorpha aerea, Ulva rigida, Ulva linza, Porphyra leucostica form* group that represents fast growing, thin filamentous and sheet - like species with high reproductive capacity and short life histories. They can grow in all environments but are abundant in highly degraded environments and disappear in highest degraded environments.
8. ESGIICb – *Cladophora sericea, Cadophora vadorum, Cladophora albida, Cadophora vagabunda, Ulva compressa, Ulva flexuosa, Ulva intestinalis, Urospora, Rhizoclonium, Bacillariophyta, Cyanobacteria* represent faster-growing, than these in ESGIICA thin filamentous and sheet-like species with high reproductive capacity and short life histories. They can grow in all environments but mostly are abundant in highly degraded environments.

*Table 2 Classification typical of Black Sea coastal benthic macroalgal taxa into Ecological Status Groups**

| **Species** | **EI class** | **ESG** |
|---|---|---|
| *Bangiadulcis atropurpurea* | IICb | ESGII |
| *Porphyra leucosticta* | IICa | ESGII |
| *Acrochaetium secundatum* | IIB | ESGII |
| *Acrochaetium virgatulum* | IIB | ESGII |
| *Stylonema alsidii* | IICb | ESGII |
| *Rhodochorton purpureum* | IC | ESGI |
| *Nemalion helminthoides* | IB | ESGI |
| *Gelidium crinale* | IIA | ESGII |
| *Gelidium spinosum* | IC | ESGII |
| *Pterocladiella capillacea* | IC | ESGI |
| *Parviphycus antipai* | IC | ESGI |
| *Phyllophora crispa* | IA | ESGI |
| *Corallina elongata* | IC | ESGI |
| *Haliptilon virgatum* | IC | ESGI |
| *Corallina officinalis* | IC | ESGI |
| *Hildenbrandia rubra* | IC | ESGI |

| | | |
|---|---|---|
| *Lomentaria clavellosa* | IB | ESGII |
| *Callithamnion corymbosum* | IICb | ESGII |
| *Callithamnion granulatum* | IICb | ESGII |
| *Antithamnion cruciatum* | IIB | ESGII |
| *Ceramium arborescens* | IIB | ESGII |
| *Ceramium circinatum* | IIB | ESGII |
| *Ceramium pedicellatum* | IIB | ESGII |
| *Ceramium virgatum* | IICa | ESGII |
| *Ceramium secundatum* | IIB | ESGII |
| *Ceramium diaphanum* | IIB | ESGII |
| *Ceramiumm diaphanum* var. elegans | IIB | ESGII |
| *Ceramium tenuicorne* | IIB | ESGII |
| *Apoglossum ruscifolium* | IA | ESGI |
| *Eupogodon spinellus* | IICb | ESGII |
| *Polysiphonia subulifera* | IIA | ESGII |
| *Polysiphonia elongata* | IIA | ESGII |
| *Polysiphonia fucoides* | IIA | ESGII |
| *Polysiphonia denudata* | IICa | ESGII |
| *Polysiphonia opaca* | IIA | ESGII |
| *Feldmannia irregularis* | IICb | ESGII |
| *Ectocarpus siliculosus* | IICb | ESGII |
| *Corynophlaea flaccida* | IIA | ESGII |
| *Corynophlaea umbellata* | IIA | ESGII |
| *Myriactula rivulariae* | IIA | ESGII |
| *Punctaria tenuissima* | IB | ESGI |
| *Stilophora tenella* | IB | ESGI |
| *Stilophora nodulosa* | IB | ESGI |
| *Zanardinia typus* | IC | ESGI |
| *Sphacelaria cirrhosa* | IIA | ESGII |
| *Cystoseira barbata* | IB | ESGI |
| *Cystoseira crinita* | IA | ESGI |
| *Bryopsis plumosa* | IICb | ESGII |
| *Bryopsis hypnoides* | IICb | ESGII |
| *Chaetomorpha aerea* | IICa | ESGII |
| *Chaetomorpha linum* | IIA | ESGII |
| *Cladophora albida* | IICb | ESGII |
| *Cladophora coelothrix* | IICb | ESGII |
| *Cladophora sericea* | IICb | ESGII |
| *Cladophora vadorum* | IICb | ESGII |

| | | |
|---|---|---|
| *Cladophora vagabunda* | IICb | ESGII |
| *Ulothrix flacca* | IICb | ESGII |
| *Ulva intestinalis* | IICb | ESGII |
| *Ulva linza* | IICa | ESGII |
| *Ulva compressa* | IICb | ESGII |
| *Ulva flexuosa* | IICb | ESGII |
| *Ulva rigida* | IICa | ESGII |
| *Ulva prolifera* | IICb | ESGII |
| *Osmundea pinnatifida* | IB | ESGI |
| Colpomenia sp. | IC | ESGI |
| | | |
| Bacillariophyta | ICb | ESGII |
| Cyanobacteria | ICb | ESGII |
| | | |
| *Zostera marina* | IA | ESGI |
| *Zostera noltei* | IB | ESGI |
| *Zannichellia palustris* | IB | ESGI |
| *Ruppia maritima* | IIB | ESGII |

**Additional species listed in Dencheva & Doncheva (2014). Changes in species classification in comparison with Dencheva & Doncheva (2014) discussed and approved by index author.*

**Calculation of EI and EI-EQR.**

The assemblage of benthic macrophytes at each transect was assessed according to the biomass (%) of species, dividing samples in the following groups: less than 100% biomass of tolerant species (ESGII), between 0 and 40% biomass of sensitive species (ESGI), 60%-80% and above 80% biomass.

The average biomass of sensitive (ESGI) and tolerant (ESGII) species from all the samples collected from replicate transects is calculated. The index is expressed as the proportion of sensitive and tolerant species average biomasses at each transect. As a value of EI, we take the biomass proportion of the most sensitive group. EI takes values in the range of 0-10, divided in five classes: 0-2 bad status, > 2-4 poor status, > 4–6 moderate, > 6-8 good and > 7.8-10 high status (equidistant division of classes).

**Ecological status group value**

The proportion each ESG group within the two main groups ESG I and ESG II was corrected with a coefficient. The criteria for this correction were distribution along the eutrophication gradient, phenotypic plasticity and growth rate.

Weight coefficients were defined for different subgroups as follows:

ESG IA–coef=1

ESGIB-coef=0.8

ESGIC-coef=0.6

ESGIIA-coef=0.6

ESGIIB-coef=0.8

ESGIIC-coef=1

After assessing the biomass proportion of every group, it is multiplied by the coefficient of this group, as follows:

**ESGI= ESGIA*1+ESGIB*0.8+ESGIC*0.6**

**ESGII=ESGIIA*0.6+ESGIIB*0.8+ESGIIC*1**

**EI calculation**

To calculate the value of EI we apply the following rules and formulas:

When ESGI = 0 - Bad status, we take the most sensitive group left from ESGII.

In case when ESGI = 0, ESGII (A+B) = 0 and ESGIICa has biomass proportion from 0 - 100%, EI takes values of 0 – 1, and is calculated with the following formula:

**(1) EI- bad (0-1) = [ESGIICa/ESGII], when ESGI=0, ESGII(A +B) = 0**

When ESGI= 0 and 0%>ESGII(A+B)≤ 100% we have EI with bad status (1-2). In this case the index is expressed as the biomass proportion of the most sensitive subgroup selected from ESGII. The following formula is used:

**(2) EI- bad (1-2)=[(ESGIIA/(ESGIIA+ESGIIB+ESGIIC))*0.6+ (ESGIIB/(ESGIIA+ESGIIB+ESGIIC)*0.8]+1**

When the proportion of sensitive species (ESGI) is between 0 - 40%, EI takes values between 2 - 4 and we have a poor status. The following formula is applied:

**(3) EI poor (2-4)=([(ESGIA/(ESGI+ESGII))*1+(ESGIB/(ESGI+ESGII))*0.8+ (ESGIC/(ESGI+ESGII))*0.6]*5)+2**

When the proportion of sensitive species is between 40-60%, EI is between 4-6. At 60-80% biomass proportion, EI is between 6 - 8 and at 80 - 100%, EI is between 8 and 10. In these three cases EI is calculated following this formula:

**(4) EI high, good, moderate (4-10)=[(ESGIA/(ESGI+ESGII))*1+ (ESGIB/(ESGI+ESGII))*0.8+(ESGIC/(ESGI+ESGII))*0.6]*10,**

For example, if the proportion of sensitive species (ESGI) is 75% or ESGI/ESGI+ESGII = 0,75, then EI high-good-moderate = 10*[ESGI/ESGI+ESGII] = 0,75*10.

When we have 39% biomass proportion of sensitive species then EI poor = 5*[ESGI/ESGI+ESGII]+2 = [5*0,39]+2.

Another example, in the case when ESGI=0 and [ESGII(A+B)/ESGII(A+B)+ESGIIC] = 48% = 0,48, then EI bad(1-2) = [ESGII(A+B)/ESGII(A+B)+ESGIIC]+1 = 0,48+1 = 1,48.

When ESGI = 0, ESGII (A+B) = 0 and [ESGIICa/ESGII] = [ESGIICa/ESGIICa+ESGIICb] = 27%, then EI bad (0-1) = [ESGIICa/ESGII] = 0,27.

To ensure comparability in accordance with the WFD (REFCOND, 2003), the EI values ranging from 0 to 10 can be transformed into Ecological Quality Ratios(EQR) from 0 to 1, i.e. the ratio between the value of the observed biological parameter and the expected value under the reference conditions, as follows:

EI EQR=(EI value/RC value) (Table 2)

Referent value is RC=9,32 (see Chapter National Reference Conditions for details)

**EI-EQR high-good-moderate=[10* (ESGI/ESGI+ESGII)]/ref value;**

**EI-EQR poor=[5*(ESGI/ESGI+ESGII]+2]//ref.value;**

**EI-EQR bad (1-2)=(ESGII(A+B)/ESGII+1)/ref.value, when ESGI=0;**

**EI-EQR bad (0-1)=(ESGIICa/ESGII)/ref. value, when ESGI=0, ESGII(A+B) = 0.**

For example, if EI bad (0-1) =0,27, then EI-EQR=EI/ref value = [ESGIICa/ESGII]/ref. value= 0,27/9,32=0,029.

In cases when EI-EQR> 1, we equate the value to 1. For example if EI= 9,7 then EI-EQR=[10* (ESGI/ESGI+ESGII)]/9,3 , or EI-EQR=9,7/9,32= 1,04 , hence we equate EI-EQR=1.

*Table 3 Biomass proportions of sensitive (ESGI) and tolerant species (ESGII), EI values and EI-EQR of macrophytobenthic communities for different status classes*

| **Biomass proportions of sensitive and tolerant species** | **EI** | **Ecological state class** | **EI-EQR** |
|---|---|---|---|
| 80-100%ESGI | 7.8-10 | High | 0.837 – 1 |
| 60-80%ESGI | 6-7.8 | Good | 0.644 – 0.837 |
| 40-60%ESGI | 4-6 | Moderate | 0.429 – 0.644 |
| 0-40%ESGI | 2-4 | Poor | 0.214 – 0.429 |
| 0%ESGI | <2 | Bad | <0.214 |

## 2.2 Sampling and data processing

*Table 4 Overview of the sampling and data processing of the national assessment methods included in the IC exercise*

| | |
|---|---|
| Sampling/survey device | Scuba divers sampling/20 by 20 cm quadrant samples. |
| How many sampling/survey occasions (in time) are required to allow for ecological quality classification of sampling/survey site or area? | Once per year in summer months. |
| Sampling/survey months | Summer season (June-September). |
| Which method is used to select the sampling /survey site or area? | Upper infralittoral (0-3 m) typical for the surveyed water body are selected for sampling. |
| How many spatial replicates per sampling/survey occasion are required to allow for ecological quality classification of sampling/survey site or area? | At least 3 samples per depth range, in three depth ranges (0-1, 1-2 and 2-3 m) are sampled from infralittoral macroalgal communities at each sampling station. Several sampling stations per water body are selected, taking into account variations in geomorphology (different exposure to wave action), local sources of pollution and other natural and anthropogenic factors. |
| 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 | 9 samples per sampling station, up to 3 sampling stations per survey area, giving a total of 9 to 27 samples per survey station. |
| Short description of field sampling/survey procedure and processing (sub-sampling) | Sampling is carried out at three depth ranges within a 10 by 10 m sampling area. At each depth range 20/20 cm random samples are collected with a metallic frame and a scraper, all plant material is placed in labelled plastic bags and transported to the lab in cooler box or fridge. Samples are then immediately processed, or preserved in freezer at – 20 C, or fixed in ethanol (75%), or in formalin (4%). Species identification of macroalgae is carried out to the lowest possible taxonomic level under a light microscope (species, genus). Each species is weighed on a digital scale with precision to the second sign after comma. Only species with 0,5 g.m$^{-2}$ and higher |

| | |
|---|---|
| | biomass are taken into account. Species are classified in Ecological Sensitivity Groups (ESGI - sensitive and ESGII - tolerant species), subdivided in 7 subcategories (see Table 2). |

## 2.3 National reference conditions

*Table 5 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 | Expert knowledge, Historical data<br>EI: The above is valid for EI.<br>For establishment of values for reference conditions, current data from a real reference site as well as some historical data is taken into account. | 2 reference zones:<br>Cape Maslen Nos area ( in High status since 1996, and before 1980 (Berov, 2013; Berov *et al.*, 2010; K. Dencheva, 2008; Petrova-Karadjova, 1975; Vasilev *et al.*, 2005; Zinova and Dimitrova-Konaklieva, 1975);<br>Sinemorets-Varvara area – in high status since first surveyed in 2007 (no prior recent data available). | Southern Bulgarian Black Sea coast (see Figure 2). | Macroalgal communities should be dominated by brown algae from the genus Cystoseira. No anthropogenic pressures are registered in the Cape Maslen Nos area since 1996, and before 1980 (Kalugina-Gutnik, 1975; Petrova-Karadjova, 1975; Dimitrova-Konaklieva, 2000; Vasilev *et al*., 2005; Bologa and Sava, 2006; Dencheva, 2008b; Berov *et al.*, 2010, 2012,2013) (pristine conditions).<br>Reference sites have been identified according to the low pressures and impacts they receive in accordance with Annex V of WFD. Criteria used:<br>• population density: no settlement with more than 1000 in/km$^2$ in the next 15 km and/or more than 100 habitats/km$^2$ in the next 3 km within that area (winter population).<br>• no more than 10% of artificial coastline<br>• no harbour (more than 100 boats) in 3 km<br>• no beach regeneration within 1 km<br>• no industries within the 3 km<br>• no fish farms within the 1 km<br>• no desalination plants within 1 km<br>• no evidence of *Cystoseira* forest regression due to other unconsidered impacts; if there is evidence of *Cystoseria* regression (for example due to overgrazing), the quality element macroalgae index may not be applied, depending on the method used). |

| Member State | Type and period of reference conditions | Number of reference sites | Location of reference sites | Reference criteria used for selection of reference sites |
|---|---|---|---|---|
| Romania | Expert knowledge, Historical data.<br>Reference conditions from reference site in Bulgaria are used. | Reference conditions from reference site in Bulgaria are used. | No real reference site is found along the Romanian Black Sea coast within the surveyed water bodies. | None of the sampling sites along the Romanian Black Sea coast meet the Annex V WFD criteria for pristine conditions. Reference conditions from reference site in Bulgaria are used. |

Reference values for the calculation of the EI-EQR ref. cond. value were determined following the IC option where we have presence of true reference sites within the IC exercise common water body type areas (Annex III of the IC guidelines). Samples with type-specific reference conditions biological communities matching the ecological description of communities in high status (see Chapter 6 Ecological Characteristics) were present in sufficient numbers in the data set used in the exercises. This data set includes 6 sampling sites, with samples from 5 different years (between 2006 and 2013), giving a total of 14 sampling events with a data set of 97 samples. The natural variation of community structure and species quantities in this dataset was determined to be small enough to establish reliable type-specific reference conditions from the available data. Samples collected from mediolittoral macroalgal communities at the 0-1 m depth interval were not included in the data set, as they are not representative for the type-specific infralittoral communities.

Extensive mapping of infralittoral communities within the referent site supports our conclusions that the selected sampling sites contain typical for the area infralittoral macroalgal communities, which are the *Cystoseira bosphorica* Sauv. and *Cystoseira barbata* communities with very little presence of ESGII species (see Berov, 2012,2013 for detailed description and mapping data).

The value of the ref. cond. was calculated using the median value of the dataset from the referent sites, giving a value of **9,32**.

## 2.4 National boundary setting

*Table 6 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 | Expert judgment<br>Boundaries are set according to biotic index (EI) and to community structure. The dominance of the late-successional species of species *Cystoseira bosphorica* and *Cystoseira barbata* form communities indicative of pristine state, which is characterized by low nutrient concentrations and clear water conditions. In terms of quantities of species, communities in high status should be strongly dominated in their average | Expert judgment on biological criteria EI=7.8: (see Table 3) | Expert judgment on biological criteria EI=6: (see Table 4) | Yes, quantitative tests |

| 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 |
|---|---|---|---|---|
| | biomass by ESGIa (*C. bosphorica* ≥ 78% of the average biomass) or ESGIb species (*C. barbata*≥ 97% of the average biomass). (See Figure 1 for a conceptual model of community change under increasing eutrophication pressures.)<br>The boundary between Good/Moderate is set by an equidistant division of the numerical space between the H/G boundary and 0. The G/M boundary value of 6 corresponds to a macroalgal community dominated in their average biomass by ESGIa (*C. bosphorica* ≥ 60% of the average biomass) and ESGIb species (*C. barbata*≥ 75% of the average biomass ) and presence of ESGII species from the genus Ulva, Cladophora, Ceramium and others in moderate quantities (30-40% of the average biomass).<br>Communities in transitioning from moderate to poor status (EI <4) are dominated by ESGII species (average biomass between 40 and 50 %) and still have some ESGI species present, but degrading (average biomass between 40 and 50%). (See Figure 1 for a conceptual model of community change under increasing eutrophication pressures.)<br>The dominance of opportunistic green and red macroalga from the genera Ulva, Cladophora, Ceramium, and Cyanobacteria films form communities indicative of degraded state, which is characterized by high nutrient concentrations, low water transparency (see (Berov, 2013; Berov *et al*., 2010; Dencheva, 2008). Communities transitioning from Poor to Bad status are those dominated by ESGIIa and ESGIIb with measureable presence of ESGIIc species (>1%). Communities in Bad status are strongly dominated by ESGIIc species. (See Figure 1 for a conceptual model of community change under increasing eutrophication pressures.) | | | |
| Romania | Same as in Bulgaria | Same as in Bulgaria | Same as in Bulgaria | Yes, quantitative tests |

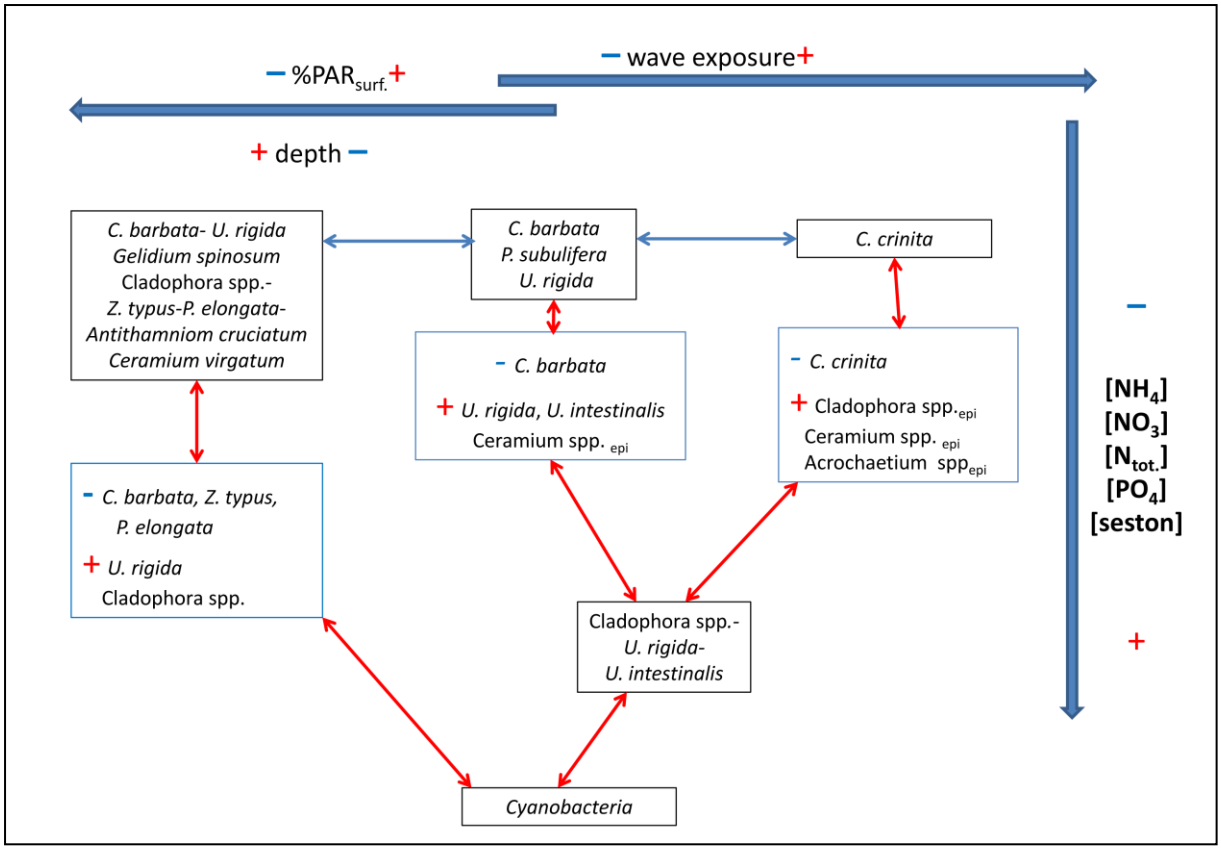


*Figure 1 Conceptual model of the change in the structure of macroalgal communities in the study area under the influence of eutrophication-related and natural environmental factors in the SW Black Sea infralittoral zon.*

*Note: Blue arrows indicate shifts between communities under the influence of 'natural' factors, red arrows – shifts triggered by the influence of eutrophication-related factors (taken from Berov, 2013).*

## 2.5 Results of WFD compliance checking

*Table 7 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 are set in line with the WFD's **normative definitions** (**Boundary setting procedure**) | Yes |
| Scope of detected pressures | LUSI ranges between 0 (reference sites) to 7,5 (in waterbodies with bad status) |
| Has the pressure-impact relationship of the assessment method been tested? | Yes |

| Compliance criteria | Compliance checking conclusions |
|---|---|
| Setting of ecological status boundaries: methodology and reasoning to derive and set boundaries | Boundaries are set according to biotic index (EI) and to community structure. The dominance of the late-successional species of the genus *Cystoseira* form communities indicative of pristine state, which is characterized by low nutrient concentrations and clear water conditions, whilst the dominance of opportunistic green and red macroalgae from the genera *Ulva, Cladophora, Ceramium*, and Cyanobacteria films form communities indicative of degraded state, which is characterized by high nutrient concentrations, low water transparency (Berov, 2013; Dencheva, 2008; Kalugina-Gutnik, 1975). The coexistence of the late-successional like *Cystoseira* spp. with opportunistic species from the generae *Ulva, Cladophora, Ceramium,* form communities that are indicative intermediate (moderate) conditions. Equidistant division of the EI and EQR (see Table 6 for details). |
| 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? | (1) See section on Pressures addressed |
| Reference and Good status community description:<br>Is a description of the communities of reference/high – good – moderate status provided? | Yes (see Section on Ecological characteristics) |
| 3. **All relevant parameters** indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole | Yes |
| Complete list of biological metric(s) used in assessment | Fresh biomass |
| Data basis for metric calculation | |
| Combination rule for multimetrics | |
| 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 | |
| Is the assessment method applied to water bodies in the whole country? | The method was applied in the common type water bodies of Romania and Bulgaria, which includes the whole Bulgarian Black Sea coast, and the Southern section of the Romanian Black Sea coast |
| Specify common intercalibration types | CW1-BL1 |

| Compliance criteria | Compliance checking conclusions |
|---|---|
| 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 | EI – habitat specific |
| Key source(s) to derive reference conditions | (Berov, 2013; Berov *et al*., 2012; Dencheva and Doncheva, 2014; Petrova-Karadjova, 1975) |
| Number of sites, location and geographical coverage of sites used to derive reference conditions | 6 C. Maslen Nos, and Varvara-Sinemorets area |
| Time period (months+years) of data of sites used to derive reference conditions | 8.2006, 9.2007, 10.2008, 7.2009, 6.2010, 9.2011, 6-7-8.2012, 8-9.2013 |
| Reference site characterisation: criteria to select them | See Table 5 |
| Is a true reference used for the definition of High status or an alternative benchmark estimation? | |
| 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 | Sample replication (min. 9 samples per site, numerous sites per water body), tests of representativity of sampling with species-area accumulation plots, seasonal sampling for establishment of natural seasonal variations |
| 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 (species level) |
| Minimum size of organisms sampled and processed | Bg and Ro: fresh biomass greater than 0,5 g.m-2 |
| Record of biological data: level of taxonomical identification – what groups to which level | Bg and Ro: most to species level, in some specific cases (the genera Ulva, Cladophora, Ceramium) – to genus level |

**General conclusion of the compliance checking:**

Compliance criteria are met. EI meets the requirements stated in the WFD IC Guidance (2.1. WFD compliance criteria). Good ecological status boundaries EI complies with the WFD normative definitions.

# 3. Results IC Feasibility checking

## 3.1 Typology

*Table 8 The intercalibration is feasible in terms of typology*

| Method | Appropriate for IC types/subtypes | Remarks |
|---|---|---|
| EI | The method was applied to infralittoral rocky coastal habitats in mesohaline Black Sea coastal waters at depths between 0 and 3 m | The method is feasible for the most common coastal water bodies typology in Bulgaria and Romania |

## 3.2 Pressures addressed

A modified version of the LUSI pressure index was applied (Flo *et al*., 2011), which was adapted to the specific conditions of the W Black Sea coast and local anthropogenic pressures on macroalgal communities (LUSI_BS). Pressure values for direct impacts were calculated based on land usage from the Corine Land Cover 2006 database (the most recent available for Bulgaria and Romania), in accordance with Flo *et al*. (2011). Taking into account the strong local influence of inputs of nutrients from land by wetlands and rivers outflow, land use was calculated for watersheds adjacent to the sampling sites along the coast. In coastal areas with no river beds and watersheds (N Bulgarian coast of Dobrudja, Romanian coast), land use was determined in land territories ~5 km around the sampling stations. In both cases LUSI_BS values were calculated based on 3 km buffers of the evaluated territories, using the scoring table of Flo *et al*. (2011) for percentage of different categories land use. Indirect impacts were assessed, adding additional scores (0 to 3), based on data for nitrogen and phosphorus inputs from point sources (waste water treatment plants, untreated waters, river inputs), proximity to major ports and touristic centers, and proximity to water bodies in degraded state. A correction number, based on the shape of the coastline and possible confinement of water circulation was also applied (as in Flo *et al*. (2011)).

The final LUSI score was calculated with the following formula:

**LUSI_BS= (score urb + score agric + score indust + score typology + others significant pressures) * correction number**

*Table 9 Values of LUSI_BS (3 km buffer of watersheds) for sites in Bulgaria and Romania included in the IC exercise*

| Site | Country | LUSI_BS |
|---|---|---|
| Burgas | BG | 7,5 |
| Byala | BG | 4 |
| Galata | BG | 7,5 |
| Irakli | BG | 1 |
| Kavarna | BG | 2 |
| Kranevo | BG | 4 |
| Krapets | BG | 4 |
| Kraymorie | BG | 7,5 |
| Nesebar | BG | 3,75 |
| Paraskeva | BG | 0 |
| Pochivka | BG | 7,5 |
| Rusalka | BG | 2 |

| | | |
|---|---|---|
| Shabla | BG | 2,25 |
| Sinemorets | BG | 1 |
| Sozopol | BG | 2 |
| Varvara | BG | 0 |
| Vatahori | BG | 0 |
| 2May | RO | 4 |
| Costinesti | RO | 5 |
| EforieSud | RO | 6 |
| Tuzla | RO | 4,5 |
| VamaVeche | RO | 3 |

*Table 10 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 | EI | LUSI_BS | LUSI_BS value as indication of direct impacts (agriculture, industry, urban areas) and indirect impacts (sewage outfall, riverine input, tourism, harbours) in coastal area | 17 sites | Linear regression ($p<0,01$) |
| Romania | EI | LUSI_BS | LUSI_BS value as indication of direct impacts (agriculture, industry, urban areas) and indirect impacts (sewage outfall, riverine input, tourism, harbours) in coastal area | 5 sites | Linear regression ($p<0,01$) |

**Relationships between the methods and the pressures**

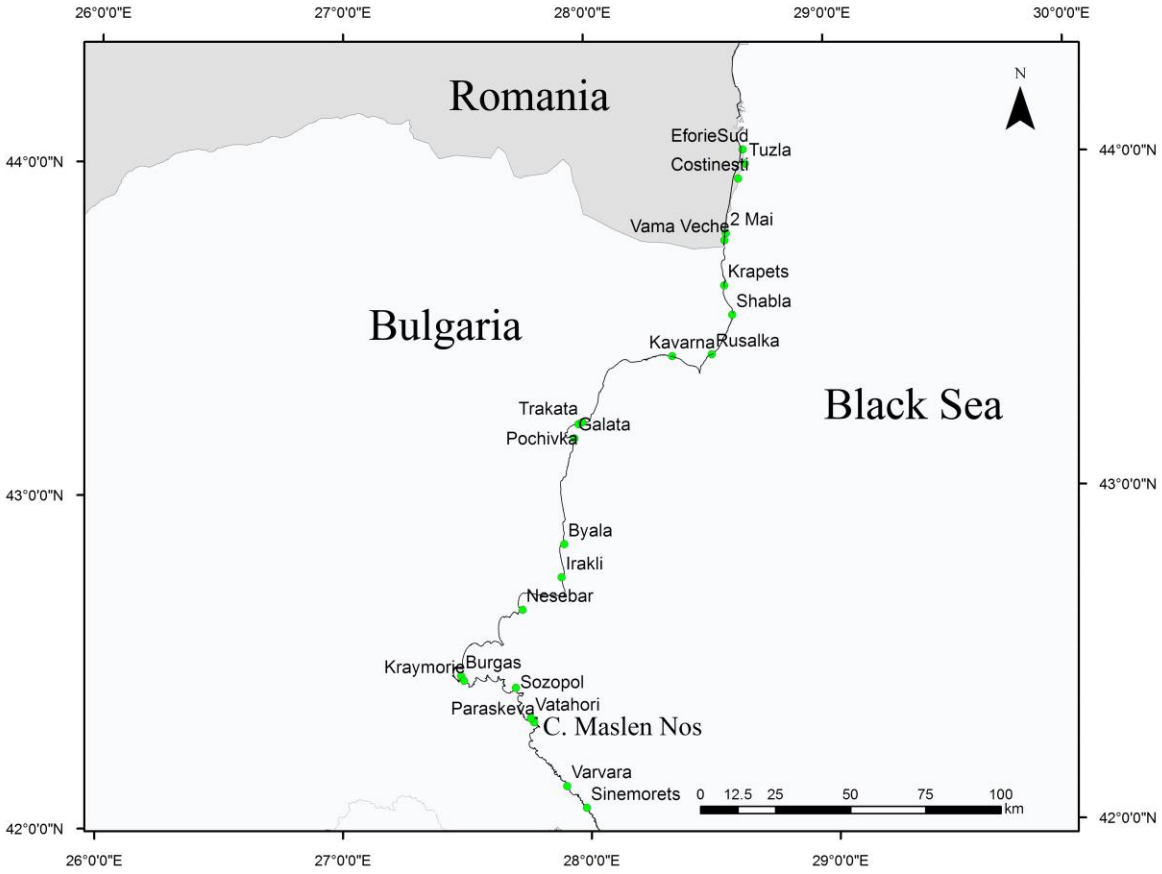


*Figure 2 Sampling sites in Romania and Bulgaria. The location of the referent site C. Maslen Nos is marke*

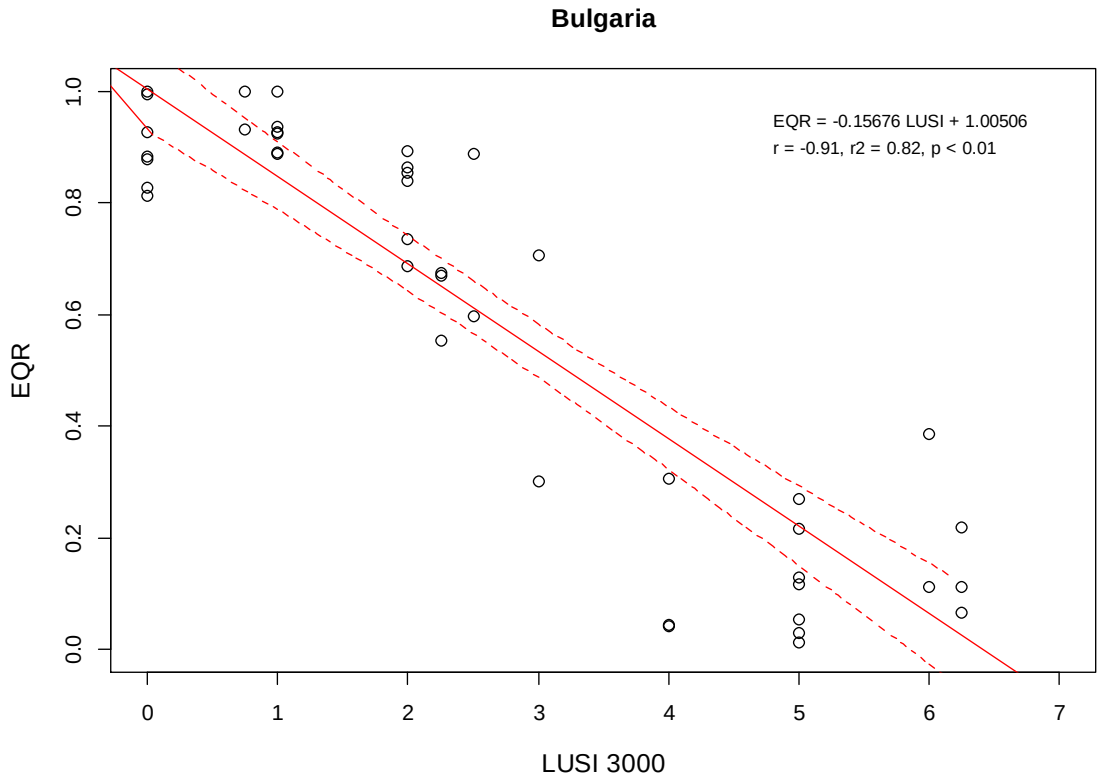


*Figure 3 Pearson linear correlation between EI EQR values and LUSI_BS (3000 m buffers) for sites in Bulgaria ($p<0,01$ $r^2=0,82$)*

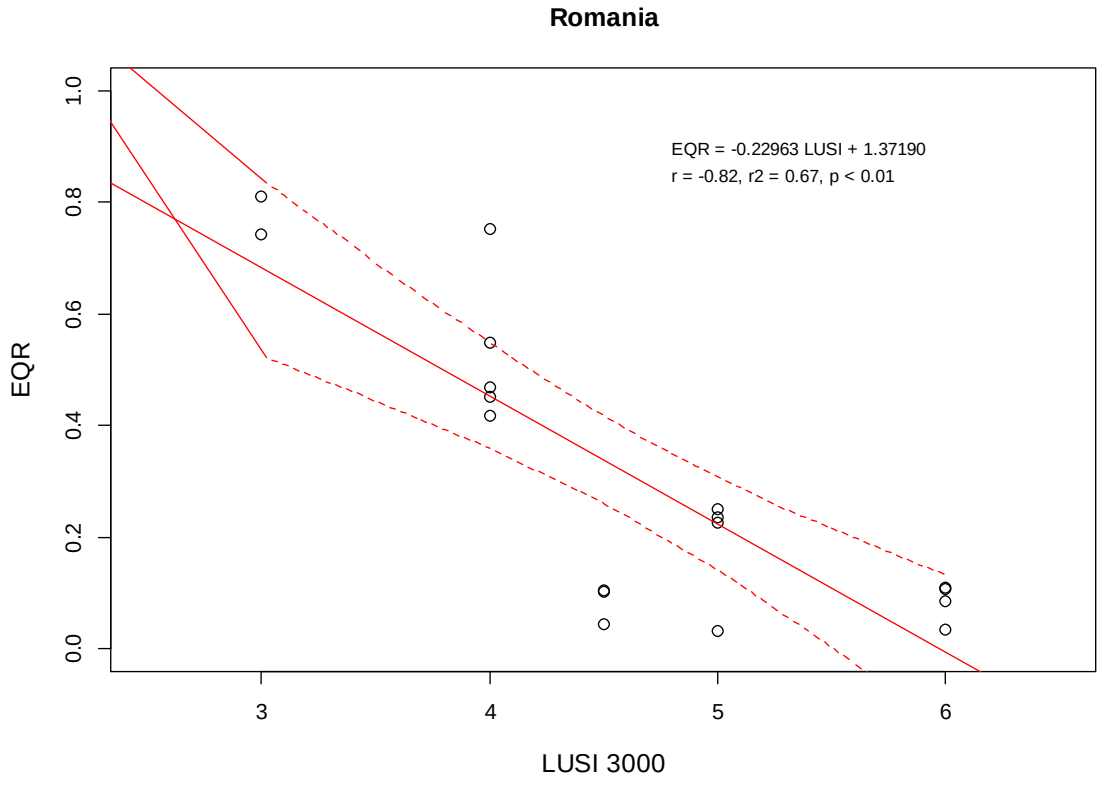


*Figure 4 Pearson linear correlation between EI EQR values and LUSI_BS (3000 m buffers) for sites in Romania ($p<0,01$ $r^2=0,67$)*

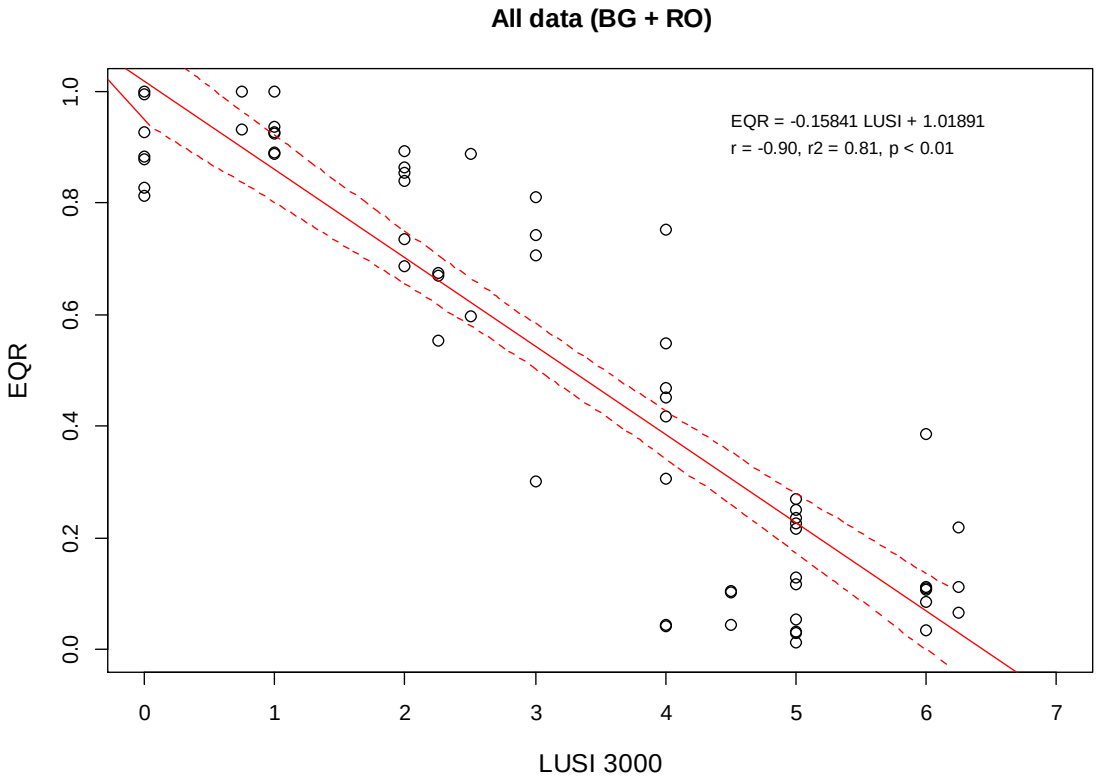


*Figure 5 Pearson linear correlation between EI EQR values and LUSI_BS (3000 m buffers) for sites in Bulgaria and Romania ($p<0,01$ $r^2=0,81$)*

| **Method** | **Pressure** |
|---|---|
| EI | eutrophication, pollution by organic matter, siltation, general habitat degradation |
| **Conclusion**<br>The Intercalibration is feasible in terms of **pressures** addressed?<br>Yes the intercalibration addresses all the above pressures. In both Romania and Bulgaria there is a good correlation between LUSI_BS and EI_EQR values. | |

## 3.4 Assessment concept

| **Method** | **Assessment concept** |
|---|---|
| EI | Late-successional vs. opportunistic species proportion |

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

The intercalibration is feasible in terms of assessment concept as the same method was used in both countries in the same water body typology, in the same zone (upper infralittoral on rocky shores), measuring coverage of macroalgae and reveal the response of benthic macrophytes to anthropogenic stress. The studied macrophyte communities have very similar species composition in both countries and exist under the same ecological conditions, reacting in the same manner to the studied anthropogenic pressures (see Figure 6).

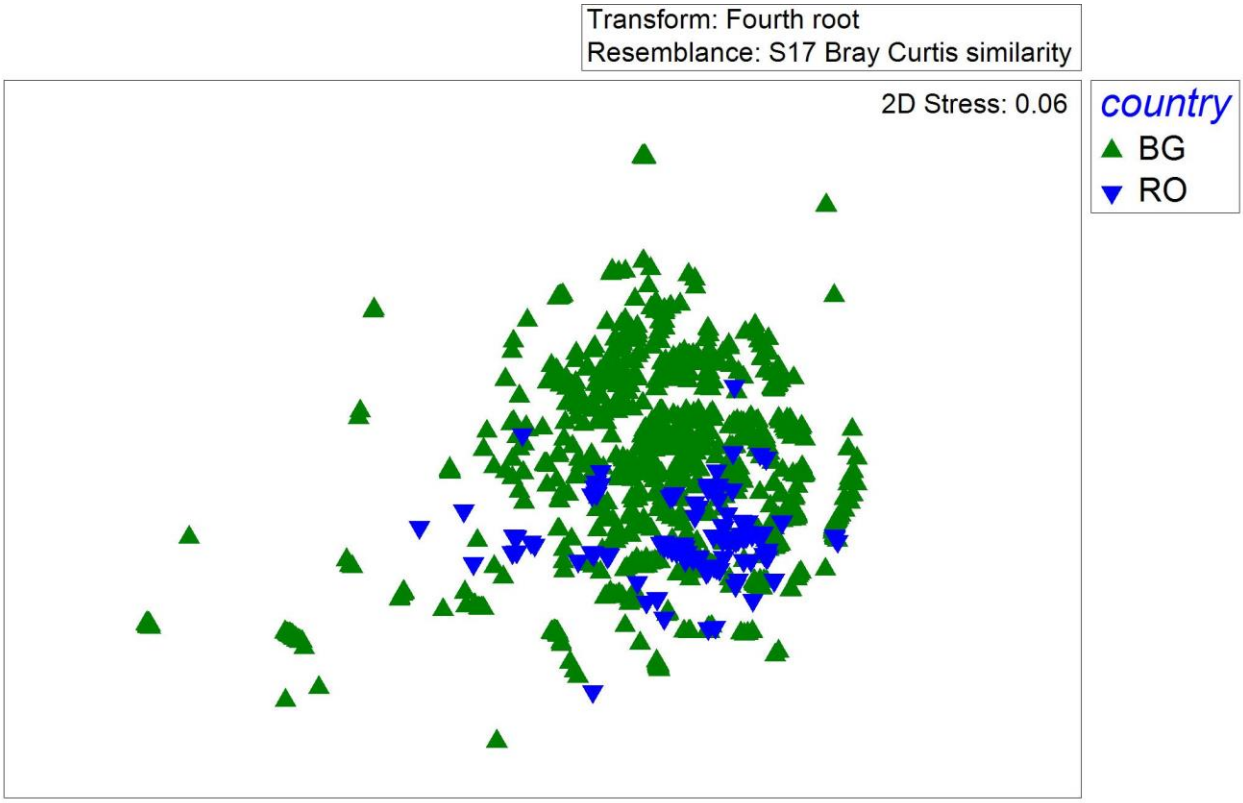


*Figure 6 MDS plot of the Bray-Curtis similarity matrix of samples from Bulgaria and Romania included in the IC data set. Dataset includes 1281 samples from Bulgaria and 172 samples from Romania*

# 4. Collection of IC dataset and benchmarking

## 4.1 Dataset description

*Table 11 Description of the data collection within the GIG*

| | |
|---|---|
| Size of common dataset: total number of sites | 24 |
| Number of Member States | 2 |
| Repackage/disaggregation of samples/WB results? | No |
| Gradient of ecological quality | Fully covered: from degraded ('bad' status) to pristine ('high' status) |
| Coverage per ecological quality class | All five ecological classes are covered for Bulgaria and Romania. In Romania the 'high' class was only established at the site Vama Veche in 2014, where a tendency for improvement of the state is present. |

*Table 12 Overview of the data set*

| **Member State** | **Number of sites or samples or data values** | | |
|---|---|---|---|
| | **Biological data** | **Physico- chemical data** | **Pressure data** |
| Bulgaria | EI(1281 samples from 19 sites) | Yes: from national monitoring programs of water quality | According to the modified LUSI_BS methodology |
| Romania | EI(172 samples from 5 sites) | Yes: from national monitoring programs of water quality | According to the modified LUSI_BS methodology |

## 4.2 Data acceptance criteria

*Table 13 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) | Sampling in both Bulgaria and Romania was carried out following the same sampling design and seasons |
| The sampling and analytical methodology | Destructive sampling with frame |
| Level of taxonomic precision required and taxalists with codes | Macroalgal species are sorted in 2 morphofunctional groups subdivided in 7 categories |
| The minimum number of sites/samples per intercalibration type | One intercalibration type, no required minimum number of sites/samples |
| Sufficient covering of all relevant quality classes per type | All quality classes are covered sufficiently |

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

Reference sites have been identified according to the low pressures and impacts they receive in accordance with Annex V of WFD.

Bulgaria -

Site1. Maslen nos  Site 2. Sinemorets  Site 3.Varvara  Site 4. Arapia  Site 5. Rezovo

Give detailed description of **setting reference condition**s (summary statistics used).

We have defined reference as sites under no  or very low pressures following the criteria:

- population density: no settlement with more than 1000 inhabitants/km2 in the next 15 km and/or more than 100 inhabitants/km2 in the next 3 km within that area (number of inhabitants is restricted to winter population)
- no more than 10% of artificial coastline
- no harbour (more than 100 boats) within 3 km
- no beach regeneration within 1 km
- no industries within 3 km
- no fish farms within 1 km
- no desalination plants within 1 km
- no evidence of *Cystoseira* forest regression due to other unconsidered impacts; if there is evidence of *Cystoseria* regression (for example due to overgrazing), the quality element macroalgae may not be applied, depending on the method used).

# 5. Comparison of methods and boundaries

## 5.1 IC option and common metrics

Both MS use the same assessment method, with same data acquisition, same numerical evaluation, and same boundaries. In fact, no comparability analyses are necessary.

# 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** | |
|---|---|

**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 | EI | 0.837 | 0.644 |
| Romania | EI | 0.837 | 0.644 |

## 6.2 Correspondence common types versus national types

It is not necessary to transform common intercalibration types and common boundaries into the national typologies/assessment systems. The results are directly applicable to the national types that belong to the common type.

# 7. Ecological characteristics

## 7.1 Description of reference or alternative benchmark communities

**Reference communities**

Bulgaria: Well-developed upper infralittoral communities of *Cystoseira crinita* f. bosphorica (=*C. bosphorica* Sauv.,(Berov *et al*., 2015)) on sites exposed to wave action and of *Cystoseira barbata* on sheltered coasts. High biodiversity of pollution- and eutrophication- sensitive brown epiphytic species from the genera Myriactula, Stilophora, and eutrophication-sensitive red epiphytic species such as *Ceramium strictum*, low biomass or complete absence of green opportunistic species from the genera Cladophora, Chaetomorpha and Ulva. Increased depth limit of distribution of *Cystoseira bosphorica* (down to 4-5 m) and of *Cystoseira barbata* (down to 10-14 m) (Berov, 2013; Berov *et al*., 2012). Spatio-temporal variability of the community's composition and abundance affected by hard substrata availability, intensity and frequency of natural disturbances, e.g. hydrodynamism, by seasonal cycle of light period and intensity, and by limiting factors like nutrients.

Romania: no true referent conditions are found in any of the sampling sites; however areas with high and good status have macroalgal communities with similar characteristics as those in the referent sites used in the exercise. These include high biomasses of *Cystoseira barbata*, presence of red macroalgal eutrophication-sensitive species, low biomass or complete absence of opportunistic green and red macroalgae from the genera *Cladophora, Ulva, Ceramium*. Main differences include low biodiversity of epiphytic brown and red macroalgae, low total number of red and brown macroalgae, shallower lower depth limit of distribution of *Cystoseira barbata* (~3 m presently, and down to 5,5 m in the 1970s).

## 7.2 Description of good status communities

Well-developed upper infralittoral communities of *Cystoseira bosphorica* Sauv. on sites exposed to wave action and of *Cystoseira barbata* on sheltered coasts. Presence of some pollution- and eutrophication- sensitive brown epyphytic species from the genera Sphacelaria, Corynophlaea, Myriactula, Stilophora, and eutrophication-sensitive red epiphytic species such as *Acrochaetium secundatum*, *Ceramium strictum*, as well as abundant presence eutrophication-tollerant red macroalgae (Gelidium spp., *Ceramium virgatum*), presence in small quantities of green opportunistic species from the genera Cladophora, Chaetomorpha and Ulva. Decreased depth limit of distribution of *Cystoseira barbata* and *Cystoseira crinita* in comparison with reference sites.

# 8. Conclusion

Romania and Bulgaria have proposed the same assessment method for the common type CW BL1: this method meets the WFD compliance criteria, and responds to the general degradation.

A proposal for common class boundaries (Table 14) has been established in basis on a common dataset built for the BQE 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 type.

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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:*

Agric: Agriculture pressure

BG: Bulgaria

EI: Ecological index

EQR: Ecological Quality Ratio

ESGI: group including sensitive species

ESGII: group including tolerant species

GIG: Geographic Intercalibration Group

GIS: Geographical Information System

IC: Intercalibration

Indust: Industrial Pressure

LUSI: Land Uses Simplified Index

LUSI_BS: LUSI index adapted to the specific conditions of the Black Sea

MS: Member State

RO: Romania

Urb: Urban pressure

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